POSSIBLE ROLE OF ADENOSINE 5'-TRIPHOSPHATE IN THE CARDIOVASCULAR SYSTEM OF THE RAT

by

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ABSTRACT

The experiments described in this thesis were designed to characterize a possible role for adenosine 5'-triphosphate (ATP) in the cardiovascular system of the conscious rat. This study assessed the role of ATP in the control of mean arterial pressure (MAP), heart rate (HR), and mean circulatory filling pressure (MCFP) by examining the effects of receptor antagonists (of α-adrenoceptors, P_1- and P_2-purinoceptors, and autonomic ganglia), chemical sympathectomy (by reserpine or guanethidine), and ATP *per se*. Furthermore, we compared the contribution of endogenous ATP and noradrenaline (NA) in basal vascular tone with that during drug-induced vasodilatation and concomittant elevation of sympathetic nerve activity.

Phentolamine (non-selective α-adrenoceptor antagonist) was found to be a more effective arterial than venous vasodilator in both basal conditions and during drug (hydralazine or nifedipine)-induced vasodilatation and reflex venoconstriction. While MCFP was not significantly decreased by phentolamine either under basal conditions or during hydralazine treatment, phentolamine did decrease MCFP in the presence of nifedipine. Following suramin treatment, the phentolamine-induced depressor effect was significantly potentiated whereas MCFP remained unchanged. Under basal conditions, mecamylamine very effectively reduced both MAP and MCFP whereas in the presence of hydralazine-induced vasodilatation and elevated venomotor tone, ganglion blockade reduced MCFP but not MAP.

Blockade of P_2-purinoceptors by suramin produced a dose-dependent increase in MAP and decrease in HR neither of which was affected by hydralazine, nifedipine, mecamylamine, reserpine, or guanethidine. Suramin failed to reduce MCFP in the presence of hydralazine, nifedipine, or guanethidine. In contrast, mecamylamine treatment revealed a significant dose-dependent decrease in MCFP by suramin, while reserpine treatment revealed a slight but significant decline in MCFP.

I.v. infusion of ATP produced profound depressor and bradycardic effects. The ATP-induced depressor effect was unaffected by mecamylamine and suramin whereas blockade of P_1-purinoceptors by 8-phenyltheophylline clearly and significantly attenuated this response. Blockade of P_{2Y}-purinoceptors by cibacron blue only slightly and insignificantly attenuated the depressor effect of ATP. ATP-induced bradycardia was not affected by mecamylamine or cibacron blue whereas 8-phenyltheophylline completely abolished this response and even revealed a slight, but insignificant, increase in HR in response to ATP. Suramin slightly but insignificantly enhanced the ATP-induced bradycardia. ATP produced a slight but insignificant depression of MCFP which was unaltered in the presence of suramin, and slightly but insignificantly enhanced both during mecamylamine-induced ganglion blockade and following 8-phenyltheophylline treatment. Cibacron blue, in contrast, revealed a slight but insignificant ATP-induced increase in MCFP.

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ABBREVIATIONS

ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
DDW	double distilled water
EDRF	endothelium-derived relaxing factor
EJC	excitatory junction current
EJP	excitatory junction potential
EtOH	ethanol
FAP	final arterial pressure
GABA	γ-aminobutyric acid
HR	heart rate
i.a.	intra-arterial
i.p.	intraperitoneal
i.v.	intravenous
IP_3	inositol-1,4,5-triphosphate
iu	international unit
L-NAME	N-nitro-L-arginine methyl ester
LDV	large dense-cored vesicle
MAP	mean arterial pressure
MCFP	mean circulatory filling pressure
MTX	methoxamine
n	number of observations
NA	noradrenaline
NANC	non-adrenergic, non-cholinergic
NO	nitric oxide
NPY	neuropeptide tyrosine
p	probability (significance level in a statistical test)
PE	polyethylene
PGI_2	prostacyclin
pK_B	-log of the dissociation constant
s.d.	standard deviation (of observed sample)
s.e.mean	standard error (of estimate mean value)

SDV	small dense-cored vesicle
UTP	uridine triphosphate
VPP	venous plateau pressure
w/v	weight by volume
≈	approximately equals

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1. INTRODUCTION

1.1. *Historical Aspects of the Concept of Sympathetic Noradrenaline-ATP Cotransmission and the Extracellular Effects of ATP in the Cardiovascular System*

1.1.1. NA as the primary sympathetic neurotransmitter

The concept of chemical neurotransmission was first explicitly proposed at the turn of the century when Elliot (1904) suggested that "sympathetic axons cannot excite the peripheral tissue except in the presence, and perhaps through the agency, of the adrenaline or its immediate precursor...", thus anticipating the modern view of sympathetic neurotransmission in which noradrenaline (NA), the immediate precursor of adrenaline, is released from sympathetic nerves and acts at specific receptors on smooth muscle near the sites of release. The view of Elliot, which was based on the similarities of the effects of sympathetic nerve stimulation and applied adrenaline, was later confirmed by Loewi (1921) who demonstrated in the perfused frog heart that tachycardia induced by sympathetic nerve stimulation was mediated by a chemical substance called "Acceleransreizstoff", which he later identified as adrenaline (Loewi 1936). As suggested by Dale (1934), postganglionic nerves were therefore termed "adrenergic". It was not until 1946, however, that Euler positively identified NA as the primary transmitter in sympathetic mammalian nerves.

The notion that neurons utilize a single transmitter substance is commonly referred to as Dale's Principle. Although a misinterpretation of Dale's original writings (see Eccles 1986), the popularization of "Dale's Principle", to a large extent by Eccles (1957), has allowed this idea to dominate thinking until the early 1980s. Nevertheless, departures from this principle can be traced back as far as 1955 when Koelle posed the following question: "... is it not likely that the terms cholinergic and adrenergic, originally proposed by Dale... might refer to the predominant rather than the exclusive types of transmitting agents of the nerve fibers, and that these... might liberate mixtures of chemical transmit-

ters?". The concept introduced by this question is currently known as cotransmission and has been a subject of rapidly growing interest since the early 1970s.

1.1.2. Development of the cotransmission hypothesis

The 1976 paper by Burnstock entitled "Do some nerve cells release more than one transmitter?" was pivotal to the early development of the cotransmission hypothesis. In this paper, it was very tentatively suggested that Dale's Principle may not have universal applicability and that, in certain instances, one nerve cell may release more than one transmitter. Today, cotransmission is a generally accepted principle and the current view is that it may apply to most neurons and thus represent the rule rather than the exception (Hokfelt *et al.* 1986; Kupfermann 1991). In their "mature" state, many neurons contain one or several members of the following three classes of putative messengers: (i) "Classic" transmitters, *i.e.* the monoamines noradrenaline, adrenaline, dopamine or 5-hydroxytryptamine, or acetylcholine, or amino acids, such as γ-aminobutyric acid (GABA), glycine or glutamate, (ii) a nucleotide, presumably often adenosine 5'-triphosphate (ATP), and (iii) neuropeptides, a rapidly expanding class of transmitter substances which includes neuropeptide tyrosine (NPY) amongst more than 50 different compounds (Hokfelt *et al.* 1986; Stjärne 1989; Kupfermann 1991).

1.1.3. Development of the NA-ATP cotransmission hypothesis

Some of the most compelling early evidence for sympathetic NA-ATP cotransmission derived from studies of adrenal chromaffin cells which were shown not only to contain very large amounts of ATP, but also to release ATP by exocytosis together with a variety of proteins, collectively referred to as chromogranins. Soon after, similar evidence was obtained from neural tissue of the sympathetic nervous system and some investigators began to propose that ATP coreleased with NA may have a physiological role. At the time, recent developments identifying ATP as the principal transmitter of "atropine-resistant" non-adrenergic, non-cholinergic (NANC) nerves of the gut, bladder, and portal vein (Burnstock 1972) gave support to such a role for cotransmitter ATP.

Studies of the vas deferens during the early and mid-1970s, most notably by Burnstock and co-workers (reviewed in Burnstock 1986, 1990d), provided the clearest evidence for NA-ATP cotransmission. The response of the vas deferens to sympathetic nerve stimulation consists of a fast (twitch) contraction, followed by a slower, more sustained contraction. A number of investigators suggested that the slow response was mediated by NA while the rapid twitch response utilized a different transmitter. The slow phase of the contraction is mimicked by exogenous NA; prazosin and other α-adrenoceptor antagonists block the response; and cocaine potentiates the response (via blockade of NA reuptake). Furthermore, it was demonstrated that adrenergic neuron blocking agents selectively eliminate the slow contraction while leaving the twitch response relatively intact. The rapid twitch contraction, on the other hand, was proposed to be mediated by ATP, since exogenous ATP was highly effective in mimicking the nerve-induced response. Although in blood vessels the contributions of ATP and NA to the mechanical response to sympathetic nerve stimulation are not as clearly separated as they are in the vas deferens, analogous studies using vascular preparations appeared to indicate that perivascular nerves also utilize ATP and NA as cotransmitters. It was these early studies of NA-ATP cotransmission, especially on the vas deferens, that laid the ground work for the ongoing investigation into the involvement of cotransmission in the cardiovascular system.

1.1.4. Other extracellular effects of ATP in the cardiovascular system

Apart from its action as an excitatory cotransmitter, ATP released intravascularly from non-neuronal stores can cause vasodilatation via its action as a local modulator of vascular tone (Burnstock 1990b). Some of the earliest observations of the inhibitory effects of purines on the cardiovascular system date from the 1920s when Drury and Szent-Gyorgyi demonstrated the potent effects of purine-containing extracts from cardiac muscle, brain, kidney and spleen on the heart and blood vessels. These observations subsequently led to the development by Berne (1963) of a general hypothesis of adenosine function in the cardiovascular system. Although early studies implicated adenosine as the

primary vasodilatatory purine, recognizing the very rapid enzymatic breakdown of ATP to adenosine in the circulation, more recently it has become apparent that ATP, *per se*, is also involved where adenosine had previously been thought to be the sole mediator (Winbury *et al.* 1953; Eikens & Wilcken 1973; Toda *et al.* 1982; Hopwood & Burnstock 1987; Hopwood *et al.* 1989). In 1981, De Mey & Vanhoutte were the first to describe the mechanism responsible for the potent vasodilatatory actions of ATP – they demonstrated that ATP, by acting on vascular endothelial cells, induces the release of endothelium-derived relaxing factor (EDRF) which subsequently causes relaxation of vascular smooth muscle. Thus, ATP is often referred to as having a dual function in the regulation of vascular tone: (i) as an intravascular mediator of endothelium-dependent vasodilatation via purinoceptors located on vascular endothelial cells, and (ii) as an excitatory cotransmitter with noradrenaline from sympathetic perivascular nerves causing vasoconstriction via purinoceptors located on smooth muscle (Burnstock & Kennedy 1986; Ralevic & Burnstock 1991).

1.2. *Classification and Mechanisms of Action of Cardiovascular Purinoceptors*

Although this thesis is concerned, in particular, with the actions of ATP, it is appropriate to discuss the receptors for both ATP and adenosine in light of the fact that extracellular ATP is susceptible to rapid enzymatic breakdown to adenosine both in the circulation and at sites of release by perivascular nerves (Gordon 1986). The interplay between ATP and adenosine in the cardiovascular system is complex: the physiological actions of one can be synergistic, antagonistic, or even modulatory with respect to the actions of the other.

1.2.1. P_1- and P_2-purinoceptors

Although the widespread and potent extracellular actions of purine nucleosides and nucleotides have been recognized for over 60 years, it was not until 1978 that an attempt was made to characterize and name the receptors mediating such actions. This classification system (Burnstock 1978) consisted of two major subtypes, P_1- and P_2-purinocep-

tors, and was based on a review of the extensive literature concerning the actions of purine nucleosides and nucleotides on a wide variety of tissues. The four criteria used in this classification were: (i) the relative potencies of ATP, ADP, AMP, and adenosine; (ii) the selective actions of antagonists, particularly methylxanthines; (iii) the activation of adenylyl cyclase by adenosine but not by ATP; (iv) the induction of prostaglandin synthesis by ATP but not by adenosine. Thus the following classification was proposed: The P_1-purinoceptors are associated with an agonist potency order of adenosine > AMP > ADP > ATP; methylxanthines such as theophylline, aminophylline, and caffeine are selective competitive antagonists with respect to these receptors; and occupation of these receptors leads to inhibition or activation of an adenylyl cyclase system with resultant changes in levels of intracellular adenosine 3',5'-monophosphate (cAMP). The P_2-purinoceptors are associated with an agonist potency order of ATP > ADP > AMP > adenosine; these receptors are not antagonized by methylxanthines and do not act via an adenylyl cyclase system. Since its proposal, it has become apparent that neither the P_1- nor the P_2-purinoceptors forms a homogeneous group and that each can be separated into at least two subtypes.

1.2.2. P_1-purinoceptor subtypes

P_1-purinoceptors were subdivided into A_1- and A_2-subtypes (Van Calker *et al.* 1979) and R_i- and R_a-subtypes (Londos *et al.* 1980). The A_1- and A_2-subtypes appear to be analogous to the R_i- and R_a-subtypes, respectively; however, the A_1/A_2 nomenclature is more widely used. The classification is based on the relative potency series of adenine analogues and according to whether adenylyl cyclase activity is increased or decreased in the presence of adenosine (A_1 decreases while A_2 increases activity). This classification of P_1-purinoceptors has recently been expanded to include four subtypes, A_1, A_{2a}, A_{2b}, and A_3 (Fredholm *et al.* 1994), all of which are G-protein-coupled and proposed to have the general structure that would place them in the rhodopsin-like group of the superfamily of G-protein-coupled receptors. Although all P_1-purinoceptors act via G-proteins, not all couple exclusively to adenylyl cyclase, but instead may couple to ion channels or

phospholipases (Fredholm *et al.* 1994). The existence of A_{1a}, A_{1b}, and A_4 subtypes has also been tentatively proposed (Tucker and Linden 1993).

Until recently, the myocardial adenosine receptor has been referred to as either A_1 or A_3 in the literature. With the cloning of a P_1 receptor distinct from established A_1 and A_2 subtypes (now referred to as A_3) (Zhou *et al.* 1992), this is no longer the case (Carruthers & Fozard 1993). Instead, it has been suggested that there exist two different A_1-like receptors, or a single receptor coupled to two different effectors, one of which mediates "direct" (cAMP-independent, atrial-specific, coupled to K^+ channels) and the other, "antiadrenergic" (cAMP-dependent, atrial- and ventricular-specific) effects in the heart (Tucker & Linden 1993). In the vasculature, it has been proposed that three P_1-purinoceptor subtypes are responsible for mediating the vasodilator effects of adenosine: a high affinity A_{2a} receptor on vascular smooth muscle cells, a low affinity A_{2b} receptor on endothelial cells (Bruns *et al.* 1987; Nees *et al.* 1987), and an A_4 receptor also on vascular smooth muscle (Tucker & Linden 1993). The A_{2b}-purinoceptor induces accumulation of cAMP in the endothelium which may, in turn, open gap junctions with adjacent smooth muscle cells to stimulate chemical (EDRF and cyclic nucleotide) and electrical communication (Greenfield *et al.* 1990a,b). The smooth muscle A_{2a} receptor acts via stimulation of adenylyl cyclase, while the putative A_4-purinoceptor on smooth muscle has been shown to activate K_{ATP} channels (Daut *et al.* 1990).

1.2.3. P_2-purinoceptor subtypes

Subtypes of the P_2-purinoceptor were first proposed by Burnstock and Kennedy (1985). P_{2X}- and P_{2Y}-purinoceptor subtypes were postulated primarily on the basis of rank order of potency of agonists in a variety of different biological systems (highly selective antagonists for P_2-purinoceptors are not available). Generally, P_{2X} and P_{2Y} activation was correlated with contraction and relaxation, respectively. Gordon (1986) extended this classification scheme to include P_{2T}- and P_{2Z}-purinoceptors, which are believed to mediate ADP-induced aggregation of platelets (Humphries *et al.* 1993) and the tetrabasic

acid ATP^{4-}-induced histamine release from rat mast cells (Dahlqvist & Diamant 1974), respectively. The P_{2Z}-purinoceptor is also found on macrophages and appears to represent the opening of a fairly nonselective type of pore (Steinberg & Silverstein 1987). It has recently become apparent that many responses to purine nucleotides do not fall into the above classification, including those that are insensitive to both 2-methylthio-ATP (P_{2Y} agonist) and α,β-methylene ATP (P_{2X} agonist), yet are sensitive to ATP (Demolle *et al.* 1988; Wilkinson *et al.* 1993). In some cases these non-P_{2X} and non-P_{2Y} responses can also be elicited by uridine triphosphate (UTP) with a similar agonist potency to ATP, which has led to the definition of the so-called "P_{2U}" or "nucleotide" or "pyrimidine" receptor (Seifert & Schultz 1989; O'Connor *et al.* 1991; Dubyak 1991). Pyrimidine receptors are coupled to phospholipase C and mediate their action via the formation of inositol-1,4,5-triphosphate (IP_3) and subsequent release of Ca^{2+} from intracellular stores. These receptors have been identified on both vascular smooth muscle (Kalthof *et al.* 1993) and endothelial cells (Wilkinson *et al.* 1993) where they mediate vasoconstriction and endothelium-dependent relaxation, respectively. Finally, there also appears to be a receptor for diadenosinetetraphosphate, designated a P_{2D} subtype (Hilderman *et al.* 1991; Castro *et al.* 1992).

The two P_2-purinoceptors responsible for mediating the "dual functions" of ATP in the regulation of vascular tone are the excitatory P_{2X} and inhibitory P_{2Y} subtypes. Although there is mounting evidence that nucleotide receptors may also be involved, the functional significance and mechanism of action of these receptors are not as well characterized. Most evidence suggests that the vascular smooth muscle P_{2X}-purinoceptor represents an intrinsic ion channel permeable to Na^+, K^+, and Ca^{2+} (Benhem & Tsien 1987; Bean 1992; Kalthof *et al.* 1993), although there have been some reports (*e.g.* von der Weid *et al.* 1993) of ATP-induced chloride currents in aortic and other vascular preparations. These depolarizing currents are directly activated by nanomolar to micromolar concentrations of extracellular ATP without the apparent involvement of G-proteins or soluble second mes-

sengers. Thus, ATP can be added to the relatively small group of physiological agonists (acetylcholine, glutamate, GABA, glycine, and 5-hydroxytryptamine) for ligand-gated ion channels. In vascular smooth muscle, activation of P_{2X}-purinoceptors by ATP increases mainly the Na^+ but also the K^+ and Ca^{2+} conductances and generates an inward current, the excitatory junction current (EJC), and a transient depolarization, the excitatory junction potential (EJP), which may or may not summate to fire a muscle action potential. The membrane depolarization activates L-type voltage-gated Ca^{2+}-channels, increases the influx of Ca^{2+}, and triggers a muscle contraction (Benham 1989). In some arterial smooth muscles, it has been claimed that the increase in intracellular Ca^{2+} is the direct result of influx through the ATP-gated channel, without a requirement for depolarization (Benham & Tsien 1987; Benham 1990). Thus, although the major cation entering through the channels is Na^+, with Ca^{2+} composing less than 10% of the total current, localized increases in intracellular Ca^{2+} concentration can be obtained. It has been suggested that these increases might directly activate contractile proteins or perhaps upregulate other Ca^{2+}-dependent enzymes modulating the contractile process and provide an enhanced source of Ca^{2+} for uptake into internal Ca^{2+} stores (Benham 1990). Bo and Burnstock (1993) used autoradiographic localization to show that specific [^{3}H]α,β-methylene ATP (P_{2X}-purinoceptor radioligand) binding sites were associated only with the smooth muscle of several different vessels in rat, guinea-pig, and rabbit. They also found that, in general, the medium- and small-sized arteries had higher densities of P_{2X}-purinoceptor than the elastic and large muscular arteries, while the veins, except for the portal vein, were sparsely labeled.

Activation of endothelial P_{2Y}-purinoceptors results in the release of nitric oxide (NO) (Kelm *et al.* 1988) and prostacyclin (PGI_2) (Pearson *et al.* 1983). Both NO and PGI_2 are vasodilators, although the latter is effective in only some vessels. *In vitro*, NO acts synergistically with PGI_2 to inhibit platelet aggregation (Radomski *et al.* 1987). The observations that PGI_2 release is rapid in onset, transient, and followed by a period of

refractoriness (Toothill *et al.* 1988), whereas NO release is also rapid, but sustained for many minutes (Martin *et al.* 1985a) have been interpreted as indicating two different cellular signalling pathways for PGI_2 and NO (Boeynaems & Pearson 1990). The initial event resulting from P_{2Y}-purinoceptor activation appears to be inositol phospholipid hydrolysis by a G-protein-coupled phospholipase C, with subsequent accumulation of IP_3 and release of intracellular Ca^{2+} stores (Forsberg *et al.* 1987; Boyer *et al.* 1990; Harden *et al.* 1990). The increase in intracellular Ca^{2+} activates phospholipase A_2 and results in PGI_2 synthesis. NO release, in contrast, requires influx of extracellular Ca^{2+} in order to maintain elevated intracellular Ca^{2+} concentration (Luckhoff *et al.* 1988). The mechanism by which extracellular Ca^{2+} enters the cell is not clear. The recent cloning of the P_{2Y}-purinoceptor (Webb *et al.* 1993) indicates that this receptor has significant homology with other G-protein-coupled receptors, yet may constitute a distinct family within the superfamily of G-protein-coupled receptors (Barnard *et al.* 1994).

There is growing evidence that, in some vessels, there is a population of P_{2Y}-purinoceptors located on the vascular smooth muscle which mediates endothelium-independent vasodilatation (rabbit mesenteric artery, Mathieson & Burnstock 1985; rabbit cerebral artery, Conde *et al.* 1991; rabbit coronary artery, Corr & Burnstock 1991; rabbit portal vein, Brizzolara *et al.* 1993). The mechanism responsible for this "direct" vasodilatation by ATP may involve the G-protein-coupled opening of K^+ channels, as demonstrated for ATP-induced relaxation of intestinal smooth muscle (Hoyle & Burnstock 1989). Shirahase *et al.* (1991) have demonstrated P_{2Y}-mediated endothelium-dependent vasoconstriction in canine basilar arteries and identified the endothelium-derived contracting factor as thromboxane A_2. P_2-purinoceptors have also been located on cardiac muscle (Burnstock 1980; Pelleg *et al.* 1990) and are believed to induce both a positive inotropy and an increase in inositol-lipid metabolism via activation of P_{2Y}-purinoceptors as has been demonstrated in atrial and ventricular preparations (Legssyer *et al.* 1988; Scamps *et al.* 1990; Mantelli *et al.* 1993).

1.2.4. Presynaptic purinoceptors

There is substantial evidence that ATP and adenosine can reduce the release of NA from perivascular sympathetic nerves; however, until recently, it was believed that these effects were mediated by a single population of P_1-purinoceptors, thus requiring the metabolism of ATP to adenosine (Katsuragi & Su 1982; Su 1983). It is now known that both adenosine and ATP *per se* are capable of prejunctional modulation of neurotransmitter release (Shinozuka *et al.* 1988; Forsyth *et al.* 1991; Fuder *et al.* 1993); however, the identity of the receptor(s) mediating these effects is currently a controversial matter. Some investigators have suggested that a novel "P_3-purinoceptor", at which adenosine and ATP are equipotent, is responsible for reducing NA release (Shinozuka *et al.* 1988; Westfall *et al.* 1990a,b; Forsyth *et al.* 1991). Stimulation of the putative P_3-purinoceptor by either P_1- or P_2-agonists has been shown to be reduced by both P_1- and P_2-receptor antagonists (Westfall et al. 1990a,b). There are other investigators, however, who have proposed the existence of two distinct presynaptic purinoceptors: a classical P_1-purinoceptor (A_1-subtype), activated mainly but not exclusively by the nucleoside adenosine, and a G-protein-linked "P_{2Y}-like"-purinoceptor activated only by nucleotides (von Kügelgen *et al.* 1989a; Kurz *et al.* 1993; von Kügelgen *et al.* 1993). Von Kügelgen *et al.* (1993) have speculated that the G-protein may couple the P_2-autoreceptor to inhibition of Ca^{2+} entry or enhancement of K^+ efflux. In contrast to NA acting at presynaptic α_2-adrenoceptors, which reduces both its own release and that of its cotransmitter ATP (Bulloch & Starke 1990; MacDonald *et al.* 1992; Msghina *et al.* 1992; Bao 1993), ATP acting at P_2-purinoceptors on sympathetic axon terminals has been shown to reduce only the release of NA (White & MacDonald 1990; Westfall *et al.* 1990a,b; von Kügelgen *et al.* 1993; Allgaier *et al.* 1994), although the possibility of autoinhibition by ATP of release of ATP itself has not been totally eliminated. In contrast, Evans and Surprenant (1992) have presented evidence indicating that ATP affects neither ATP nor NA release. Interestingly, several groups (Miyahara & Suzuki 1987; Shinozuka *et al.* 1992; Ishii *et al.* 1993a,b; Ishii *et al.* 1994) have

demonstrated enhanced release of NA following activation by ATP of P_2- or, possibly, P_3-purinoceptors on perivascular sympathetic nerve terminals.

1.3. *Sources of Extracellular ATP in the Cardiovascular System*

ATP is well-known as a ubiquitous intracellular constituent and is synthesized in cells by glycolysis and by mitochondrial oxidative phosphorylation. Because of its ubiquitous occurrence in all cell types (at cytosolic concentrations of 2-3 mM), total tissue contents of ATP are unlikely to reveal possible extracellular functions. Knowledge of both the release and source of ATP is therefore a much more important criterion to be satisfied if one wishes to establish specific functions for extracellular ATP in a particular system. Furthermore, the receptor subtypes mediating ATP-induced responses in the cardiovascular system are often determined by the cellular source of ATP. Plasmatic ATP, which is believed to be the primary mediator of endothelium-dependent vascular responses, may be released from a number of different cellular sources. Neuronally-released ATP, in contrast, is believed to act directly by activation of purinoceptors located on the vascular smooth muscle. Unlike ATP derived from neurons, platelets, and the adrenal medulla, which is released via exocytosis, other cell-derived or extra-neuronal ATP (*e.g.* from vascular endothelial and smooth muscle cells) is believed to be released in a non-secretory fashion (Gordon 1986). These latter cells lack storage granules containing ATP, and the ATP is apparently released from the cytoplasm (Pearson & Gordon 1979).

1.3.1. ATP from nerves

ATP has been found to occur together with NA in both small dense-cored vesicle (SDV) and large dense-cored vesicle (LDV) fractions of the homogenates of sympathetically innervated tissues (Lagercrantz 1971; Klein 1982; Lagercrantz & Fried 1982). The ATP:NA ratio in large LDVs is three to five times higher than in SDVs. As the vesicles are transported down the axon to the nerve terminal, there is an increase in the content of NA but not of ATP, as the molar ratio of NA:ATP increases from 4:1 to as much as 50:1 (Klein

1982; Lagercrantz & Fried 1982). Thus, some have suggested that the low proportion of ATP in the mature vesicles argues against a cotransmitter role for ATP in light of the finding that exogenous ATP and NA are equipotent as smooth muscle spasmogens (Fredholm *et al.* 1982).

When attempts have been made to measure release of transmitter ATP from vascular sympathetic nerves, results have proved especially controversial. Numerous studies using "overflow" methods have demonstrated the release of radiolabeled purines during stimulation of sympathetic nerves in blood vessels (Su 1983); however, such results have been criticized because most of the radiolabel appears as adenosine rather than ATP. Although it has been assumed that released ATP is rapidly degraded to adenosine by ectonucleotidases, it is possible that adenosine itself is released during the sympathetic nerve stimulation. (Fredholm *et al.* 1982; Pons *et al.* 1980; White & MacDonald 1990). Further complications arise from the inability of overflow methods to distinguish between "transmitter ATP", presumably originating from sympathetic vesicles, and "non-transmitter ATP" released from the cytosol of neuronal and non-neuronal cells in response to field stimulation (Shinozuka *et al.* 1991; Msghina *et al.* 1992; Ishii *et al.* 1993b). The magnitude of this problem is illustrated by the finding (in rabbit aorta) that less than 3% of the field stimulation-induced overflow of ATP was derived from sympathetic nerves, with the remainder originating from smooth muscle (7%) and endothelium (90%) (Sedaa *et al.* 1990). Although the release of ATP, and possibly adenosine, from both neuronal and non-neuronal stores far exceeds that of NA (by factors of 350 and 2000 in the rabbit aorta and pulmonary artery, respectively) (Sedaa *et al.* 1990; Mohri *et al.* 1993), the ratio of NA to ATP released from nerves *per se* is approximately 1 – which is estimated to provide a physiologically active concentration of ATP once released (Sedaa *et al.* 1990). It has been demonstrated that both the endothelial and smooth muscle "overflow" ATP is released via α_1-adrenoceptor stimulation (Katsuragi & Su 1982, Buxton *et al.* 1990; Sedaa *et al.* 1990; Starke *et al.* 1992; Shinozuka *et al.* 1992, Ishii *et al.* 1993b). Interestingly, nicotine, long

known to induce exocytotic NA release via activation of nicotinic receptors on sympathetic nerve terminals (Loffelholz 1970), releases ATP in addition to NA in vascular preparations (Bultmann *et al.* 1991a).

One of the currently debated issues with respect to storage and release of cotransmitter ATP concerns the possible "dissociation" of nerve impulse-induced release of ATP and NA as reflected by the variability in ratios of released NA and ATP. In other words, it is unclear as to whether ATP released by nerve stimulation originates from purely purinergic nerves or from sympathetic nerves and, if it comes from sympathetic nerves, whether from vesicles containing both NA and ATP, or ATP alone. According to the conventional view of sympathetic cotransmission, NA and ATP are stored together in vesicles and nerve impulses release them by exocytosis in the same proportions as they are stored (Stjärne 1989). Numerous observations of "non-parallel" modulation of nerve-released NA and ATP under various stimulation parameters or pharmacological treatments (Kennedy *et al.* 1986b; White & MacDonald 1990; Bulloch & Starke 1990; Sjöblom-Widfeldt & Nilsson 1990; Starke *et al.* 1992; Evans & Cunnane 1992; Driessen *et al.* 1993) have cast doubt as to whether NA and ATP are stored in the same transmitter vesicle and released via exocytosis as a mixed multimolecular packet. For example, Sjöblom-Widfeldt and Nilsson (1990) demonstrated in small mesenteric arteries of the rat that extracellular Ca^{2+} concentration differentially affected the adrenergic and purinergic components of the contractile response to sympathetic nerve stimulation, suggesting the possibility that NA and ATP are regulated separately. Although both components of the response were abolished by chemical sympathectomy with 6-hydroxydopamine, these authors suggested that this did not necessarily indicate that only one type of adrenergic nerve was responsible for the release of NA and ATP since it is possible, though highly speculative, that subpopulations of vesicles, or even of adrenergic nerves, with varying ATP:NA ratios may exist (Sjöblom-Widfeldt 1990). An alternative, and perhaps more likely, explanation for the apparent differential regulation of NA and ATP is that transmitter release, instead of occurring via

complete (all-or-none) exocytosis which would require NA and ATP to be release in a fixed ratio, may occur via a non-exocytotic ion-exchange mechanism capable of partial and selective release of vesicle contents (Uvnäs *et al.* 1989). Evidence in support of corelease has been presented by Msghina and coworkers (Msghina *et al.* 1992; Msghina & Stjärne 1993) who have demonstrated (in rat tail artery) "parallel" modulation of NA and ATP release by both presynaptic α_2-autoreceptors and tetanic stimulation. Similarly, Sjöblom-Widfeldt *et al.* (1990; see also Sjöblom-Widfeldt 1990) showed that both adrenergic and purinergic components of the neurogenic contractile response in rat mesenteric arteries are potentiated to the same extent by tetanic stimulation.

1.3.2. ATP from blood cells

Erythrocytes, leukocytes, neutrophils, and platelets contain significant amounts of ATP which can be released following exposure to damaging stimuli. For example, release of ATP from human erythrocytes in response to hypoxia and other haemodynamic stresses has been demonstrated (Forrester 1990). Blood platelets, in particular, are a rich source of plasmatic ATP (Detwiler & Feinman 1973) as they contain ATP, together with 5-hydroxytryptamine, in "dense granules" which have been compared to storage vesicles in some neurons (Sneddon 1973). Platelets contain approximately 40 nmol of ATP and ADP per mg of protein (Da Prada *et al.* 1981). The plasma concentration of ATP following platelet activation by thrombin *in vitro* can reach concentrations up to 20 μM (Ingerman *et al.* 1979; Born & Kratzer 1984) while that *in vivo*, where platelet degranulation occurs following localized platelet aggregation, can approach the high micromolar range since the concentration within storage granules is of the order of 1 M (Ugurbil 1981; Meyers *et al.* 1982). In addition to thrombin, adrenaline, collagen, and ADP are known to induce ATP release (reviewed in Colman 1990). Degranulation may also occur when platelets interact, perhaps transiently, with the vascular wall (George 1985) and this could result in a smaller, more localized release of nucleotides.

1.3.3. ATP from the adrenal medulla

Cells of the adrenal medulla, like neurons and platelets, contain ATP in storage granules and discharge them, together with catecholamines, via degranulation (Winkler *et al.* 1987). ATP represents approximately 15% of the dry weight of adrenal granules (Hillarp 1959) and is stored with catecholamines in the ratio of about 4 moles amine:1 mole ATP (Winkler & Westhead 1980). Although the exact functions of this released ATP have not yet been established, it is possible that the adrenal medulla may contribute significantly to local release of ATP into the plasma (White 1988).

1.3.4. ATP from the vessel wall and other tissues

As mentioned previously, there are certain cells which release ATP from a cytosolic pool rather than from vesicle-bound stores. These ATP sources include skeletal (Abood *et al.* 1962) and cardiac muscle cells (Paddle & Burnstock 1974; Forrester 1990), and vascular smooth muscle and endothelial cells (Pearson & Gordon 1979; Buxton *et al.* 1990). Given its molecular mass and anionic nature, there is generally little permeation of ATP (or MgATP) across the plasma membrane. The mechanism of release from cytosolic stores is unclear, although Forrester (1990) has demonstrated that hypoxia-induced release of cytosolic ATP from cardiac myocytes and human erythrocytes is substantially attenuated by inhibitors of either anion transporters or nucleoside transporters. Recently, Abraham *et al.* (1993) described very interesting observations which suggest that the multidrug resistance (*mdr1*) gene product (or P glycoprotein) can function as a channel for ATP. This raises the possibility that other proteins belonging to the "ABC" transporter superfamily may also act as conduits for ATP release in response to physiological or pathological stimuli.

Using cultured endothelial and smooth muscle cells, Pearson and Gordon (1979) demonstrated that ATP release was not accompanied by any evidence of cell damage, as indicated by the lack of extracellular lactate dehydrogenase activity and absence of staining of cells with trypan blue, and therefore was not the result of a nonspecific increase in

membrane permeability such as induced by cell lysis. Furthermore, the absence of compartmentalization of endothelial ATP excluded the possibility of a secretory process involving degranulation. The selective release of ATP from cultured endothelium appears to be a cellular response to potentially damaging stimuli. Mechanisms have not yet been identified; however, effective stimuli include neutral proteinases such as trypsin and thrombin, which require an active catalytic site of the enzyme (Lollar & Owen 1981), and cationic proteins or polymers (LeRoy *et al.* 1984). In nonpathological states, it is believed that endothelial ATP is liberated by NA released during increased sympathetic nerve activity since exogenous NA has been shown to stimulate release of ATP from cultured cardiac endothelial cells via a prazosin-sensitive, yohimbine-insensitive, α_1-adrenoceptor-mediated, mechanism (Buxton *et al.* 1990). Recently, Yang *et al.* (1994) presented evidence supporting the ability of ATP to stimulate ATP release from cardiac endothelial cells via an action at P_{2Y}-purinoceptors.

The release of ATP from vascular smooth muscle has been shown to be an α_1-adrenoceptor-mediated process (Katsuragi & Su 1982, Sedaa *et al.* 1990; Starke *et al.* 1992; Shinozuka *et al.* 1992, Ishii *et al.* 1993b) and, as described previously, contributes to the ATP "overflow" observed following electrical nerve stimulation. An ATP-evoked ATP release system, analogous to that found in cardiac endothelial cells (Yang *et al.* 1994), has been demonstrated in vas deferens and ileal longitudinal smooth muscles (Katsuragi *et al.* 1991; von Kügelgen & Starke 1991b), raising the possibility that such a system may also operate in vascular smooth muscle. The functional significance of ATP released postsynaptically from vascular smooth muscle and endothelium may rest in its ability to modulate NA release by an action at presynaptic nerve terminals (reduced NA release, Shinozuka *et al.* 1991; enhanced NA release, Ishii *et al.* 1993b).

1.4. *Metabolism of Extracellular ATP*

The enzymes responsible for removal of ATP from the extracellular space are ectonucleotidases. Ectonucleotidases, though primarily located in large numbers on the luminal surface of vascular endothelial cells, are widely distributed amongst other tissues and isolated cells such as skeletal, cardiac, and smooth muscle, blood platelets, and leukocytes (Ryan 1982; Pearson & Gordon 1985; Zimmerman *et al.* 1992). The efficiency of metabolism of ATP in the circulation has been known since 1950 when Binet and Burstein demonstrated that a bolus of ATP is virtually all removed by a single passage through the pulmonary vasculature. Sollevi *et al.* (1984) demonstrated that ATP intravenously administered to anaesthetized dogs is virtually all removed during its passage through the lung circulation. In addition, Ryan and Smith (1971) demonstrated that the mean transit time of ATP and its metabolites was indistinguishable from that of a vascular space marker (blue dextran or albumin) in the perfused rat lung, and that the half-life of nucleotides in the perfused lung is ~ 0.2 s or less. In comparison, the half-life for ATP incubated in cell-free plasma or whole blood at 37 °C is 20-30 min and 10 min, respectively (Jorgensen 1956; Trams *et al.* 1980). The heart is an additional site of efficient ATP metabolism – 99% of a bolus of ATP can be removed on a single passage through the coronary vasculature (Paddle & Burnstock 1974).

The metabolic pathway involves sequential dephosphorylation, with ATP being converted to ADP, then to AMP, then to adenosine, which is taken up by active transport and rephosphorylated or further metabolized to inosine and hypoxanthine. Unlike endothelial cells, vascular smooth muscle cells lack the high-affinity adenosine transport system (Pearson *et al.* 1978) and convert very little of the adenosine produced extracellularly to metabolites (Slakey *et al.* 1990). The three separate enzymes catabolizing ATP $\rightarrow$ ADP $\rightarrow$ AMP $\rightarrow$ adenosine are ATPase, ADPase, and monophosphatase (5'-nucleotidase), respectively (Pearson & Gordon 1985). Non-specific phosphatases do not contribute to nucleotide metabolism at the endothelial surface (Pearson *et al.* 1980).

Of particular importance to the metabolism of ATP released either intravascularly as a local modulator, or abluminally as a cotransmitter are the ectonucleotidases located on the vascular endothelium and smooth muscle, respectively. Slakey *et al.* (1990) have suggested that regulation of the time course of adenine nucleotide hydrolysis in the endothelium and vascular smooth muscle differs in light of the fact that the site of action and extent of phosphorylation can profoundly modify the physiologic effect of these nucleotides. They demonstrated, that for smooth muscle cells, the rate of production of adenosine is regulated predominantly by delivery of substrates to the cell surface, while for endothelial cells "feed-forward" inhibition leads to a pronounced lag in production of adenosine. This lag, it was speculated, serves to insure that proaggregatory ADP remains in the extracellular milieu long enough to allow thrombus formation, after which antiaggregatory adenosine is produced. Cotransmitter ATP, in contrast, is rapidly metabolized to adenosine which subsequently acts at presynaptic autoinhibitory P_1 (or P_3) receptors on sympathetic nerve terminals (Katsuragi & Su 1982). In effector cells expressing both P_1 and P_2 receptors, the production of adenosine from ATP may serve to either attenuate or potentiate the biological responses triggered by the initial release of ATP (Dubyak & El-Moatassim 1993).

1.5. *Regulation of Vascular Tone by ATP*

1.5.1. Dual regulation of blood vessel tone by ATP: vasoconstriction and vasodilatation

The effect of ATP on blood vessels depends on a number of factors, the most important of which are: (i) the source of ATP, (ii) the integrity of the endothelium, and (iii) the activity of ectonucleotidases. Numerous studies have demonstrated that ATP induces vasodilatation in endothelium-intact blood vessels but vasoconstriction once the endothelium has been removed (Kennedy et al, 1985; Machaly *et al.* 1988; Read *et al.* 1993), or that at low basal tone ATP causes vasoconstriction while at high tone vasodilatation is observed (Furchgott 1966; Kennedy & Burnstock 1985b; White *et al.* 1985; Mathieson

and Burnstock 1985; Burnstock & Warland 1987a; Ralevic *et al.* 1991). Other studies have shown the asymmetry in vascular responses to intraluminal and abluminal administration of ATP (Cohen *et al.* 1984; Chen & Suzuki 1991; Kaul *et al.* 1992). Such studies have shown that intraluminal administration produces endothelium-dependent vasodilatation while abluminal administration tends to produce vasoconstriction. Kaul and coworkers (1992) demonstrated in the perfused rabbit carotid arteries that ADP administered intraluminally and abluminally elicited endothelium-dependent vasodilatation in vessels preconstricted with phenylephrine and α,β-methylene-ATP (a potent P_{2X}-purinoceptor agonist), respectively, but not when applied abluminally to phenylephrine-constricted arteries in the presence of an ADPase inhibitor. These results are representative of the generally accepted finding that the response of a vessel to ADP, ATP, and their analogues is a balance between opposing effects mediated by P_{2X}- and P_{2Y}-purinoceptors. Thus, ATP generated by endothelial cells, platelets, and erythrocytes will act predominantly at P_{2Y}-purinoceptors located on the endothelium, promoting the release of EDRF and producing vasodilatation, whereas ATP released from sympathetic nerve endings at the abluminal surface will act primarily at P_{2X}-purinoceptors on smooth muscle to promote constriction, perhaps with a synergistic interaction with noradrenaline (Kennedy & Burnstock 1986a; Ralevic and Burnstock 1990; Corr & Burnstock 1991; Witt *et al.* 1991; Bultmann *et al.* 1991a). Thus, in the presence of endothelial dysfunction, unopposed stimulation of P_{2X}-purinoceptors on smooth muscle by intravascularly-derived ADP and ATP may produce vasoconstriction. In addition, the observations of Kaul *et al.* (1992) suggest that abluminal application may result in preferential activation of smooth muscle P_{2X}-purinoceptors since occupation of these by α,β-methylene-ATP unmasked a P_{2Y}-purinoceptor vasodilatation. These investigators also showed that the so-called "diffusion barrier" (*i.e.* the smooth muscle and endothelial ectonucleotidases encountered by purine nucleotides in transit from the abluminal to intraluminal side, or *vice versa*), though somewhat reducing the endothelium-dependent vasodilatation produced by abluminal ADP in the pres-

ence of α,β-methylene-ATP, may not be as great a factor in the response to abluminal nucleotides as the preferential activation of smooth muscle P_{2X}-purinoceptors and concomitant "masking" of the opposing P_{2Y}-mediated effect.

1.5.2. Pharmacological tools used to characterize P_2-purinoceptors

Unfortunately, a lack of selective competitive antagonists for P_2-purinoceptors has hampered the characterization of these receptors in the cardiovascular system by necessitating a reliance on "relative agonist potency orders" (see Kennedy 1990; Fedan & Lamport 1990). As a result, investigators have resorted to using a procedure, first introduced by Kasakov and Burnstock (1982), which utilizes the slowly degradable P_{2X}-purinoceptor agonist α,β-methylene-ATP as a selective desensitization agent for P_{2X}-purinoceptors. The use of α,β-methylene-ATP as an antagonist remains the most widely used method for characterizing P_{2X}-purinoceptors and for distinguishing P_{2X}- from P_{2Y}-mediated responses; however, its long-lasting and selective effects *in vitro* (except in immature rat basilar artery, Byrne & Large 1986) have been very difficult to reproduce *in vivo* (Flavahan *et al.* 1985; Bulloch & McGrath 1988b; Taylor & Parsons 1989; Schlicker *et al.* 1989; Daziel *et al.* 1990). A further complication of the use of α,β-methylene-ATP *in vivo* is its cardiodepressant effect (Delbro *et al.* 1985; Flavahan *et al.* 1985). Reactive blue 2 (also known as cibacron blue), an anthraquinone sulfonic acid dye, was first introduced by Kerr and Krantis (1979) as a new class of P_2-purinoceptor antagonist and was later shown to be a noncompetitive antagonist selective for P_{2Y}-purinoceptors (Burnstock & Warland 1987a; Hopwood *et al.* 1989; Taylor *et al.* 1989), although competitive activity has also been demonstrated (Houston *et al.* 1987). Although reactive blue 2 appears to display a degree of selectivity for P_{2Y}-purinoceptors at low concentrations, nonspecific effects such as antagonism of acetylcholine or adenosine (Burnstock & Warland 1987b; Taylor *et al.* 1989) are not uncommon at higher concentrations, thus limiting the usefulness of the compound.

The most recent and promising candidate demonstrating P_2-purinoceptor antagonism is the trypanocidal drug suramin which, interestingly, has also been utilized in the treatment of cancer and acquired immunodeficiency syndrome (reviewed in Voogd *et al.* 1993). Dunn and Blakely (1988) initially introduced suramin as a potential P_{2X}-purinoceptor antagonist in the mouse vas deferens; however, it has since been demonstrated in a number of tissues that suramin inhibits not only P_{2X}- but also P_{2Y}-mediated effects (Den Hertog *et al.* 1989; Hoyle *et al.* 1990; von Kügelgen & Starke 1991c). Leff and coworkers (1990; 1991) conducted a quantitative pharmacological analysis of suramin's actions and concluded that, indeed, suramin is a genuine competitive antagonist at P_{2X}-receptors although its affinity for these receptors is low ($pK_B = 5.17$) and it requires a relatively long time to achieve equilibrium *in vitro*. Similar studies have yet to be carried out for antagonism of P_{2Y}-purinoceptors by suramin. Reports demonstrating that suramin competitively antagonizes responses to UTP acting on vascular smooth muscle nucleotide (P_{2U}) receptors (Kalthof *et al.* 1993) and to ADP acting on platelet P_{2T}-purinoceptors (Hourani *et al.* 1992) seem to indicate that suramin cannot distinguish between the proposed subtypes of P_2-purinoceptors. In the rabbit saphenous artery, suramin has been shown to inhibit responses not only to ATP and α,β-methylene-ATP but also to histamine and 5-hydroxytryptamine, thus raising doubts about its selectivity (Nally & Muir 1992; see also Schlicker *et al.* 1989).

1.5.3. P_{2X}-purinoceptor-mediated vasoconstriction of vascular smooth muscle

In many *in vitro*, and relatively fewer *in vivo*, preparations, the mechanical and electrical responses to perivascular nerve stimulation are partially resistant to α-adrenoceptor antagonists (Glick *et al.* 1967; Holman & Surprenant 1980; Suzuki & Kou 1983; Hirst & Neild 1980; Hirst & Lew 1987). This knowledge, together with the observation that sympathetic nerve stimulation is associated with the release of purines (Su 1975, 1983), eventually led to the proposal that sympathetic NA-ATP cotransmission operates in blood vessels (reviewed in Westfall *et al.* 1990c; Burnstock 1990c,d; von Kügelgen & Starke 1991a).

1.5.3.1. Pharmacological dissection of the mechanical response to sympathetic perivascular nerve stimulation

The sympathetic nerve-mediated electrical and mechanical responses have been pharmacologically dissected into two distinct components: the adrenergic component, which is sensitive to block by α-adrenoceptor antagonists such as prazosin, and the purinergic component, which is susceptible to desensitization by α,β-methylene-ATP or antagonism by suramin. Studies conducted using the rabbit saphenous artery exemplify the experimental approach taken in a number of other vessels (Burnstock & Warland 1987b; Warland & Burnstock 1987). In this particular vessel, only 28% of the vasoconstrictor response to sympathetic nerve stimulation is blocked by prazosin while the remainder is abolished by α,β-methylene-ATP. Furthermore, all contractions can be eliminated by either blocking the nerve action potential with tetrodotoxin or destroying sympathetic nerves with guanethidine or 6-hydroxydopamine, thus indicating a sympathetic nerve origin for both NA and ATP and suggesting corelease (Burnstock & Warland 1987b; Warland & Burnstock 1987). Reserpine treatment, which depletes sympathetic nerves of their catecholamine content, failed to abolish neurogenic contractions despite a 95.7% reduction in NA content of the tissue. The response remaining after reserpine treatment, however, could be eliminated by desensitization with α,β-methylene-ATP but not prazosin (Warland & Burnstock 1987). A very similar approach has also been used to identify ATP as a sympathetic cotransmitter in a number of other vessels including the rabbit mesenteric (von Kügelgen & Starke 1985), hepatic (Brizzolara & Burnstock 1990), jejunal (Evans & Cunnane 1992), and saphenous arteries (Nally & Muir 1992), the guinea-pig submucosal arterioles (Evans & Surprenant 1992), the dog basilar (Muramatsu *et al.* 1981) and mesenteric arteries (Muramatsu 1987), and the rat mesenteric arteries (Sjöblom-Widfeldt *et al.* 1990). It should also be noted that chemical sympathectomy, with either 6-hydroxydopamine or guanethidine, has also been used to ascertain whether α-adrenoceptor antagonist-resistant nerve-mediated responses are due to ATP released from sympathetic

nerves as a cotransmitter or ATP released as the primary transmitter from NANC nerves. For example, in the rabbit portal vein, in which NANC neurotransmission is well-characterized, chemical sympathectomy fails to abolish the response to perivascular nerve stimulation, nerve-mediated release of tritiated purines, and quinacrine fluorescence (which allows histochemical localization of ATP) (Burnstock *et al.* 1979; Burnstock *et al.* 1984).

1.5.3.2. Pharmacological dissection of the electrical response to sympathetic perivascular nerve stimulation

Electrophysiological studies have demonstrated that not only is there an adrenoceptor antagonist-resistant component of the contractile response to sympathetic nerve stimulation, but there is also an analogous component to the smooth muscle electrical response (Cheung 1982; Kuriyama 1983; Sneddon & Burnstock 1985; Allcorn *et al.* 1985; Kennedy *et al.* 1986b; Sjöblom-Widfeldt 1990; Nally & Muir 1992). This response is the EJP and is generally characterized as a short-latency, rapid membrane depolarization (~ 10 mV) of brief duration (~ 1 s) (reviewed in Hirst & Edwards 1989). Similar EJPs have been recorded from many systemic arteries after sympathetic stimulation and are typically followed by a slow depolarization of ~ 2 mV that lasts for many seconds and is associated with an α-adrenoceptor antagonist-sensitive contraction. With brief stimuli, the EJP is not associated with a muscle contraction. However, increasing stimulus strength results in summation of EJPs which activates Ca^{2+} channels, consequently introducing an associated component in the contractile response. Although the slow α-adrenoceptor-mediated depolarization is larger under these conditions, it is not sufficient to initiate an action potential. With a further increase in stimulus strength, the EJP still triggers an action potential and associated contraction, but the α-adrenoceptor-mediated depolarization is now sufficiently large to trigger an action potential as well, thereby increasing the associated tension response. Here, the α-adrenoceptor-mediated constriction precedes the α-adrenoceptor-mediated depolarization, suggesting that a membrane potential change is not required for tension development, presumably because Ca^{2+} has been released from

intracellular stores. The majority of blood vessels produce EJPs; however, not all are followed by a neurogenic α-adrenoceptor-mediated depolarization and it is not uncommon for low concentrations of NA to produce large contractions in the absence of any detectable membrane change (Hirst & Edwards 1989). Nevertheless, it is very unusual for *neuronally*-released NA to evoke constriction without causing a membrane potential change at some stage (Hirst & Edwards 1989).

The suggestion that the EJP is mediated by ATP released from sympathetic nerves as a cotransmitter has met with some controversy. Although it has been demonstrated that the EJP can be mimicked by exogenous ATP (Suzuki 1985), is abolished by chemical sympathectomy (guanethidine or 6-hydroxydopamine) but not reserpine (Cheung 1982; Sneddon & Burnstock 1985; Kennedy *et al.* 1986b; Cunnane & Evans 1989), and is blocked by suramin or desensitization with α,β-methylene-ATP (Allcorn et al 1985; Sneddon & Burnstock 1985; Kennedy *et al.* 1986b; Ramme *et al.* 1987; Nally & Muir 1992), some have proposed that NA may, in fact, be responsible for the EJP in spite of its resistance to α-adrenoceptor antagonists. This alternate hypothesis assumes that NA activates specialized receptors (γ-adrenoceptors) which are restricted to regions near sympathetic nerve varicosities (Hirst & Neild 1981; Luff *et al.* 1987). This is based on the observation that very large concentrations of noradrenaline (> 1 mM), in the absence (Hirst & Neild 1980; Hirst *et al.* 1982) or presence (Laher *et al.* 1986; Bevan *et al.* 1987) of α-adrenoceptor blockade, mimic the initial, rapid electrical and mechanical responses observed during sympathetic nerve stimulation. In addition, it has been suggested that postjunctional α-adrenoceptors are found only in extra-junctional areas and that antagonism of neuronal responses by prazosin is due to non-specific depression of smooth muscle function rather than α_1-receptor blockade (Hirst & Neild 1981; Neild & Zelcer 1982). However, the γ-adrenoceptor hypothesis is difficult to reconcile with the observation that reserpine pretreatment, which greatly diminishes the release of NA, abolishes the second, slow onset depolarization but has no effect on the initial generation of EJPs (Suzuki *et al.* 1984).

More recently, Hirst and Jobling (1989) showed that α,β-methylene-ATP eliminates EJPs caused by perivascular nerve stimulation but has no effect on arterial responses supposedly mediated by γ-adrenoceptors, thus supporting the notion that ATP is responsible for the generation of EJPs. Interestingly, this same study found that neither EJPs nor γ-adrenoceptor responses could be elicited in veins even though veins responded to applied ATP with a contractile response, suggesting that the distribution of P_2-purinoceptors has no correlation with the ability of sympathetic nerves to initiate an EJP.

1.5.3.3. Influence of the parameters of nerve stimulation

There are two types of inconsistency in the literature which appear to indicate a large degree of variability in the role of ATP as a cotransmitter and cast in doubt the possible physiological role for NA-ATP cotransmission. First, in some vessels, the EJPs but not neurogenic contractions are resistant to α-adrenoceptor blockade (*e.g.* rabbit ear artery: Allcorn *et al.* 1985), while in others both the EJP and contractile response are totally abolished by α,β-methylene-ATP (Ramme *et al.* 1987). Second, some investigators have reported α-adrenoceptor antagonist-resistant contractile responses to nerve stimulation (*e.g.* rabbit ear artery: Kennedy *et al.* 1986) whereas, in the same tissue, others have demonstrated contractions which are virtually abolished by α-adrenoceptor blockade (*e.g.* rabbit ear artery: Allcorn *et al.* 1985). Most commonly, there are reports of elimination of both the EJP and a significant proportion of the contractile response by α,β-methylene-ATP (*e.g.* Sjöblom-Widfeldt 1990). It is becoming more and more apparent that the source of these discrepancies rests in the variations in stimulation parameters used to elicit neurotransmission and contraction. It appears that contractions evoked by short trains of low frequency stimuli are predominantly purinergic, whereas longer periods of stimulation and/or higher frequencies are usually required to reveal a substantial noradrenergic component of contraction (Kennedy *et al.* 1986b; Sjöblom-Widfeldt *et al.* 1990; Sjöblom-Widfeldt & Nilsson 1990; Evans & Cunnane 1992). Thus, the stimulation frequency-dependence of the proportions of ATP and NA contributing to the contractile response is probably re-

sponsible for observations in the rabbit saphenous (Burnstock & Warland 1987) and ileocolic arteries (Bulloch & Starke 1990) – short periods of electrical stimulation elicited monophasic, α,β-methylene-ATP-sensitive vasoconstriction, whereas longer periods produced biphasic vasoconstriction of which the first phase was α,β-methylene-ATP-sensitive while the second phase was prazosin-sensitive. Based on the observation that NA, in addition to ATP, is released during low frequency nerve stimulation, and that yohimbine (α_2-adrenoceptor antagonist) enhanced α,β-methylene-ATP-sensitive EJPs, Ramme *et al.* (1987) concluded that NA released by low frequency stimulation and/or short trains of stimuli does not reach the threshold concentration required to elicit contraction through postjunctional α_1-adrenoceptors, but is sufficient to activate prejunctional α_2-adrenoceptors. Therefore, stimulation at higher frequencies for longer durations must release sufficient NA to activate enough postjunctional α_1-adrenoceptors to elicit a contraction.

Although this hypothesis also explains findings in the dog basilar artery (Muramatsu *et al.* 1981) and rabbit saphenous artery (Burnstock & Warland 1987b), Evans and Surprenant (1992) were unable to demonstrate an α-adrenoceptor-sensitive component of the contractile response of guinea-pig submucosal arterioles even at stimulation parameters known to elicit substantial noradrenergic contractions in all arteries studied to date. In this particular study, the fact that reserpine had virtually no effect on neurogenic contractions eliminated a possibility of γ-adrenoceptor involvement, thus suggesting that ATP is the sole mediator of vasoconstriction in this vessel while NA modulates transmitter release via presynaptic α_2-receptors. The estimation that submuscosal arterioles contribute up to 40% of the total mesenteric splanchnic resistance (Parks & Jacobson 1987) and contribute significantly to the maintenance of systemic blood pressure, together with the findings of Evans and Surprenant (1992), suggest an important physiological role for ATP. Burnstock (1990d) has speculated upon the physiological significance of the dependence of relative rates of cotransmitter release on stimulation parameters (*i.e.* functional activity of the nerve terminal) in proposing that NA may be the most important component of

sympathetic cotransmission during activities such as gentle exercise, while ATP might be the more important component during stress when short burst frequencies occur in sympathetic nerves. Indeed, the *in vivo* activity of sympathetic nerves is highly irregular in both humans and animals (Delius *et al.* 1972; see also Nilsson *et al.* 1985).

1.5.4. P_{2Y}-purinoceptor-mediated vasodilatation of vascular smooth muscle

ATP-induced vasodilatation can occur via P_{2Y}-purinoceptors located on the vascular smooth muscle or endothelium (see section 1.2.3.). Smooth muscle P_{2Y}-purinoceptors are activated by ATP released from either sympathetic or NANC nerves, whereas intraluminally-released ATP (*e.g.* ATP from platelets and endothelium) is believed to act on endothelial P_{2Y}-purinoceptors.

1.5.4.1. P_{2Y}-purinoceptors and sympathetic cotransmission

Blood vessels in which P_{2Y}-purinoceptors have been identified on vascular smooth muscle cells, though few in comparison to those with endothelial P_{2Y}-purinoceptors, include rabbit mesenteric (Mathieson & Burnstock 1985), coronary (Corr & Burnstock 1991; Keef *et al.* 1992), and hepatic (Brizzolara & Burnstock 1991) arteries, guinea-pig coronary artery (Keef *et al.* 1992), and cat middle cerebral artery (Conde *et al.* 1991). It has been suggested that these vessels relax in response to ATP released as a cotransmitter with NA from sympathetic nerves. In the rabbit coronary artery (Corr & Burnstock 1991) NA coreleased with ATP from sympathetic nerves has been shown to cause dilatation via β-adrenoceptors which is consistent with the synergistic nature of P_{2X}-purinoceptor- and α_1-adrenoceptor-mediated vasoconstrictor responses seen in other vessels with sympathetic NA-ATP cotransmission.

1.5.4.2. P_{2Y}-purinoceptors and NANC transmission

The best example of a blood vessel receiving NANC transmission is the rabbit portal vein. This was the vessel originally used by Su (1975) to provide evidence for the release of ATP from perivascular nerves. He showed that although this release was abolished by tetrodotoxin, a significant proportion was resistant to blockade by guanethidine, suggesting

that this component arose from ATP released from a nonsympathetic source. Other evidence supporting these observations include: (i) the inability of sympathectomy to affect quinacrine fluorescence (Burnstock *et al.* 1984); (ii) mimicry by ATP of the rapid vasodilatation produced by stimulation of the perivascular nerves in the presence of guanethidine and atropine (Kennedy & Burnstock 1985a); (iii) reduction by reactive blue 2 of vasodilatation mediated by perivascular nerves or produced by exogenous ATP (Reilly *et al.* 1987). Similar evidence for "direct" purinergic vasodilatation has been found for rat intrapulmonary arteries (Inoue & Kannan 1988), skeletal muscle blood vessels (Shimada & Stitt 1984), and some coronary and cerebral resistance vessels (Burnstock 1990c).

1.5.4.3. P_{2Y}-purinoceptors and endothelium-dependent vasodilatation

Endothelial cell P_{2Y}-purinoceptors are believed to be activated by blood-borne ATP – usually that derived from platelets during aggregation, or from the endothelium itself as a result of increased blood flow (Bodin *et al.* 1991; Ralevic *et al.* 1992; Hassessian *et al.* 1993), ischaemia, hypoxia (Hopwood *et al.* 1989; Buxton *et al.* 1990), or physical damage (Pearson & Gordon 1979). It was recently shown, however, that hypoxia may not stimulate ATP release from rat cerebral cortex capillary endothelium (Phillis *et al.* 1993), as previously believed, which is in contrast to what occurs in the ischaemic or hypoxic heart (Borst & Schrader 1991; Headrick *et al.* 1992). ATP itself has been shown to induce release of ATP from cardiac endothelial cells via an action at P_{2Y}-purinoceptors (Yang *et al.* 1994). Numerous *in vitro* studies have demonstrated release of PGI_2 and EDRF following exposure of cultured endothelial, but not vascular smooth muscle, cells to ATP (De Mey & Vanhoutte 1981; Pearson & Gordon 1989; Pearson & Carter 1990). Furthermore, the EDRF released in response to ATP has been identified as NO (Palmer *et al.* 1987), derived from L-arginine (Palmer *et al.* 1988), and can be inhibited by haemoglobin, methylene blue (Ralevic *et al.* 1991), hydroquinone (Hopwood *et al.* 1989), and derivatives of L-arginine (Mathie *et al.* 1991; Kaul *et al.* 1992; Toda *et al.* 1993). Other studies have demonstrated the ability of ATP to produce endothelium-dependent relaxation even

when the capacity of endothelial cells to synthesize PGI_2 is blocked (Gordon & Martin 1983). Therefore, it has been proposed that ATP-induced vasodilatation is largely a consequence of NO under most conditions (Gordon 1990). It appears that the vasodilator effects of PGI_2 are only seen in response to larger amounts of ATP (Fleetwood & Gordon 1987). As discussed previously, although ATP can cause vasoconstriction via P_{2X}-purinoceptors, the net effect of intraluminal ATP is usually vasodilatation under normal conditions when the endothelium is intact (Cohen *et al.* 1984; Kaul *et al.* 1992). Thus, in the absence of endothelium (mechanical or chemical removal) (Kennedy *et al.* 1985; Machaly *et al.* 1988; Ralevic & Burnstock 1991b; Ralevic *et al.* 1992) or in the presence of reactive blue 2 (Taylor *et al.* 1989) a vasoconstrictor effect is often unmasked. The eventual metabolites of ATP, ADP and adenosine, can reinforce the vasodilatatory effects of ATP by activating P_{2Y}- and P_1-receptors located (predominantly) on the endothelium and smooth muscle, respectively.

1.6. *Actions of ATP in Vascular Beds and in Whole Animals*

The majority of studies investigating the vascular actions of ATP have been conducted in isolated tissues *in vitro*. Although *in vitro* observations are useful in the characterization and identification of receptor subtypes, closer approximations of physiological effects of ATP are obtained from studies using vascular beds and whole animals. Very little has been published on the effects of ATP and its analogues using vascular bed preparations; however, it appears that effects are variable not only among different vascular beds but also among different species. There are even fewer studies on the effects of ATP in whole animals. Nevertheless, evidence to date appears to implicate an important role for ATP and related purines in vasoregulation and haemostasis.

1.6.1. Vascular beds

Since vascular beds comprise small resistance arteries and arterioles, observations obtained from these preparations are physiologically more relevant to the control of

peripheral vascular resistance than are those from isolated vessels. Vasodilator and vasoconstrictor responses of ATP have been demonstrated in a number of vascular beds, including the coronary, hepatic, mesenteric, intestinal, pancreatic, renal, hindlimb, foetal, and facial/nasal.

1.6.1.1. Coronary vascular bed

Although the action of adenosine in heart function and tone has been the subject of intense investigation since Berne (1963) identified adenosine as a physiological regulator of cardiac blood flow, the cardiac actions of ATP have not received much attention until recently due to the general belief that ATP exerted its actions indirectly, following break-down to adenosine. Indeed, ATP's negative inotropic and chronotropic effects, anti-adrenoceptor effect, and inhibition of adrenergic neurotransmission are mediated by stimulation of P_1-purinoceptors since they are antagonized by the non-selective adenosine receptor antagonist, 8-phenyltheophylline (Burnstock 1980; Pelleg *et al.* 1990). Likewise, part of the vasodilatatory response to ATP has been attributed to adenosine (Ribeiro & Lima 1985), although the potency of adenosine as a coronary vasodilator is only about 25% that of ATP (Burnstock 1980). There is accumulating evidence implicating ATP *per se* not only as a coronary vasodilator (Olsson & Pearson 1990; Pelleg *et al.* 1990), but also as a mediator of positive inotropy (Hoyle & Burnstock 1986; Legssyer *et al.* 1988; Scamps *et al.* 1990; Pelleg *et al.* 1990).

A recent study (Mantelli *et al.* 1993) demonstrated that the depressant effect of ATP on atrial contractility is converted to a positive inotropic effect in the presence of either 1,3-dipropyl-8-cyclopentylxanthine (A_1 receptor antagonist) or 8-phenyltheophylline. In addition, these authors showed that suramin and reactive blue 2 concentration-dependently reduced the positive inotropic effect of ATP. Similarly, Legssyer *et al.* (1988) have shown that 8-phenyltheophylline enhances ATP-induced positive inotropy in rat papillary and ventricular muscles.

The rat coronary vascular bed responds to ATP by producing a biphasic response, consisting of an initial increase followed by a decrease in perfusion pressure, which is mediated by P_{2X}- and P_{2Y}-purinoceptors, respectively (Hopwood & Burnstock 1987). Furthermore, it has been demonstrated that ATP can mediate both endothelium-dependent (Hopwood *et al.* 1989) and endothelium-independent (Corr & Burnstock 1991) vasodilatation via action at endothelial or smooth muscle P_{2Y}-purinoceptors, respectively, in coronary vessels. It has been suggested that the vasodilatatory effect of ATP may contribute to myocardial reactive hyperaemia (Giles & Wilcken 1977; Olsson & Pearson 1990).

1.6.1.2. Hepatic vascular bed

In the isolated perfused rabbit liver at basal tone, ATP and its analogues produced vasoconstriction with a potency order consistent with an action at P_{2X}-purinoceptors (Ralevic *et al.* 1991). In the same study, raising vascular tone with NA revealed ATP-induced vasodilator responses with a rank order of potency of ATP analogues consistent with an action at P_{2Y}-purinoceptors. Furthermore, the ability of methylene blue (which antagonizes smooth muscle guanylyl cyclase and, possibly, inactivates EDRF) to antagonize responses to ATP, and the inability of 8-phenyltheophylline to attenuate ATP responses remaining after antagonism with methylene blue, demonstrated that the ATP-induced vasodilatation was due to a direct action on endothelial P_{2Y}-purinoceptors and subsequent release of EDRF, and not due to adenosine acting at P_1-purinoceptors following ectoenzymatic breakdown of ATP. The attenuation of relaxations to ATP but not those to adenosine by inhibitors of the L-arginine to NO pathway, N-monomethyl-L-arginine and N-nitro-L-arginine methyl ester (L-NAME), further confirms that ATP-induced vasodilatation of the hepatic arterial vascular bed is mediated by endothelial P_{2Y}-purinoceptors (Mathie *et al.*. 1991). Thus, it appears that the role of ATP in the hepatic vasculature is two-fold: constriction via smooth muscle P_{2X}-purinoceptors following release as a cotransmitter from sympathetic nerves (Brizzolara & Burnstock 1990), and relaxation via endothelial P_{2Y}-purinoceptors following local release. It has also been proposed that ATP may partici-

pate in the compensatory hepatic arterial vasodilatation in response to reduced portal blood flow (*i.e.* the "buffer response") (Ralevic *et al.* 1991).

1.6.1.3. Mesenteric vascular bed

Studies similar to those conducted in the hepatic vascular bed have also been undertaken in the isolated perfused rat mesenteric arterial bed. Ralevic and Burnstock (1991b) have demonstrated vasoconstrictor responses to ATP in preparations with basal tone and L-NAME-sensitive vasodilator responses in those with raised tone. The vasodilator response was abolished by removal of the endothelium with sodium deoxycholate. In the same study, evidence was presented for the existence of "pyrimidinoceptors" on the vascular smooth muscle and endothelium which mediate vasoconstriction and vasodilatation, respectively. In addition, it has been shown that both ATP and α,β-methylene-ATP, at subthreshold and supra-threshold doses, produce potentiation of vasoconstrictor responses to NA, thus indicating a postjunctional synergistic action between NA and ATP via action at α-adrenoceptors and P_{2X}-purinoceptors, respectively (Ralevic & Burnstock 1990b). This finding is supported by *in vitro* experiments using rat mesenteric arteries in which ATP, at doses not producing contraction, potentiates NA-induced contraction (Sjöblom-Widfeldt 1990). Sympathetic cotransmitter ATP has also been implicated in the augmented pressor response to transmural field stimulation during moderate cooling in the rat mesenteric vasculature, since the enhanced constrictor effect seen at 24 °C, but not 37 °C, is abolished by α,β-methylene-ATP in the presence of prazosin (Yamamoto *et al.* 1992).

1.6.1.4. Pancreatic vascular bed

Dual effects of ATP have been demonstrated in the isolated perfused rat pancreas (Chapal & Loubatieres-Mariani 1983; Hillaire-Buys *et al.* 1991). In this preparation, the effect of ATP at basal tone was concentration-dependent, with P_{2Y}-mediated vasodilatation occurring at low doses and P_{2X}-mediated vasoconstriction occurring at high doses. Also, blockade of P_{2Y}-purinoceptors revealed a vasoconstrictor response to ATP at a concentration without effect *per se*, while blockade of P_{2X}-purinoceptors enhanced ATP-in-

duced vasodilatation. Furthermore, theophylline (P_1-purinoceptor antagonist) failed to modify the vasodilator effect of ATP, thus indicating that vasodilator responses to ATP were not mediated by adenosine derived from the breakdown of ATP.

1.6.1.5. Intestinal vascular bed

P_{2X}-mediated vasoconstriction and P_{2Y}-mediated vasodilatation, in preparations with low and high perfusion pressures, respectively, have been demonstrated in the autoperfused intestinal circulation of anaesthetized cats (Taylor *et al.* 1989). Interestingly, α,β-methylene-ATP was found to be a more powerful vasoconstrictor in post-capillary capacitance vessels than in pre-capillary resistance vessels, and produced tachyphylaxis of P_{2X}-purinoceptors in the latter, but not the former (Taylor & Parsons 1991). In the same preparation, Taylor and Parsons (1989) demonstrated a prazosin- and yohimbine-resistant neurogenic vasoconstrictor response. The residual vasoconstrictor response was abolished by α,β-methylene-ATP desensitization; however, a small, slower onset, more sustained vasoconstriction persisted, the cause of which was not determined although it was suggested that high local concentrations of NA may have overcome α-adrenoceptor blockade or that there may have been an increase in the concentration of other transmitter substances (*e.g.* peptides). α,β-methylene-ATP desensitization also attenuated neurogenic vasoconstriction in cats not treated with α-adrenoceptor antagonists, with a 74% decrease in response elicited by 1 Hz but only a 31% reduction in the response to 8 Hz. This observation is consistent with *in vitro* findings of the frequency- and train length-dependency of the relative contributions of NA and ATP to the stimulation-induced vasoconstrictor response in rat mesenteric arteries (Sjöblom-Widfeldt 1990).

1.6.1.6. Renal vascular bed

α-adrenoceptor antagonist-resistant vasoconstrictor responses have been demonstrated in the isolated rat kidney such that the α_1-adrenoceptor antagonists, prazosin and corynanthine, and the nonselective α-adrenoceptor antagonist, phentolamine, did not significantly reduce vasoconstrictor responses to low frequency but attenuated the responses

to high frequency periarterial nerve stimulation (Schwartz & Malik 1989). The responses to low frequency stimulation and the α-adrenoceptor antagonist-resistant responses at higher frequencies were abolished or significantly reduced after desensitization of P_{2X}-purinoceptors with α,β-methylene-ATP. In comparison, prazosin alone dramatically reduced the neurogenic vasoconstriction at all frequencies in the rabbit kidney, suggesting species differences in renal vascular responses to periarterial nerve stimulation. Churchill and Ellis (1993) have demonstrated both P_{2X}-mediated vasoconstriction and P_{2Y}-mediated endothelium-dependent vasodilatation in response to 2-methylthio-ATP and α,β-methylene-ATP which are specific agonists for the P_{2Y}- and P_{2X}-purinoceptors, respectively.

1.6.1.7. Hindlimb vascular bed

In the rat hindlimb, a biphasic response to ATP is produced, with vasoconstriction preceding a transient vasodilatation, although the pressor response is likely mediated by 5-hydroxytryptamine (Sakai *et al.* 1979). In the hindlimb of the cat, ATP and ADP caused vasodilatation, and the rank order of potency of ATP analogues was consistent with an action at P_{2Y}-purinoceptors (Taylor *et al.* 1989). This vasodilatation was reduced by 88% by gossypol, a potent and irreversible inhibitor of EDRF (Dudel & Forstermann 1988). In the rabbit hindlimb *in vivo*, Shimada and Stitt (1984) determined that the increase in blood flow to skeletal muscle produced by hypothalamic stimulation (the "defence reaction") is mediated by ATP acting at P_2-purinoceptors.

1.6.2. Whole animals

Bolus injection or infusion of ATP in animals has long been known to induce a profound reduction in blood pressure often associated with bradycardia. Gillespie (1934) reported one of the earliest studies demonstrating this effect in anaesthetized cats. Following bolus injection in the anaesthetized rat the onset of vasodilatation is typically rapid and the duration of the peak depressor response is transient (Delbro & Burnstock 1987). In contrast, the blood pressure response to a bolus injection of ATP in the pithed rat was reported to consist of an initial rise followed by a decrease and a second increase (Schlicker

et al. 1989). Measurement of cardiac output revealed that peak pressure changes to bolus injections of ATP and several ATP analogues were at least 80% due to changes in systemic vascular resistance (Sollevi *et al.* 1984; Delbro & Burnstock 1984) which confirms the early findings of Rowe *et al.* (1962). The observation that dipyridamole, an inhibitor of adenosine uptake, significantly prolonged and potentiated the depressor response to ATP injection or infusion suggests that P_1-purinoceptors are involved as a result of metabolism of ATP to adenosine (Sollevi *et al.* 1984; Delbro & Burnstock 1987). These findings are confirmed by comparison of the arterial and venous ATP and adenosine levels during inferior vena cava infusion of ATP in dogs – very low ATP levels in arterial samples indicated that virtually all of the nucleotide had been metabolized to adenosine before reaching the arterial section of the vasculature, whereas in venous samples collected simultaneously, only small amounts of adenosine were present, suggesting that metabolism occurs during passage through the pulmonary and coronary circulation (Sollevi *et al.* 1984). Consistent with this observation, the same study demonstrated that infusion of adenosine and ATP produced equal decreases in systemic vascular resistance.

There are very few studies which have attempted to characterize the receptor subtypes responsible for ATP effects *in vivo*, and even fewer have addressed the functional significance of NA-ATP cotransmission. This, however, appears to be a technical problem since many of the pharmacological tools used successfully *in vitro* have not performed similarly in intact animals. For example, α,β-methylene-ATP desensitization of P_{2X}-purinoceptors has been shown to lack selectivity *in vivo* (Schlicker *et al.* 1989; Daziel *et al.* 1990). In addition, α,β-methylene-ATP exerts cardiotoxic effects at doses required to produce desensitization, and even when desensitization is produced by administering the dose of α,β-methylene-ATP incrementally (to avoid cardiac arrest), receptor desensitization rapidly declines within a few minutes (Schlicker *et al.* 1989; Tarasova & Rodionov 1992). As a metabolically-stable agonist, α,β-methylene-ATP cannot be infused due to rapid tachyphylaxis and cardiotoxicity and, as a bolus injection, has been found to pro-

duce unpredictable vascular and cardiac effects which vary according to the route of administration (Delbro *et al.* 1985). The recently identified P_2-purinoceptor antagonist, suramin, has shown promise as a useful pharmacological tool *in vivo*. Urbanek *et al.* (1990) demonstrated that suramin produces a 6-fold parallel shift to the right of the dose-response curve for α,β-methylene-ATP in the pithed rat, while Schlicker *et al.* (1989), in the same preparation, showed that suramin decreased not only the response to α,β-methylene-ATP but also the initial rise in blood pressure (but not the subsequent decrease) elicited by ATP or electrical stimulation of the thoracolumbar sympathetic outflow. Nevertheless, it was suggested that the ability of suramin to reduce the electrically-evoked pressor response may be the result of inhibition of neurotransmitter release via a presynaptic site rather than blockade of postsynaptic P_{2X}-purinoceptors (Schlicker *et al.* 1989). Therefore, further characterization of the action of suramin is required before the use of this drug can provide substantive evidence of the role of cardiovascular purinoceptors *in vivo*.

An α-adrenoceptor antagonist-resistant response to sympathetic nerve stimulation, analogous to that observed in isolated vessels and vascular beds, has been identified in the pithed rat (Flavahan *et al.* 1985). This vasopressor response was abolished by 6-hydroxydopamine or guanethidine, indicating a sympathetic nerve origin, and was found to constitute approximately half the control response. However, since the response remaining after reserpine treatment was smaller than the prazosin-plus-rauwolscine-resistant response, it was suggested that part of the α-adrenoceptor antagonist resistant pressor response could be mediated, in part, by γ-adrenoceptors. In this study, desensitization with α,β-methylene-ATP reduced the nerve-mediated pressor response only after α-blockade or reserpine pretreatment, but not in drug-free controls; this was interpreted as indicating a relatively minor role for purinergic cotransmission in rat vasculature. Subsequently, the use of a novel administration regime for α,β-methylene-ATP resulted in a 60% reduction of the nerve-evoked pressor response in the absence of α-adrenoceptor blockade, and completely abolished the residual response after α-adrenoceptor antagonism

(Bulloch & McGrath 1988b). The previous inability of α,β-methylene-ATP to affect the pressor response before α-antagonism, or to abolish the residual response after α-antagonism completely (Flavahan *et al.* 1985), was attributed to the transient nature of its action. Thus, the effects of α,β-methylene-ATP and α-antagonists appeared to be additive, with 40% of the pressor response attributable to α-adrenoceptor (mainly α_1) stimulation and the remainder to P_2-purinoceptor stimulation (Bulloch & McGrath 1986, 1988a). Similarly, Daziel *et al.* (1990) demonstrated that the pressor response to sympathetic nerve stimulation in the pithed rat was attenuated by approximately 80% following α,β-methylene-ATP desensitization; however, unlike Bulloch and McGrath (1988b) who deemed the desensitization procedure "selective" by virtue of its failure to block responses to NA, Daziel and coworkers (1990) found that α,β-methylene-ATP treatment also attenuated the pressor responses to NA, angiotensin II, and vasopressin.

In the pithed rat, it has been demonstrated that the purinergic component of the sympathetic pressor response can be attenuated by nifedipine, which blocks L-type Ca^{2+} channels, whereas the adrenergic component is largely resistant to Ca^{2+} channel blockade (Bulloch & McGrath 1988a). Nifedipine also attenuated the vasopressor response produced by intravenous bolus administration of α,β-methylene-ATP. These observations appear to provide an *in vivo* correlate for *in vitro* findings which indicate electromechanical coupling (*i.e.* involving the EJP and voltage-dependent Ca^{2+} channels; see section 1.5.3) as the mechanism of ATP-induced vasoconstriction.

Recently, purinergic transmission was found to contribute significantly to the pressor sinocarotid reflex but only negligibly to the pressor response resulting from stimulation of somatic afferents (Tarasova & Rodionov 1992). This study demonstrated that the pressor responses to sciatic nerve stimulation, and to asphyxia, were strongly depressed by phentolamine and dihydroergotamine, while the average magnitude of the response to carotid artery occlusion remained unchanged in the presence of α-adrenoceptor blockade. In comparison, ganglion blockade with hexamethonium brought about a pronounced

decrease in all three reflexes (*i.e.* those to carotid artery occlusion, sciatic nerve stimulation, and asphyxia). Interestingly, the ability of α-adrenoceptor antagonism to attenuate the sinocarotid reflex was quite variable, with responses ranging from a 70% decrease in pressor response to only a slight decrease or even augmentation of the response. Desensitization by α,β-methylene-ATP attenuated the pressor responses resistant to α-adrenoceptor blockade, and recovery from desensitization (in 3 - 5 min) was accompanied by restoration of the sinocarotid reflex. Another interesting finding from this study is that the rate of blood pressure elevation, but not the magnitude, in response to sciatic nerve stimulation was lowered in the presence of α,β-methylene-ATP. This observation is consistent with studies on isolated vessels which reveal that the initial, phasic component of the response to nerve stimulation is preferentially inhibited by α,β-methylene-ATP (Sjöblom-Widfeldt 1990).

1.7. *Aims of the Thesis*

The vascular effects of ATP and its role in the regulation of blood pressure in whole animals are not well characterized for reasons discussed above. Although some studies have identified α-adrenoceptor antagonist-resistant vascular responses in whole animals, very few have sought to determine whether these responses have a purinergic component (Flavahan *et al.* 1985; Bulloch & McGrath 1988a,b; Schlicker *et al.* 1989; Daziel *et al.* 1990; Tarasova & Rodionov 1992). Therefore, the present thesis is an attempt to study the cardiovascular effects and possible venoregulatory role of ATP in the conscious rat.

The specific aims of the experiments described in this thesis are:

(i) To demonstrate resistance to α-adrenoceptor antagonism in the venous system, as reflected by mean circulatory filling pressure;

(ii) To determine whether purinergic neurotransmission is involved in the control of basal and reflex venous tone;

(iii) To determine whether sympathetic NA-ATP cotransmission contributes to the maintenance of venous tone;

(iv) To characterize the arterial and venous effects of exogenous ATP.

2. METHODS & MATERIALS

2.1. *Methods*

2.1.2. Animals

Male Sprague-Dawley rats (360 ± 30 g, mean ± s.d., and median = 360 g) obtained from Charles River, Canada were used in this study. Rats were kept in the Department of Pharmacology and Therapeutics of the University of British Columbia and given free access to Purina Rat Chow and water. Recommendations from the Canada Council of Animal Care and internationally accepted principles in the care and use of experimental animals were adhered to.

2.1.3. Surgical preparation of animals for use in MCFP studies

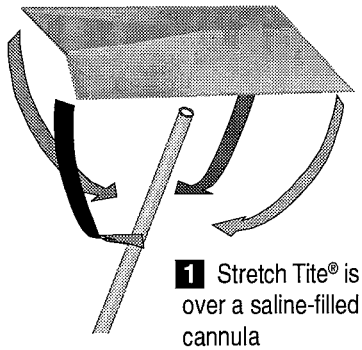
2.1.3.1. Standard preparation

Mean circulatory filling pressure (MCFP) was determined by the method of Yamamoto *et al.* (1980). Animals were prepared while anaesthetized with halothane vaporized in air (5% for induction, 1.5-2.5% for maintenance). PE 50 cannulae (Intramedic, NJ, USA) were inserted into the left iliac artery and right iliac vein for the measurement of blood pressure by a pressure transducer (model P23XL, P23ID or P23D8, Gould Statham, CA, USA) and the administration (bolus injection or infusion with Harvard Apparatus infusion pump model 975, Harvard Apparatus, MA) of drugs, respectively. These cannulae were advanced approximately 2-3 cm into each vessel. An additional PE 50 cannula was inserted into left iliac vein and advanced 4-5 cm into the inferior vena cava to record central venous pressure (CVP) with a pressure transducer (model P23XL, P23ID or P23D8, Gould Statham, CA, USA). Gentle tapping on the ventral mid-abdominal region of the rat produced a large, transitory increase in central venous pressure if the iliac central venous cannula was positioned correctly.

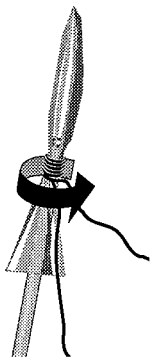
A saline-filled, balloon-tipped cannula was placed in the right atrium through the right external jugular vein, and proper positioning of the balloon was initially assessed by care-

fully shaking the cannula. Shaking the cannula produced arrhythmias if the balloon was positioned correctly. Proper positioning was further verified by testing the ability of the inflated balloon to stop the circulation completely as described in section 2.1.4. This was shown by a simultaneous, smooth increase in venous pressure and a decrease in mean arterial pressure (MAP) to less than 25 mmHg. Atrial balloons were constructed from PE 50 tubing, 5-0 silk suture and Stretch Tite® plastic wrap as shown below. All cannulae were filled with heparinized saline (25 iu ml^{-1} in 9% w/v NaCl) and tunneled with a trochar to the back of the neck where they were exteriorized, secured, flame-sealed, and colour coded. Incisions were closed and rats were placed in individual cages, with free access to food and water, to recover from surgery for 18-24 h before being used in experiments.

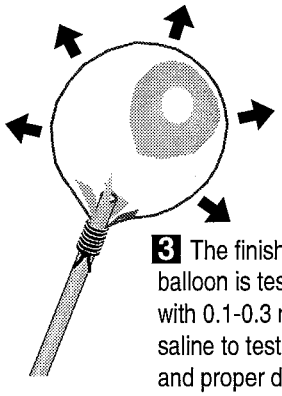
Construction of Atrial Balloons



1 Stretch Tite® is folded over a saline-filled cannula



2 Silk suture is lashed around the Stretch Tite® and the cannula several times then secured; excess Stretch Tite is cut away



3 The finished atrial balloon is test-inflated with 0.1-0.3 ml of saline to test for leaks and proper deflation

After recovery, arterial and central venous lines were reconnected to pressure transducers attached to a Grass multichannel polygraph (model 79D, Grass Instrument Co., MA, USA). Mean arterial and central venous pressures were obtained by electronic averaging. Heart rate (HR) was obtained either manually from the upstroke of the blood pressure recording or electronically using a Grass tachograph (model 7P44C or 7P4G, Grass Instrument Co., MA, USA). At the conclusion of experiments, rats were sacrificed using i.v. pentobarbitone and post-mortem dissection of rats was used to confirm that the atrial

balloon and central venous line were positioned properly. Results from rats in which these were improperly positioned were discarded.

2.1.3.2. Modifications to the standard preparation: experiments involving suramin

PE 50 cannula was inserted into the right carotid artery for the injection of suramin or its vehicle (saline). Care was taken not to damage the vagus nerve.

2.1.4. Measurement and calculation of MCFP

MCFP was determined in conscious, unrestrained rats by inflating the implanted atrial balloon with ≈ 0.5 ml of saline in order to arrest circulation. Within 5 sec after circulatory arrest, MAP decreased to a final arterial pressure (FAP) of 25 mmHg or less and central venous pressure simultaneously increased from baseline to a plateau value. The difference between the baseline and plateau venous pressures is referred to as venous plateau pressure (VPP). MCFP was calculated using equation 1, taken from Samar and Coleman (1978), and using a value of 1/60 for the arterial to venous compliance ratio (Yamamoto *et al.* 1980).

$$MCFP = VPP + \frac{1}{60}(FAP - VPP) \qquad \textit{equation 1}$$

2.2. *Experimental Design, Statistical Analysis, and Presentation of Results*

2.2.1. Experimental design

All experiments followed a randomized, split plot factorial design with repeated measures on dose-dependent factors. Groups of experiments were arranged as replicated 2 x 2 x χ factorials, with 2 pretreatment levels, 2 treatment levels and χ repeated measures on the treatment (5 or 7 sequential doses). Repeated measurements were conducted on MAP, HR and central venous pressure. Plateau central venous pressures during circulatory arrest were converted to MCFP using the equation defined in section 2.1.4. In addi-

tion, control values were measured before and after pretreatment and are referred to as "Pretreatment Control" and the "Post-treatment Control", respectively.

2.2.2. Statistical analysis

Analyses were performed separately on control values and dose-response relationships. Control values were analyzed without transformation, whereas dose-response analysis was performed on data expressed as percentage of post-treatment control in order to reduce variance introduced by the control covariate.

i. Analysis of variance with replication was used to test the effect of pretreatment on pre- and post-treatment control means.

ii. To test the between groups pretreatment and treatment main effects and the pretreatment x treatment interaction on the average response, standard repeated-measures analysis of variance was used. Between groups *a priori*, multiple comparisons were made using the Tukey (hsd) procedure.

iii. Within groups effects were analyzed with standard repeated measures analysis of variance to test main and interaction effects. The Huynh-Feldt epsilon correction for multisample asphericity was used to adjust probabilities. Within groups *a priori* hypotheses were tested using multivariate profile/trend analysis (referred to as curve analysis in Results). Profile/trend analysis was used to compare dose-dependency of responses. The F-value was estimated using Wilks' lambda. Bonferonni layering was used to adjust probabilities when multiple comparisons were made.

The appropriate corrections were made for unequal sample sizes. A $p < 0.05$ was preselected as the criterion for statistical significance. The statistical package, SYSTAT 5.2.1 for Macintosh (SYSTAT Inc., IL), was used to analyze all data.

2.2.3. Presentation of results

All values are expressed as mean ± s.e.mean with the number of observations given in brackets, if provided. Values in all tables are referenced to their respective figures.

Points in figures are mean % of post-treatment control with error bars representing ± s.e.mean. Vehicle-treated time controls are presented in the Controls section of the Results and in the appropriate figures as shaded lines and symbols. The shaded, control data is repeated in figures along with the solid black, drug-treated data so that main and interactive effects can be distinguished.

2.3. *Experimental Protocol*

The general design used in all experiments is presented in section 2.2.1.

2.3.1. Phentolamine and mecamylamine

i. Rats were pretreated with either saline (1.8 μl min^{-1}) or hydralazine (0.3 μmol kg^{-1} min^{-1}) which was continuously infused through the central venous line for the remainder of the experiment. After a plateau response to the pretreatment was attained (20 min), a dose-response curve to either saline (1.8 - 100 μl min^{-1}) or phentolamine (0.035 - 1.9 μmol kg^{-1} min^{-1}) was constructed. Saline and phentolamine were infused through the right iliac venous line until a plateau response was attained (7 min), after which MCFP was measured. Rats were allowed 3 min to recover after each dose, so that MCFP measurements were made at 10 min intervals. MCFP measurements were also taken prior to the administration of the pretreatment (Pretreatment Control) and after a steady state response to the pretreatment was attained (Post-treatment Control). MAP and HR were continuously monitored.

ii. Rats were pretreated with a continuous infusion of either 30% ethanol in double-distilled water (37 μl min^{-1}) or nifedipine (0.3 μmol kg^{-1} min^{-1}) for 20 min followed by construction of dose-response curves to either saline (1.8 - 100 μl min^{-1}) or phentolamine (0.035 - 1.9 μmol kg^{-1} min^{-1}) infused through the right iliac venous line. As a precaution arising from the light-sensitivity of nifedipine, all bottles, syringes and cannulae containing nifedipine were covered with aluminium foil. Haemodynamic measurements were taken as described in 2.3.1.(i).

iii. Rats were pretreated with a continuous infusion of either saline (1.8 μl min^{-1}) or hydralazine (0.3 μmol kg^{-1} min^{-1}) for 20 min followed by the construction of a cumulative dose-response curve to either saline (0.3 ml $dose^{-1}$) or mecamylamine (0.18 - 180 μmol kg^{-1}) injected through the right iliac venous line. Haemodynamic measurements were taken as described in 2.3.1.(i).

iv. In preliminary experiments, the dose range of mecamylamine was selected by measuring the ability of each dose of mecamylamine (0.18 - 180 μmol kg^{-1}) to inhibit the reflex bradycardia produced by a methoxamine (80 nmol kg^{-1})-induced increase in MAP. The left iliac artery and vein were cannulated for measurement of MAP and HR, and administration of drugs, respectively. Methoxamine was i.v. injected at 10 min intervals, 7 min following i.v. injection of each dose of mecamylamine which allowed 3 min for haemodynamic readings to recover from methoxamine.

2.3.2. Suramin I

i. Rats were pretreated with continuous infusion (through the central venous line) of either saline (1.8 μl min^{-1}) or hydralazine (0.3 μmol kg^{-1} min^{-1}) followed by construction of a cumulative dose-response curve to either saline (0.3 ml $dose^{-1}$) or suramin (25 - 400 μmol kg^{-1}). Saline and suramin were given as bolus injections through the carotid arterial line 7 min before MCFP was measured, not including the time required for injection. Preliminary studies demonstrated that i.a. injection substantially reduced the severe cardiotoxic effects observed when suramin was administered i.v. High doses of suramin (100 - 400 μmol kg^{-1}) were injected slowly over a 2-3 min period to minimize suramin's severe cardiotoxic and convulsive effects. Rats were allowed 3 min to partially recover from the effects of each dose, so that MCFP measurements were made at ≈ 10 min intervals. MCFP measurements were also taken prior to and 20 min after the start of the infusion of hydralazine, which was at the plateau phase of the response to hydralazine. MAP and HR were continuously monitored.

ii. Pretreatments were administered similarly to that described in 2.3.2.(i) except that the continuous infusion consisted of either 30% ethanol in double-distilled water (37 μl min^{-1}) or nifedipine (0.3 μmol kg^{-1} min^{-1}). Administration of saline or suramin and measurement of haemodynamic variables were the same as described in 2.3.2.(i). As a precaution arising from the light-sensitivity of nifedipine, all bottles, syringes and cannulae containing nifedipine were covered with aluminium foil.

iii. Rats were pretreated with either saline (0.3 ml) or mecamylamine (18 μmol kg^{-1}) i.v. injected through the central venous line, followed (20 min later) by construction of dose-response curves to either saline or suramin. Administration of saline or suramin and measurement of haemodynamic variables were the same as described in 2.3.2.(i). The degree of mecamylamine-induced ganglionic blockade was assessed for each experiment by comparing the reflex tachycardia induced following i.v. injection of a depressor dose of acetylcholine (2 μg) before and after (at the end of the experiment) administration of mecamylamine. MAP and HR were continuously monitored.

2.3.3. Phentolamine

i. Rats were pretreated with either saline (0.3 ml) or suramin (200 μmol kg^{-1}) which was injected slowly through the carotid arterial line. After a plateau response to the pretreatment was attained (10 min), a dose-response curve to i.v. infusion of either saline (1.8 - 100 μl min^{-1}) or phentolamine (0.035 - 1.9 μmol kg^{-1} min^{-1}) at dose-intervals of 10 min was constructed. MCFP measurements were also taken prior to and 10 min after pretreatment with suramin and 7 min after the infusion of each dose of phentolamine or saline.

2.3.4. Suramin II

i. Rats were pretreated with either 6% citrate dissolved in double-distilled water (0.4 ml) or reserpine (3 mg kg^{-1}) which was injected i.p. 24 h before the start of the experiment. On the next day, a cumulative dose-response curve to either saline (0.3 ml $dose^{-1}$) or suramin (25 - 400 μmol kg^{-1}) was constructed. Administration of suramin

and all haemodynamic measurements were the same as described in 2.3.2.(i), except that a pretreatment control measurement could not be taken.

ii. Rats were pretreated with either saline (26 μl min^{-1} for 10 min followed by 1.8 μl min^{-1} for the duration) or guanethidine (loading dose of 7.2 μmol kg^{-1} min^{-1} for 10 min followed by a maintenance dose of 0.5 μmol kg^{-1} min^{-1}) which was infused through the central venous line for the remainder of the experiment. At the plateau response to the pretreatment (1 hr), a cumulative dose-response curve to either saline or suramin was constructed. Administration of suramin and all haemodynamic measurements were the same as described in 2.3.2.(i).

2.3.5. ATP

i. Rats were pretreated with either saline (0.3 ml) or mecamylamine (18 μmol kg^{-1}) injected through the central venous cannula. Attainment of a plateau response to pretreatment (20 min) was followed by construction of a dose-response curve to either saline (3.5 - 200 μl min^{-1}) or ATP (0.29 - 16.8 μmol kg^{-1} min^{-1}) infused through the right iliac venous line at dose-intervals of 10 min. MCFP measurements were also taken prior to and 20 min after pretreatment with mecamylamine and 7 min after the infusion of each dose of ATP or saline. Administration of saline or suramin and measurement of haemodynamic variables were the same as described in 2.3.2.(i). The degree of mecamylamine-induced ganglionic blockade was assessed for each experiment by comparing the reflex tachycardia induced following i.v. injection of a depressor dose of acetylcholine (2 μg) before and after (at the end of the experiment) administration of mecamylamine. MAP and HR were continuously monitored.

ii. Rats were pretreated with either saline (0.3 ml) or suramin (200 μmol kg^{-1}) which was injected slowly through the carotid arterial line. After a plateau response to the pretreatment was attained (10 min), a dose-response curve to infusion of either saline or ATP was constructed. Administration of ATP and all measurements of MCFP were identical to those described in 2.3.5.(i).

iii. Rats were pretreated with either saline (0.3 ml) or cibacron blue (13 μmol kg^{-1}) injected through the central venous cannula. After a plateau response to the pretreatment was attained (20 min), a dose-response curve to either saline or ATP was constructed. Administration of ATP and all measurements of MCFP were identical to those described in 2.3.5.(i).

iv. Rats were pretreated with either saline (0.5 ml) or 8-phenyltheophylline (27 μmol kg^{-1}) which was injected through the central venous cannula. After a plateau response to the pretreatment was attained (20 min), a dose-response curve to either saline or ATP was constructed. Administration of ATP and all measurements of MCFP were identical to those described in 2.3.5.(i).

2.4. *Drugs*

All drugs were prepared fresh daily and dissolved or diluted with normal saline (9% NaCl w/v in double-distilled water), except where noted. Sonication was used to dissolve hydralazine, mecamylamine, and phentolamine in vehicle. Acetylcholine chloride, adenosine 5'-triphosphate disodium, cibacron blue 3GA, guanethidine, hydralazine HCl, mecamylamine HCl, α,β–methylene ATP Li salt, nifedipine, and 8-phenyltheophylline were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Citric acid and sodium chloride were obtained from Fisher Scientific Co. (ON, Can). Sodium suramin was obtained from CB Chemicals (Woodbury, CT, USA). Methoxamine, sodium pentobarbitone, phentolamine, and reserpine were obtained from Burroughs Wellcome Inc (ON, Can), MTC Pharmaceuticals (ON, Can), CIBA Geigy (ON, Can) and Carl Roth (Germany), respectively. Nifedipine was dissolved in 100% ethanol then diluted with double-distilled water to 30% ethanol. Doses of 8-phenyltheophylline were prepared as individual aliquots and suspended in saline. Reserpine was dissolved in 6% citrate in double-distilled water.

3. RESULTS

3.1. *Controls: Time and Volume Effects of Vehicle in the Absence and Presence of Various Drug Treatments*

Pre- and post-treatment control values of MAP, HR, and MCFP for time/volume control experiments in vehicle- or drug-treated groups are presented in Table 1. The data shown in Table 1 and Figures 1.1 to 1.6.3 is presented in this section in order to illustrate the time and volume effects of vehicle in the absence and presence of drug treatment. The dose-response curves illustrated in the figures of this section reappear (as shaded lines and symbols) for the purpose of comparison along with the appropriate groups in sections 3.2 to 3.6 in which the time/volume effects are described.

Table 1. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and after treatment (Post-treatment Control) with either vehicle or drug in conscious, unrestrained Sprague-Dawley rats.

		Pretreatment Controls			*Post-treatment Controls*			
Figure Reference	*Treatment Group*	*HR (beats/min)*	*MAP (mmHg)*	*MCFP (mmHg)*	*HR (beats/min)*	*MAP (mmHg)*	*MCFP (mmHg)*	*n*
1.1	Saline	404 ± 20	101 ± 1	5.4 ± 0.1	396 ± 18	100 ± 2	5.2 ± 0.1	7
	Hydralazine	414 ± 12	106 ± 2	5.5 ± 0.2	477 ± 8	78 ± 2	6.7 ± 0.2	7
	Suramin	412 ± 10	105 ± 3	5.6 ± 0.1	355 ± 11	114 ± 2	5.9 ± 0.2	6
1.2	DDW (30% EtOH)	410 ± 10	109 ± 2	5.8 ± 0.2	420 ± 12	102 ± 3	5.2 ± 0.2	6
	Nifedipine	380 ± 8	104 ± 2	5.3 ± 0.2	420 ± 8	70 ± 2	6.5 ± 0.3	9
1.3	Saline	383 ± 12	107 ± 3	5.2 ± 0.1	371 ± 9	107 ± 4	5.1 ± 0.1	6
	Hydralazine	414 ± 12	106 ± 2	5.5 ± 0.2	477 ± 8	78 ± 2	6.7 ± 0.2	7
1.4	Saline	408 ± 11	101 ± 2	5.3 ± 0.2	418 ± 15	102 ± 2	5.1 ± 0.3	6
	Hydralazine	421 ± 14	104 ± 2	5.4 ± 0.2	475 ± 17	77 ± 3	6.5 ± 0.2	6
	Mecamylamine	418 ± 18	103 ± 3	5.2 ± 0.2	393 ± 15	76 ± 4	3.6 ± 0.1	6
	Guanethidine	425 ± 17	104 ± 3	5.6 ± 0.2	393 ± 10	79 ± 2	5.4 ± 1.1	5
1.5	DDW (6% Citrate)	416 ± 12	104 ± 2	5.3 ± 0.2	403 ± 15	103 ± 3	5.2 ± 0.1	6
	Reserpine	–	–	–	357 ± 17	83 ± 3	5.4 ± 0.2	6
1.6	Saline	383 ± 18	98 ± 2	5.3 ± 0.1	377 ± 16	102 ± 2	5.1 ± 0.2	6
	Suramin	432 ± 17	103 ± 3	5.3 ± 0.1	351 ± 13	112 ± 5	4.6 ± 0.2	6
	Mecamylamine	406 ± 12	106 ± 3	5.7 ± 0.3	362 ± 6	72 ± 2	4.4 ± 0.3	5
	Cibacron Blue	375 ± 8	100 ± 2	5.6 ± 0.1	383 ± 15	103 ± 2	5.7 ± 0.2	7
	8-Phenyltheophylline	396 ± 19	102 ± 2	5.5 ± 0.1	391 ± 14	105 ± 3	5.5 ± 0.2	7

Values are mean ± s.e.mean. DDW = double distilled water, EtOH = ethanol, – = not measured. Figures referenced show the volume- or infusion rate-response curves as % of control value for each treatment. Doses of drugs were as follows: cibacron blue (13 μmol kg^{-1}), guanethidine (7.2 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}), hydralazine (0.3 μmol kg^{-1} min^{-1}), mecamylamine (18 μmol kg^{-1}), nifedipine (0.3 μmol kg^{-1} min^{-1}), 8-phenyltheophylline (27 μmol kg^{-1}), reserpine (3 mg kg^{-1} 24 h prior to study), suramin (200 μmol kg^{-1}).

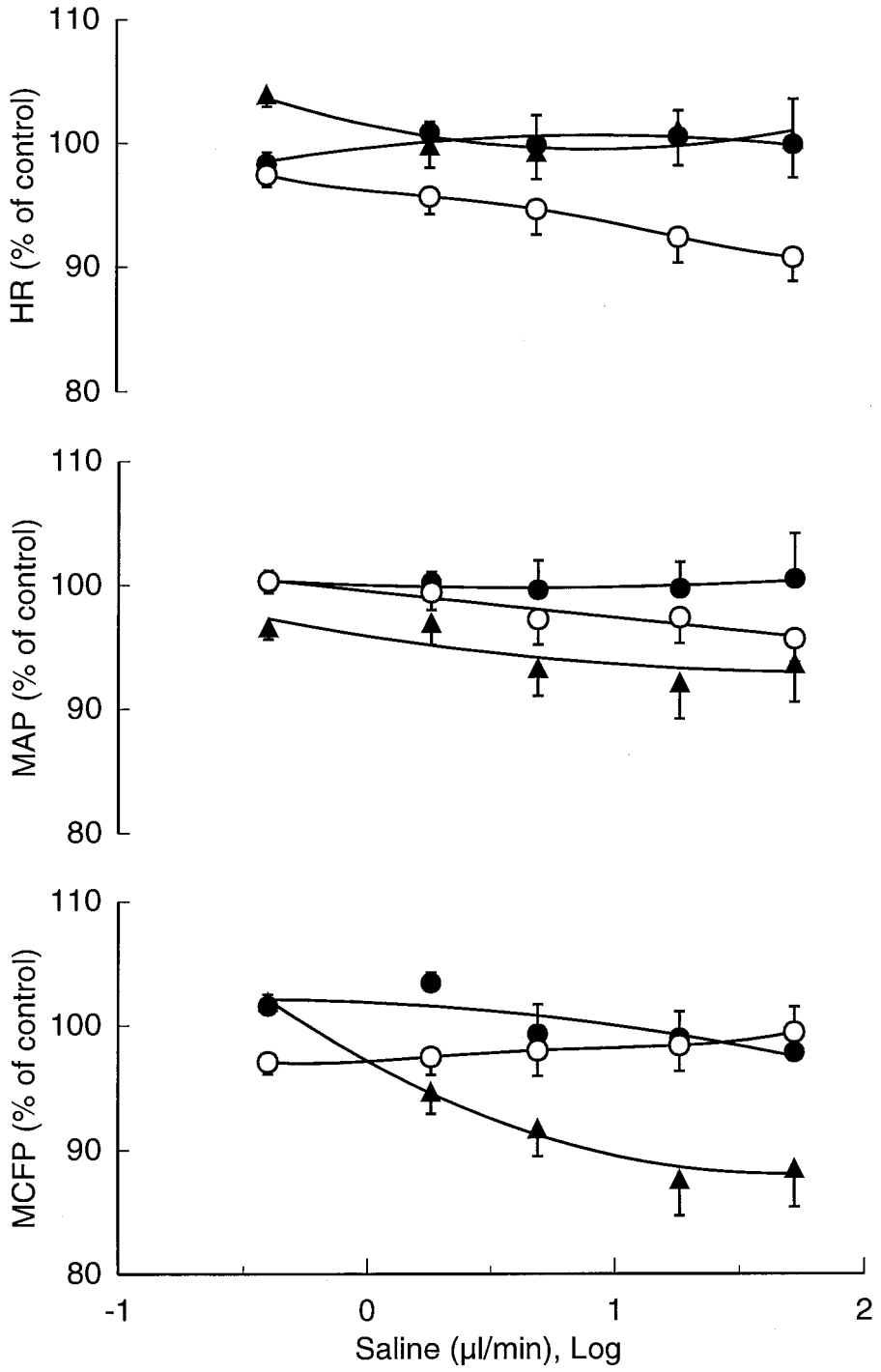


Figure 1.1. Dose-response curves of the effects of saline on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with saline (●), hydralazine (0.3 µmol kg^{-1} min^{-1}) (O) or suramin (200 µmol kg^{-1}) (▲); each point represents mean ± s.e.mean, $n \geq 6$.

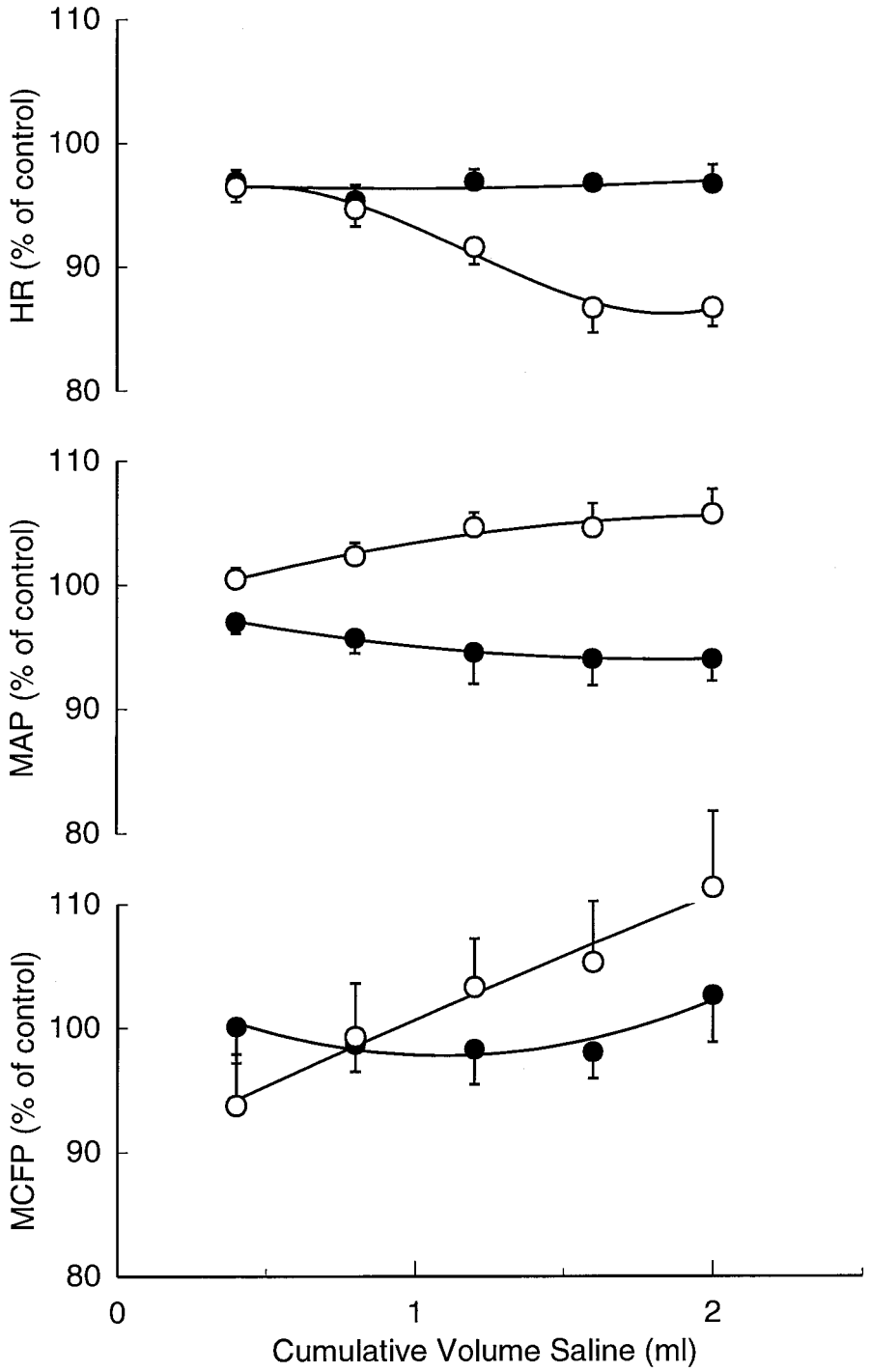


Figure 1.2. Dose-response curves of the effects of saline on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with vehicle (30% ethanol in double-distilled water) (●) or nifedipine (0.3 μmol kg^{-1} min^{-1}) (O); each point represents mean ± s.e.mean, $n \geq 6$.

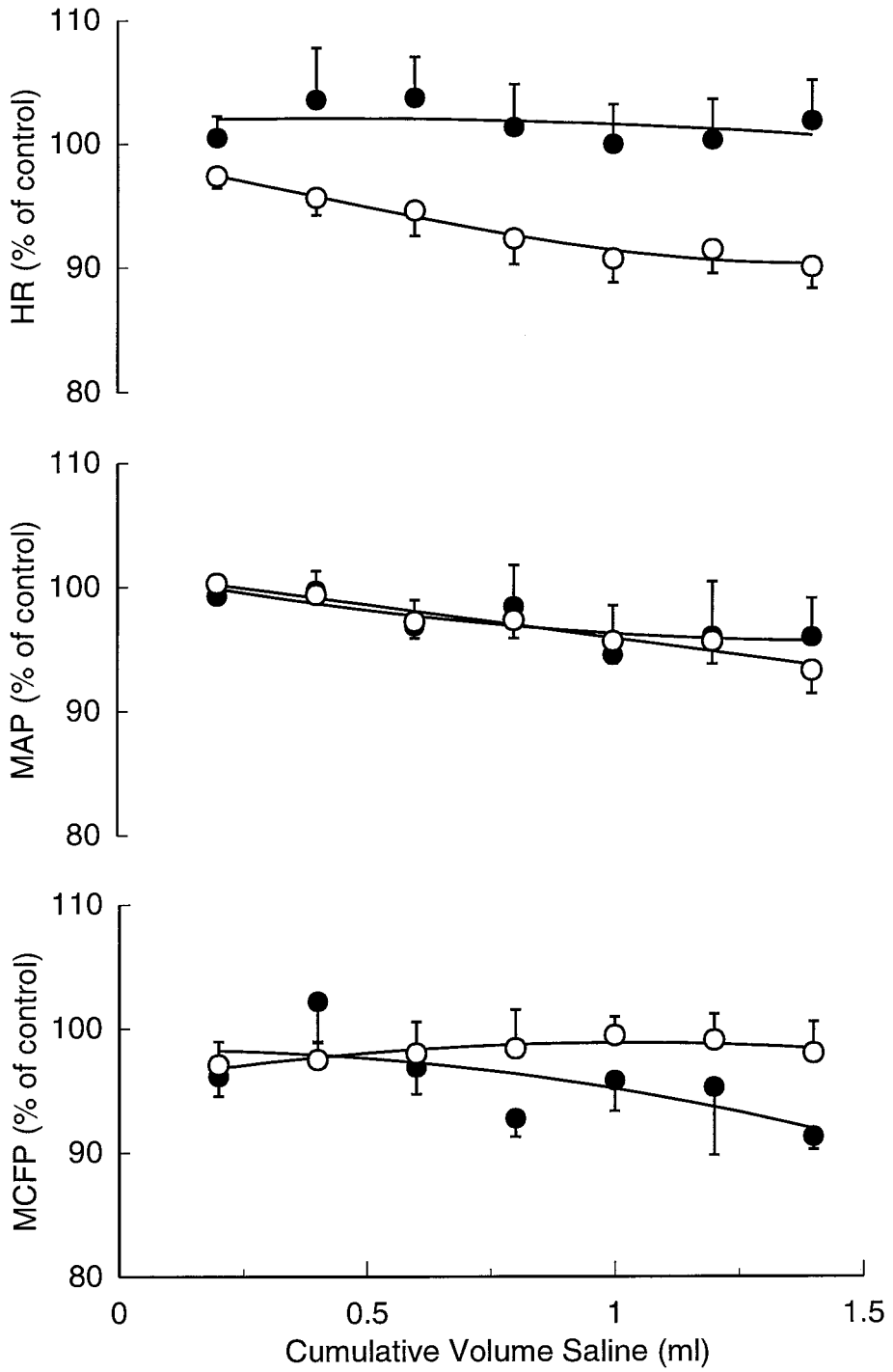


Figure 1.3. Dose-response curves of the effects of saline on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with saline (●) or hydralazine (0.3 μmol kg^{-1} min^{-1}) (O); each point represents mean ± s.e.mean, $n \geq 6$.

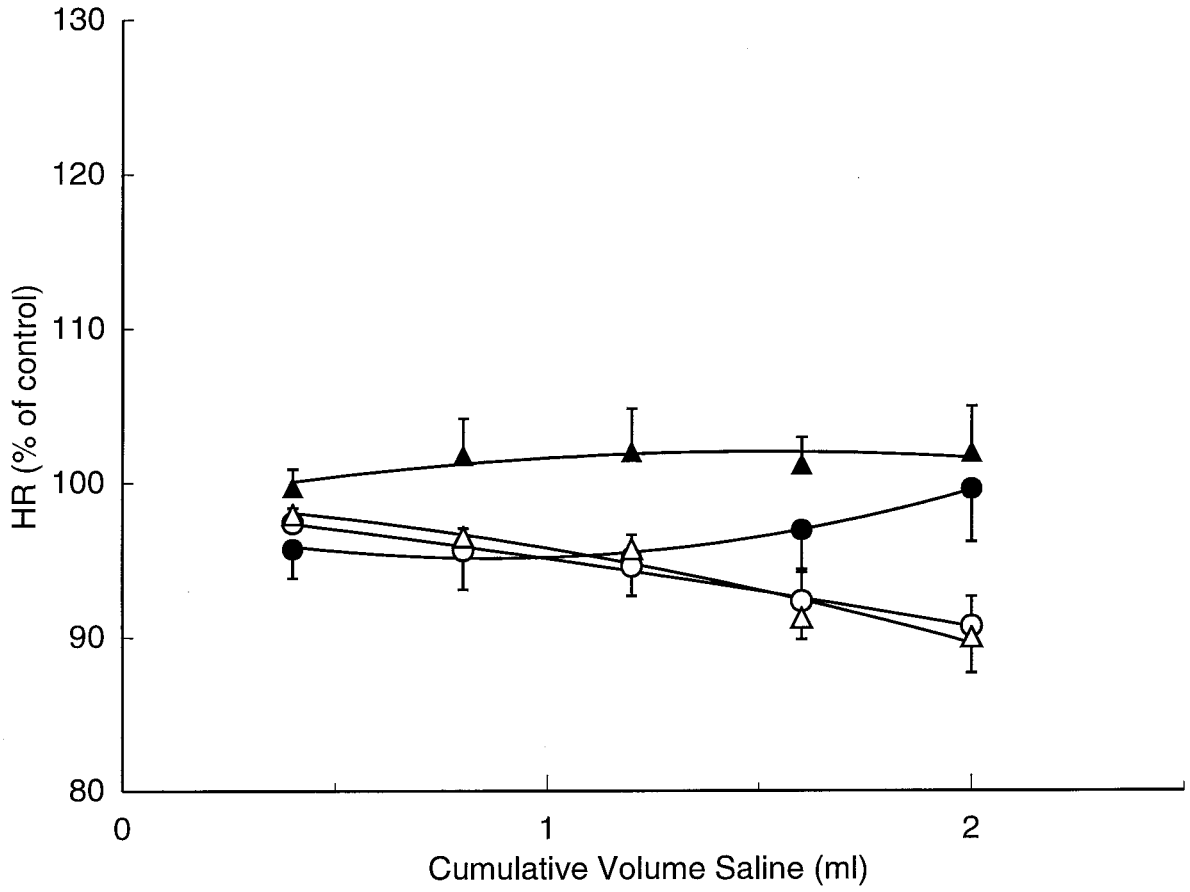


Figure 1.4.1. Dose-response curves of the effects of saline on heart rate (HR) in conscious, unrestrained rats treated with saline (●), hydralazine (0.3 μmol kg^{-1} min^{-1}) (O), mecamylamine (18 μmol kg^{-1}) (▲) or guanethidine (3 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}) (△); each point represents mean ± s.e.mean, $n \geq 5$.

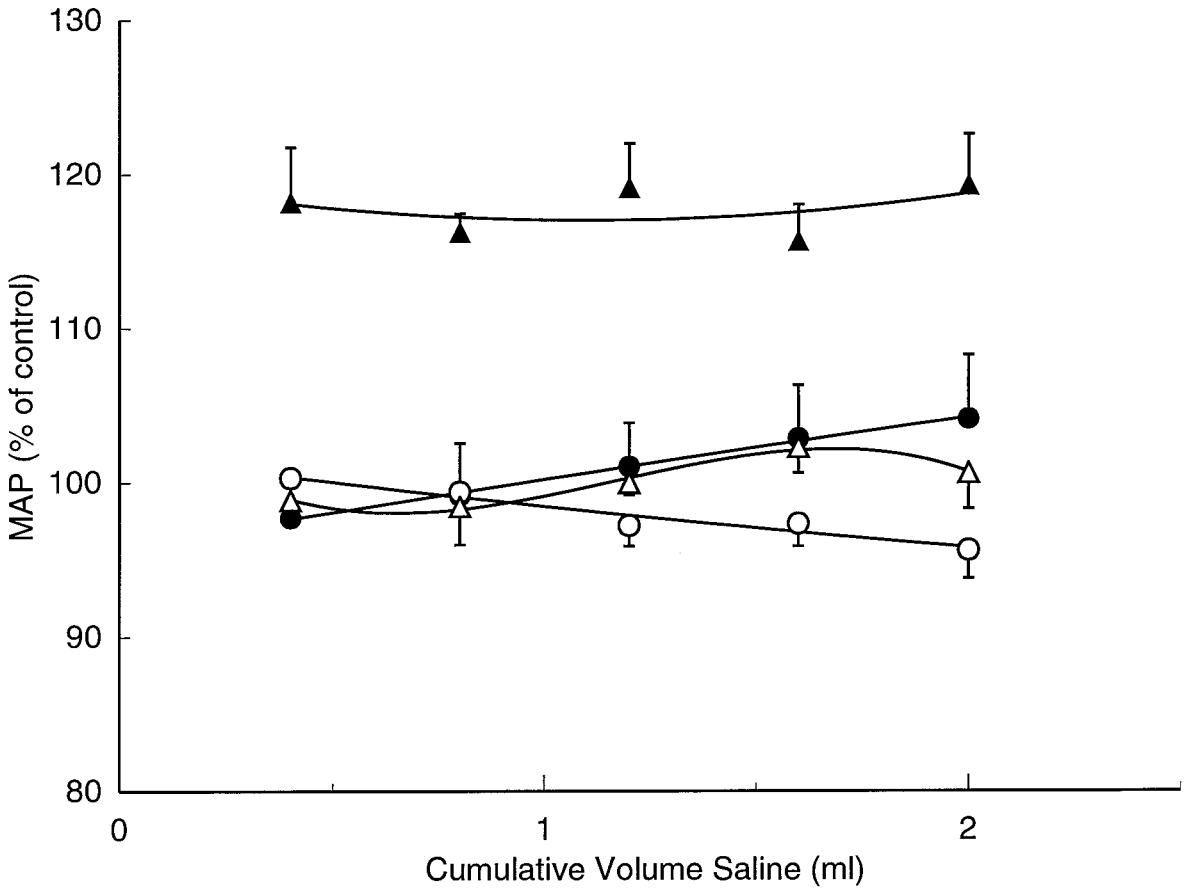


Figure 1.4.2. Dose-response curves of the effects of saline on mean arterial pressure (MAP) in conscious, unrestrained rats treated with saline (●), hydralazine (0.3 μmol kg^{-1} min^{-1}) (O), mecamylamine (18 μmol kg^{-1}) (▲) or guanethidine (3 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}) (Δ); each point represents mean ± s.e.mean, $n \geq 5$.

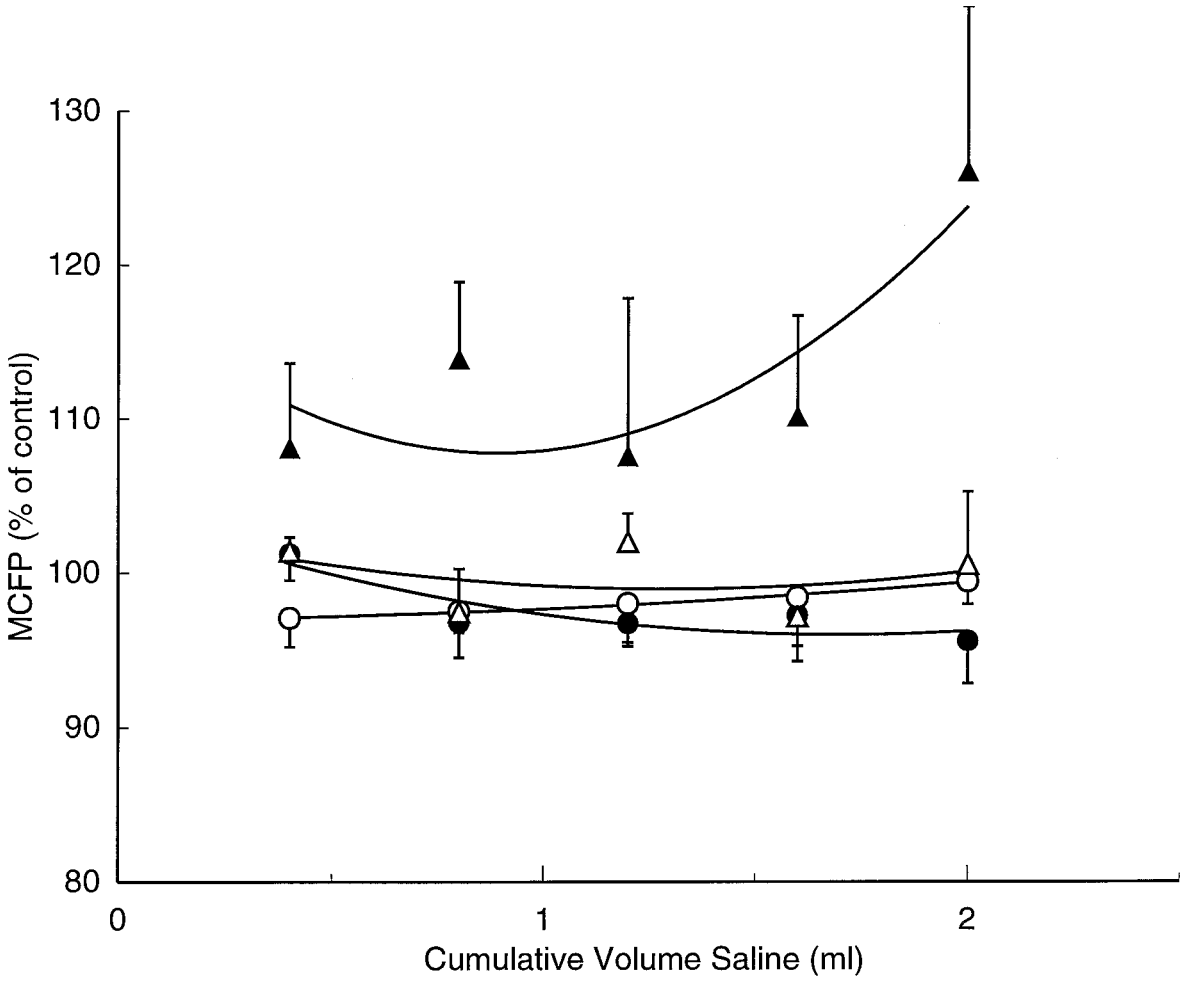


Figure 1.4.3. Dose-response curves of the effects of saline on mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with saline (●), hydralazine (0.3 μmol kg^{-1} min^{-1}) (○), mecamylamine (18 μmol kg^{-1}) (▲) or guanethidine (3 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}) (△); each point represents mean ± s.e.mean, $n \geq 5$.

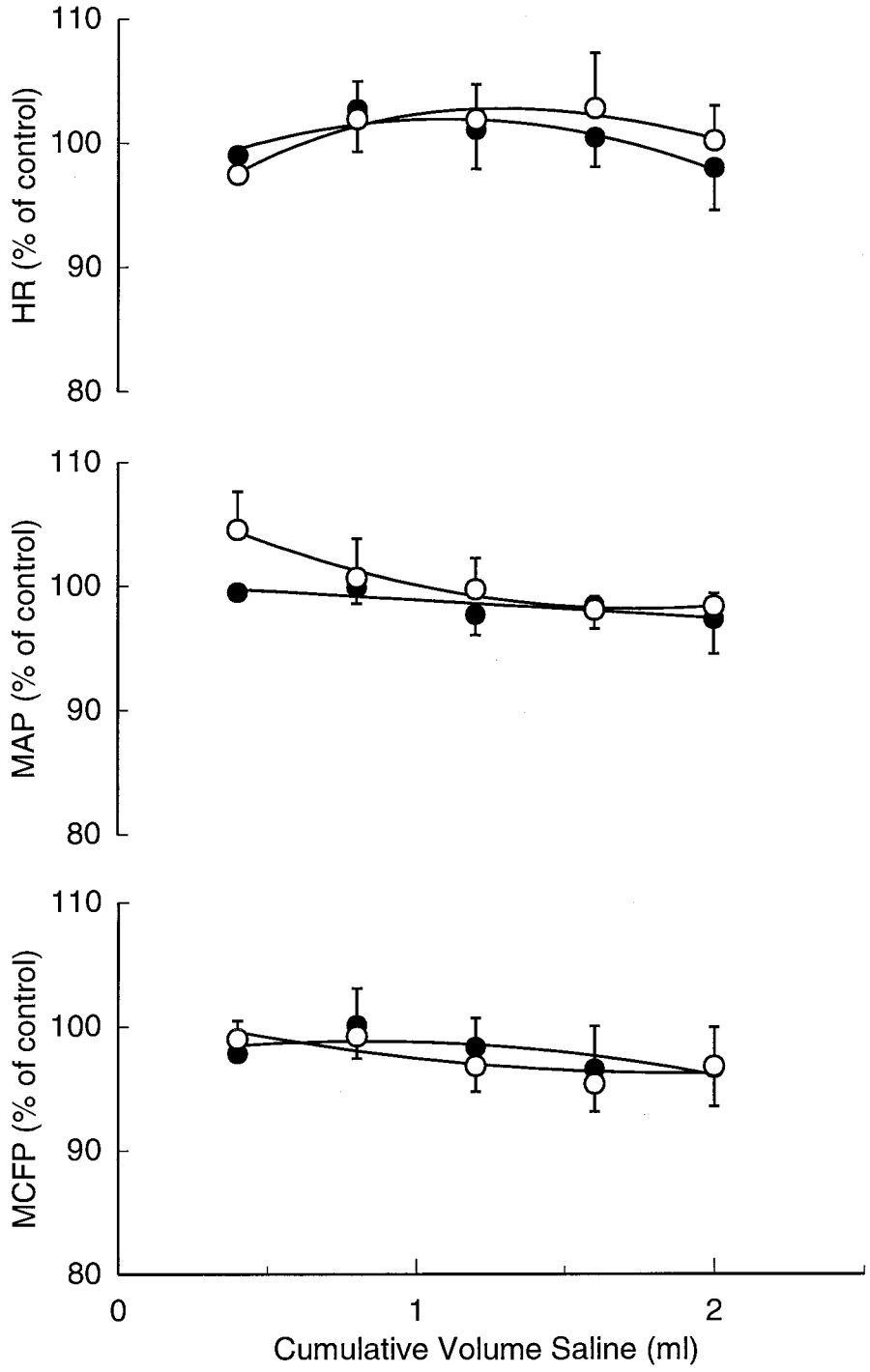


Figure 1.5. Dose-response curves of the effects of saline on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with DDW (6% citrate) (●) or reserpine (3 mg kg^{-1} 24 h prior to study) (O); each point represents mean ± s.e.mean, $n = 6$.

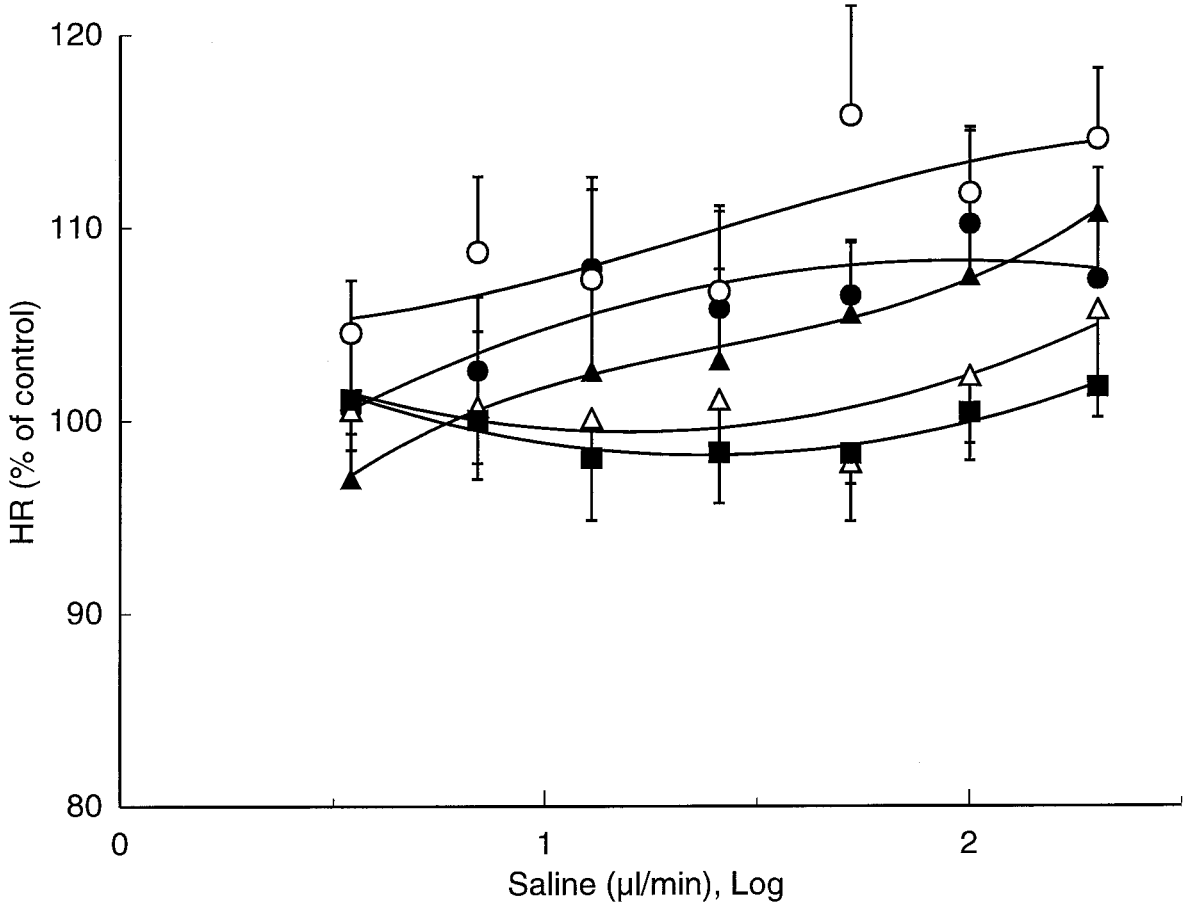


Figure 1.6.1. Dose-response curves of the effects of saline on heart rate (HR) in conscious, unrestrained rats treated with saline (●), suramin (200 µmol kg^{-1}) (O), mecamylamine (18 µmol kg^{-1}) (▲), cibacron blue (13 µmol kg^{-1}) (△) or 8-phenyltheophylline (27 µmol kg^{-1}) (■); each point represents mean ± s.e.mean, $n \geq 5$.

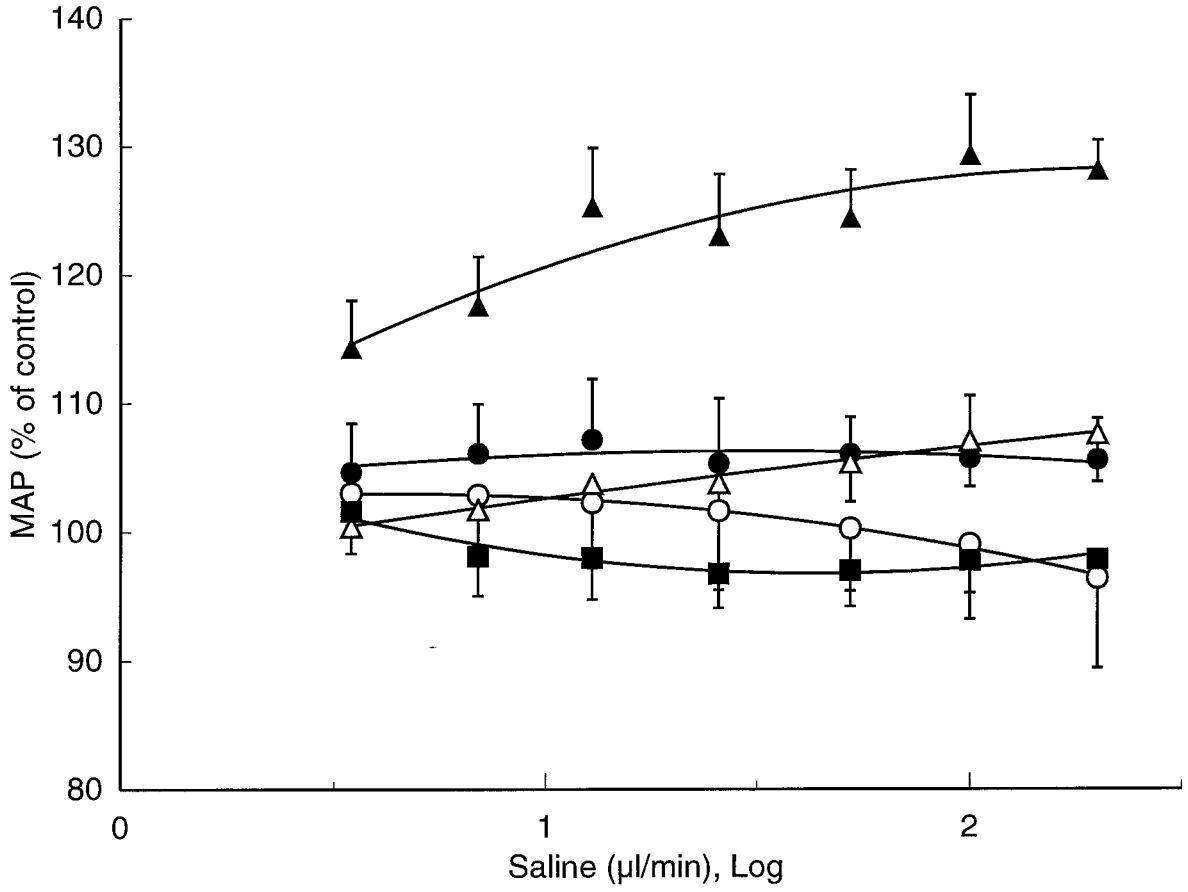


Figure 1.6.2. Dose-response curves of the effects of saline on mean arterial pressure (MAP) in conscious, unrestrained rats treated with saline (●), suramin (200 µmol kg^{-1}) (○), mecamylamine (18 µmol kg^{-1}) (▲), cibacron blue (13 µmol kg^{-1}) (△) or 8-phenyltheophylline (27 µmol kg^{-1}) (■); each point represents mean ± s.e.mean, $n \geq 5$.

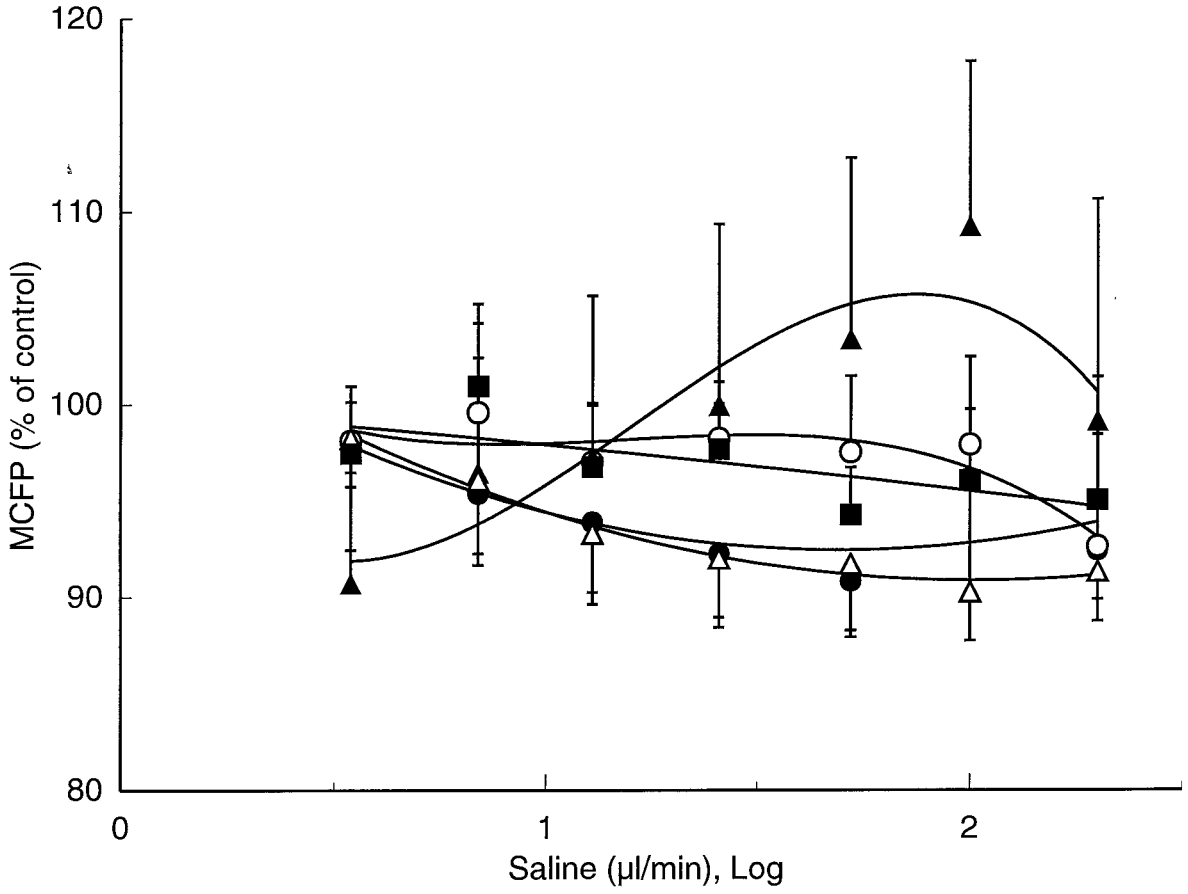


Figure 1.6.3. Dose-response curves of the effects of saline on mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with saline (●), suramin (200 µmol kg^{-1}) (O), mecamylamine (18 µmol kg^{-1}) (▲), cibacron blue (13 µmol kg^{-1}) (△) or 8-phenyltheophylline (27 µmol kg^{-1}) (■); each point represents mean ± s.e.mean, $n \geq 5$.

3.2. *Resistance of MCFP to α-Adrenoceptor Antagonism in Rats with Normal and Reflexly-Increased Venous Tone*

3.2.1. Effect of phentolamine in the absence and presence of hydralazine (Figure 2.1)

The control values for haemodynamic variables are given in Table 2. Saline did not significantly alter MAP, HR, or MCFP whereas hydralazine decreased MAP (23 ± 1%) and increased both HR (19 ± 2%) and MCFP (31 ± 2%) compared to post-saline control values ($n = 14$). Over time, continuous hydralazine infusion produced no further significant changes in MAP, HR, or MCFP.

Phentolamine, in the absence of hydralazine, clearly and significantly decreased MAP and increased HR in a dose-dependent manner, but had no effect on MCFP. Curve analysis of the MAP curves revealed a significant difference between phentolamine groups in the absence and presence of hydralazine in addition to a significant hydralazine-phentolamine interaction which, together with visual inspection of the curves, suggest that hydralazine potentiated the depressor effect of low doses of phentolamine. In the presence of hydralazine, phentolamine produced dose-dependent bradycardia which was not significantly different from the decrease in HR observed in the hydralazine-treated control group. However, a significant hydralazine-phentolamine interaction suggests that hydralazine converted the phentolamine-induced tachycardia into bradycardia. In the presence of hydralazine, phentolamine produced a very modest, but insignificant, decline in MCFP.

3.2.2. Effect of phentolamine in the absence and presence of nifedipine (Figure 2.2)

The control values for haemodynamic variables are given in Table 2. Vehicle (30% ethanol in double-distilled water) did not significantly alter MAP, HR, or MCFP. Nifedipine treatment significantly decreased MAP (27 ± 2%) and increased MCFP (19 ± 3%), but did not affect HR compared to the post-treatment control values of vehicle-treated groups ($n = 15$). During continuous nifedipine infusion, MAP increased slightly but significantly over

time. In contrast, HR and MCFP were not significantly affected, although HR showed a slight, gradual decrease and MCFP a slight, gradual increase.

In the vehicle-treated group, phentolamine dose-dependently and significantly decreased MAP and increased HR, but had no effect on MCFP. The phentolamine-induced depressor effect was not significantly different in the presence of nifedipine compared to that observed in the absence of nifedipine. Phentolamine produced a very modest decrease in HR in the presence of nifedipine; however, this effect was insignificant when compared to the effect of saline in the nifedipine-treated control group. A significant interaction between nifedipine and phentolamine suggests that nifedipine converted the phentolamine-induced tachycardia into bradycardia. A moderate but significant phentolamine-induced decrease in MCFP was revealed in the presence of nifedipine.

3.2.3. Effect of mecamylamine in the absence and presence of hydralazine (Figure 2.3)

The dose-response effect of mecamylamine-mediated blockade of the reflex bradycardia accompanying a methoxamine-induced increase in MAP was examined. In the absence of mecamylamine, methoxamine increased MAP by 61 ± 5 mmHg and decreased HR by 182 ± 11 beats min^{-1} ($n = 6$). At the dose range tested (0.18 - 180 μmol kg^{-1}), mecamylamine potentiated the pressor effect of methoxamine by 0 to 65 ± 16% ($n = 6$) and inhibited methoxamine-induced bradycardia by 8 to 100 ± 1% (Figure 2.4).

The control values for haemodynamic variables are given in Table 2. Saline had no significant effect on MAP, HR, or MCFP. Hydralazine treatment significantly decreased MAP (27 ± 3%) and increased both HR (22 ± 2%) and MCFP (19 ± 3%) compared to the respective post-treatment control values in saline-treated groups ($n = 14$). During continuous hydralazine infusion, HR declined slightly but significantly over time, while MAP and MCFP were stable.

In the absence of hydralazine, mecamylamine markedly decreased HR, MAP, and MCFP in a dose-dependent and significant manner. The mecamylamine-induced decrease in MCFP was not significantly altered by hydralazine. In comparison, hydralazine

treatment considerably attenuated the mecamylamine-induced depressor effect such that the response was no longer significantly different from that in the hydralazine-treated control rats given saline infusion. Curve analysis indicated a significant hydralazine-mecamylamine interaction, suggesting that hydralazine potentiated the bradycardic effect of mecamylamine.

Table 2. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and following treatment (Post-treatment Control) with either vehicle, hydralazine (0.3 μmol kg^{-1} min^{-1}) or nifedipine (0.3 μmol kg^{-1} min^{-1}) in conscious, unrestrained Sprague-Dawley rats.

Figure Ref.	Treatment Group		Pretreatment Controls HR (beats/min)	Pretreatment Controls MAP (mmHg)	Pretreatment Controls MCFP (mmHg)	Post-treatment Controls HR (beats/min)	Post-treatment Controls MAP (mmHg)	Post-treatment Controls MCFP (mmHg)	n
2.1	Phentolamine	Saline[1.1]	404 ± 20	101 ± 1	5.4 ± 0.1	396 ± 18	100 ± 2	5.2 ± 0.1	7
		Hydralazine[1.1]	414 ± 12	106 ± 2	5.5 ± 0.2	477 ± 8	78 ± 2	6.7 ± 0.2	7
		Saline	401 ± 6	102 ± 2	5.3 ± 0.1	395 ± 8	101 ± 2	5.2 ± 0.1	6
		Hydralazine	425 ± 18	102 ± 2	5.6 ± 0.2	467 ± 16	78 ± 1	7.0 ± 0.1	7
	Saline Pooled		403 ± 11	101 ± 1	5.4 ± 0.1	396 ± 10	101 ± 1	5.2 ± 0.1	13
	Hydralazine Pooled		419 ± 10	104 ± 1	5.6 ± 0.1	472 ± 9 [a]	78 ± 1 [a]	6.8 ± 0.1 [a]	14
2.2	Phentolamine	DDW (30% EtOH)[1.2]	410 ± 10	109 ± 2	5.8 ± 0.2	420 ± 12	102 ± 3	5.2 ± 0.2	6
		Nifedipine[1.2]	380 ± 8	104 ± 2	5.3 ± 0.2	420 ± 8	70 ± 2	6.5 ± 0.3	9
		DDW (30% EtOH)	401 ± 6	102 ± 2	5.3 ± 0.1	395 ± 7	101 ± 2	5.2 ± 0.1	6
		Nifedipine	413 ± 17	107 ± 3	5.2 ± 0.2	434 ± 12	79 ± 1	6.0 ± 0.2	6
	DDW (30% EtOH) Pooled		406 ± 5	103 ± 2	5.6 ± 0.1	407 ± 8	101 ± 2	5.2 ± 0.1	12
	Nifedipine Pooled		394 ± 9	105 ± 1	5.3 ± 0.1	424 ± 7	74 ± 1 [a]	6.2 ± 0.2 [a]	15
2.3	Mecamylamine	Saline[1.3]	383 ± 12	107 ± 3	5.2 ± 0.1	371 ± 9	107 ± 4	5.1 ± 0.1	6
		Hydralazine[1.3]	414 ± 12	106 ± 2	5.5 ± 0.2	477 ± 8	78 ± 2	6.7 ± 0.2	7
		Saline	398 ± 19	104 ± 5	5.7 ± 0.3	394 ± 23	103 ± 6	5.6 ± 0.4	6
		Hydralazine	376 ± 12	104 ± 1	5.3 ± 0.2	451 ± 11	76 ± 3	6.1 ± 0.2	7
	Saline Pooled		391 ± 11	105 ± 3	5.4 ± 0.2	382 ± 12	105 ± 3	5.4 ± 0.2	12
	Hydralazine Pooled		387 ± 11	105 ± 1	5.4 ± 0.1	464 ± 8 [a]	77 ± 2 [a]	6.4 ± 0.2 [a]	14

Values are mean ± s.e.mean. DDW = double distilled water, EtOH = ethanol. Figures referenced show the dose-response curves as a percentage of the Post-treatment Control for each group. Treatments followed by superscript numbers are the respective vehicle-treated time controls and are also referenced to the figures showing the time-control curves.

[a] significant difference from saline control, $p < 0.05$

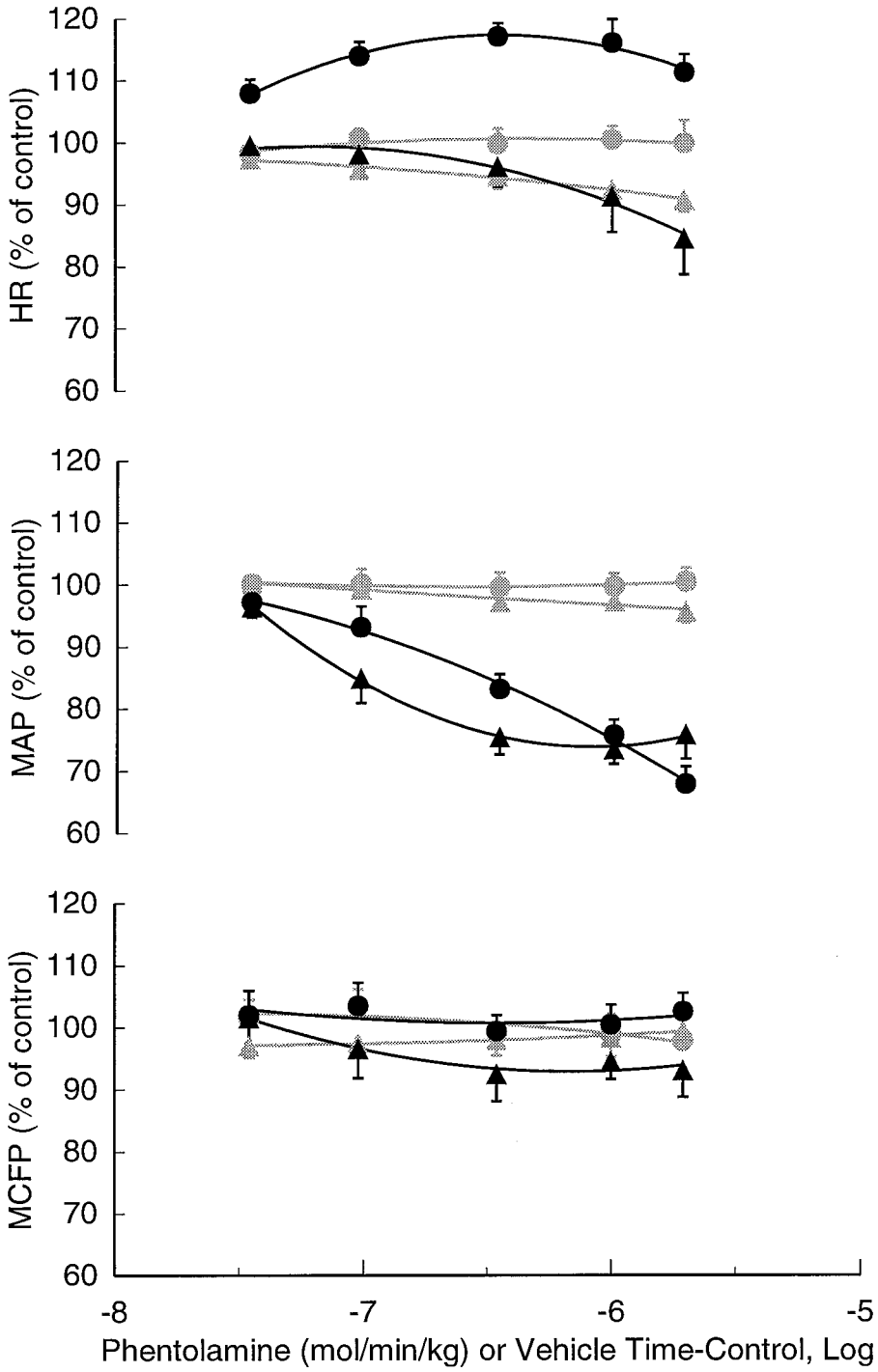


Figure 2.1. Dose-response curves of the effects of phentolamine (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either saline (circles) or hydralazine (0.3 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

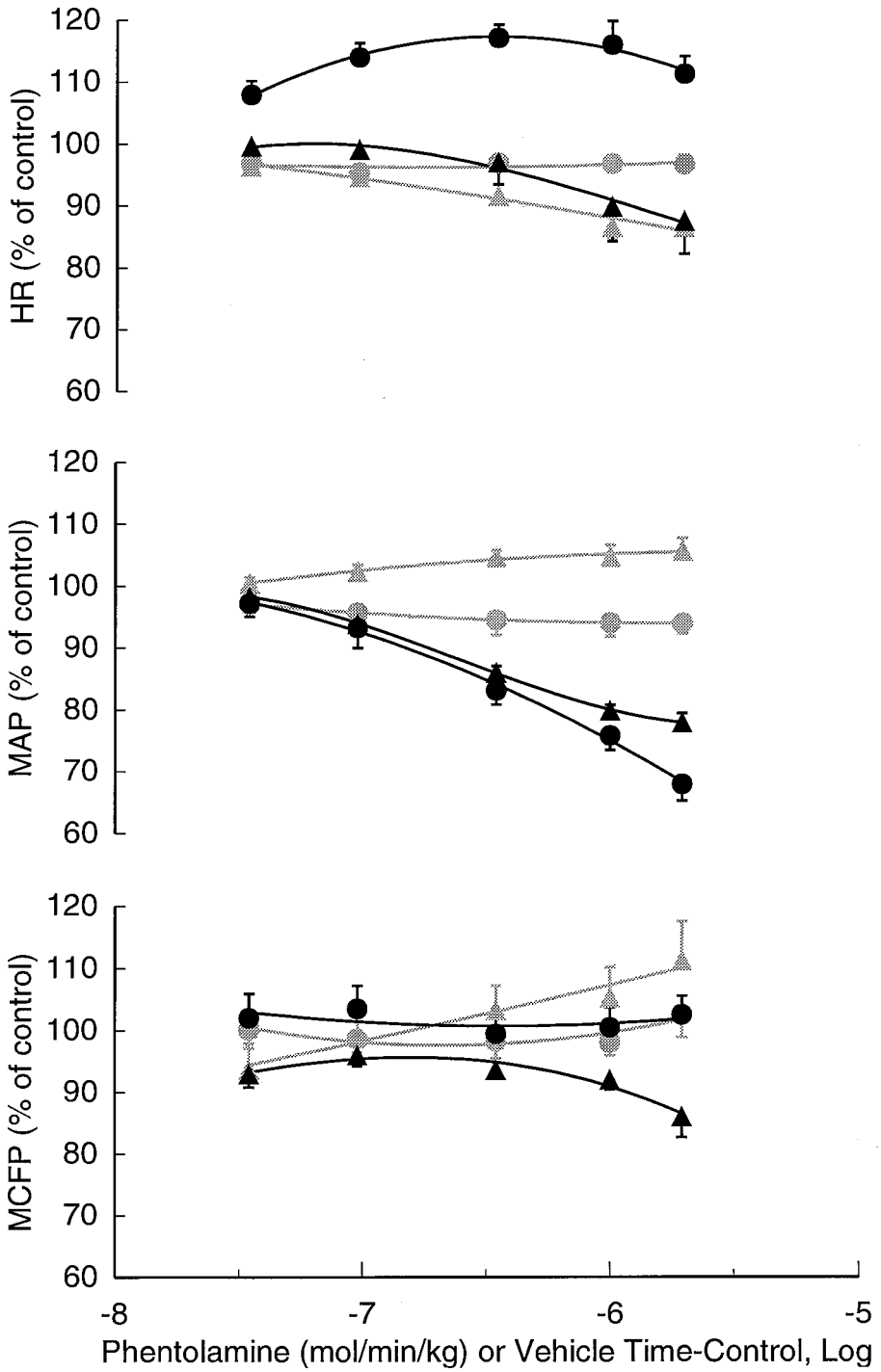


Figure 2.2. Dose-response curves of the effects of phentolamine (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either vehicle (30% ethanol in double-distilled water) (circles) or nifedipine (0.3 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

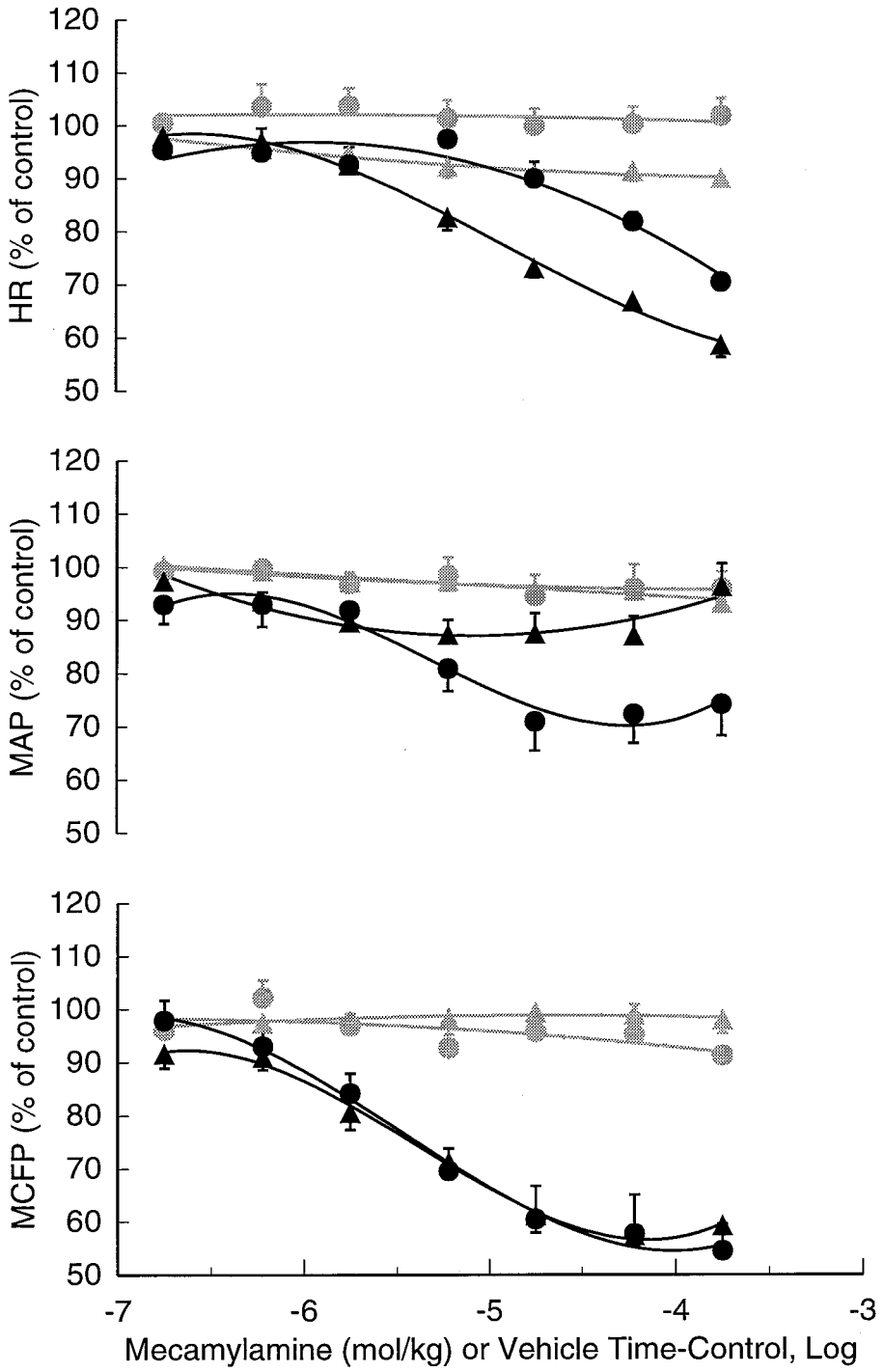


Figure 2.3. Dose-response curves of the effects of mecamylamine (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either saline (circles) or hydralazine (0.3 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

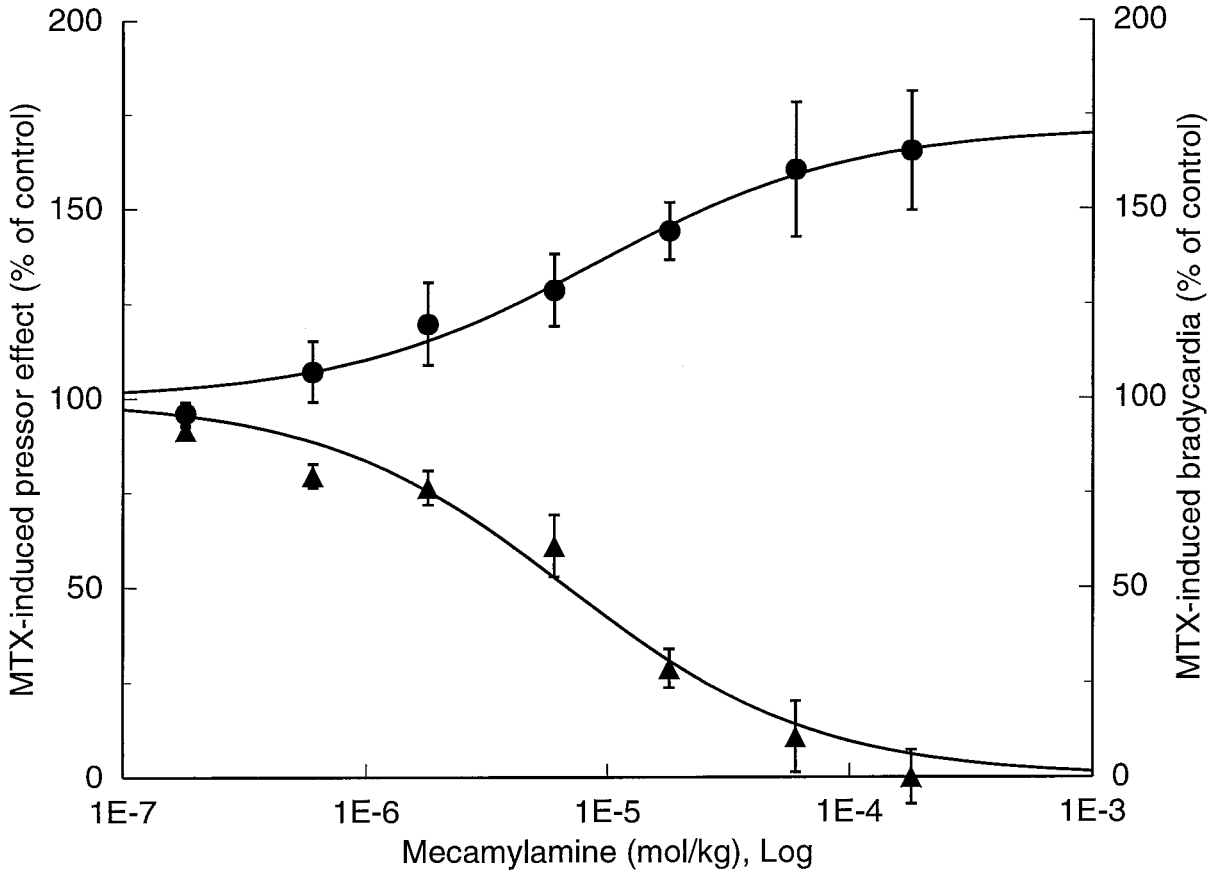


Figure 2.4. Dose-response curves of the effects of mecamylamine on changes in heart rate (HR) (▲) and mean arterial pressure (MAP) (●) produced by a test dose of methoxamine (80 nmol kg^{-1}) in conscious, unrestrained rats; each point represents mean ± s.e.mean, $n = 6$.

3.3. *Possible Role of Purinergic Neurotransmission in Basal and Reflexly-Increased Venous Tone*

3.3.1. Effect of suramin in the absence and presence of hydralazine (Figure 3.1)

The control values for haemodynamic variables are given in Table 3. Saline did not alter MAP, HR, or MCFP whereas hydralazine treatment decreased MAP (28 ± 2%) and increased both HR (18 ± 2%) and MCFP (25 ± 2%) compared to post-treatment control values of saline-treated groups (n = 16). Neither MAP, HR, nor MCFP changed significantly over time during continuous hydralazine infusion.

In both saline- and hydralazine-treated groups, suramin significantly and dose-dependently decreased HR, but had no significant effect on MCFP. Suramin produced dose-dependent pressor effects in the absence and presence of hydralazine which, according to curve analysis, were significantly different from the corresponding saline control curves. The MAP curves for suramin in the absence and presence of hydralazine were also significantly different from each other which suggests that hydralazine attenuated the suramin-induced pressor effect at low doses of suramin.

3.3.2. Effect of suramin in the absence and presence of nifedipine (Figure 3.2)

The control values for haemodynamic variables are given in Table 3. Vehicle treatment (30% ethanol in double-distilled water) did not significantly alter MAP, HR, or MCFP whereas nifedipine treatment produced a significant decrease in MAP (33 ± 2%) and increased both HR (6 ± 2%), and MCFP (25 ± 3%) (n = 16). During continuous nifedipine infusion, there were only a slight, gradual but insignificant increase in both MAP and MCFP, and a slight, gradual but insignificant decrease in HR over time.

In the vehicle-treated group, suramin produced a significant dose-dependent increase in MAP which was accompanied by a dose-dependent, but insignificant, decrease in HR and no change in MCFP. Nifedipine did not significantly alter the suramin-induced pressor effect. Similarly, the suramin-induced bradycardia also persisted in the presence of nifedipine but was significantly greater than that occurring in the absence of nifedipine –

this did not appear to be the result of potentiation, since no significant nifedipine-suramin interaction occurred, but rather the result of the time-dependent slight bradycardic effect of nifedipine. According to curve analysis, nifedipine treatment revealed a slight but significant increase in MCFP when compared to either the nifedipine-treated control or the vehicle-treated suramin group.

3.3.3. Effect of suramin in the absence and presence of mecamylamine (Figure 3.3)

The control values for haemodynamic variables are given in Table 3. Saline did not significantly affect MAP, HR, or MCFP whereas mecamylamine treatment significantly decreased both MAP (31 ± 3%) and MCFP (30 ± 2%), and insignificantly decreased HR (6 ± 3%), compared to post-treatment control values from saline-treated groups ($n = 12$). The dose of mecamylamine used in these studies inhibited acetylcholine-induced tachycardia by 79 ± 9% ($n = 12$). In the presence of mecamylamine, HR did not change significantly over time; however, MAP and MCFP each exhibited a marked and significant increase, immediately following the balloon inflation for the post-treatment control response, which was maintained for the duration of the experiment.

In the absence of mecamylamine, suramin significantly and dose-dependently decreased HR and increased MAP, but had no effect on MCFP. Mecamylamine treatment did not significantly alter the suramin-induced bradycardia observed in the absence of mecamylamine, whereas it revealed a significant dose-dependent decrease in MCFP. The suramin-induced pressor effect persisted in the presence of mecamylamine but was insignificant compared to the respective mecamylamine-treated control group, although it was significantly greater than the vehicle control in the absence of mecamylamine.

Table 3. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and following treatment (Post-treatment Control) with either vehicle, hydralazine (0.3 μmol kg^{-1} min^{-1}), nifedipine (0.3 μmol kg^{-1} min^{-1}) or mecamylamine (18 μmol kg^{-1}) in conscious, unrestrained Sprague-Dawley rats.

Figure Ref.	*Treatment Group*		*Pretreatment Controls* HR (beats/min)	MAP (mmHg)	MCFP (mmHg)	*Post-treatment Controls* HR (beats/min)	MAP (mmHg)	MCFP (mmHg)	*n*
3.1	Suramin	Saline[1.4]	408 ± 11	101 ± 2	5.3 ± 0.2	418 ± 15	102 ± 2	5.1 ± 0.3	6
		Hydralazine[1.4]	421 ± 14	104 ± 2	5.4 ± 0.2	475 ± 17	77 ± 3	6.5 ± 0.2	6
		Saline	396 ± 11	111 ± 3	5.3 ± 0.1	390 ± 10	111 ± 2	5.3 ± 0.1	13
		Hydralazine	416 ± 7	102 ± 1	5.1 ± 0.1	462 ± 9	77 ± 2	6.5 ± 0.2	9
		Saline Pooled	400 ± 8	108 ± 2	5.2 ± 0.1	399 ± 9	108 ± 2	5.3 ± 0.1	19
		Hydralazine Pooled	415 ± 6	104 ± 1	5.3 ± 0.1	469 ± 6 [a]	78 ± 1 [a]	6.6 ± 0.1 [a]	16
3.2	Suramin	DDW (30% EtOH)[1.2]	410 ± 10	109 ± 2	5.8 ± 0.2	420 ± 12	102 ± 3	5.2 ± 0.2	6
		Nifedipine[1.2]	380 ± 8	104 ± 2	5.3 ± 0.2	420 ± 8	70 ± 2	6.5 ± 0.3	6
		DDW (30% EtOH)	409 ± 18	111 ± 4	5.8 ± 0.2	410 ± 14	105 ± 5	5.4 ± 0.2	7
		Nifedipine	378 ± 8	104 ± 1	5.4 ± 0.2	460 ± 12	70 ± 2	6.9 ± 0.3	7
		DDW (30% EtOH) Pooled	410 ± 10	110 ± 2	5.8 ± 0.1	414 ± 9	104 ± 3	5.3 ± 0.1	13
		Nifedipine Pooled	381 ± 5	104 ± 1	5.4 ± 0.2	440 ± 8 [a]	70 ± 1 [a]	6.6 ± 0.2 [a]	13
3.3	Suramin	Saline[1.4]	408 ± 11	101 ± 2	5.3 ± 0.2	418 ± 15	102 ± 2	5.1 ± 0.3	6
		Mecamylamine[1.4]	418 ± 18	103 ± 3	5.2 ± 0.2	393 ± 15	76 ± 4	3.6 ± 0.1	6
		Saline	396 ± 11	111 ± 3	5.3 ± 0.1	390 ± 10	111 ± 2	5.3 ± 0.1	13
		Mecamylamine	393 ± 23	102 ± 5	5.5 ± 0.2	357 ± 13	75 ± 6	3.9 ± 0.2	6
		Saline Pooled	396 ± 11	111 ± 3	5.2 ± 0.1	399 ± 9	108 ± 2	5.3 ± 0.1	19
		Mecamylamine Pooled	405 ± 15	103 ± 3	5.4 ± 0.1	375 ± 11	75 ± 4 [a]	3.7 ± 0.1 [a]	12

Values are mean ± s.e.mean. DDW = double distilled water, EtOH = ethanol. Figures referenced show the dose-response curves as a percentage of the Post-treatment Control for each group. Treatments followed by superscript numbers are the respective vehicle-treated time controls and are also referenced to the figures showing the time-control curves.
[a] significant difference from saline control, $p < 0.05$

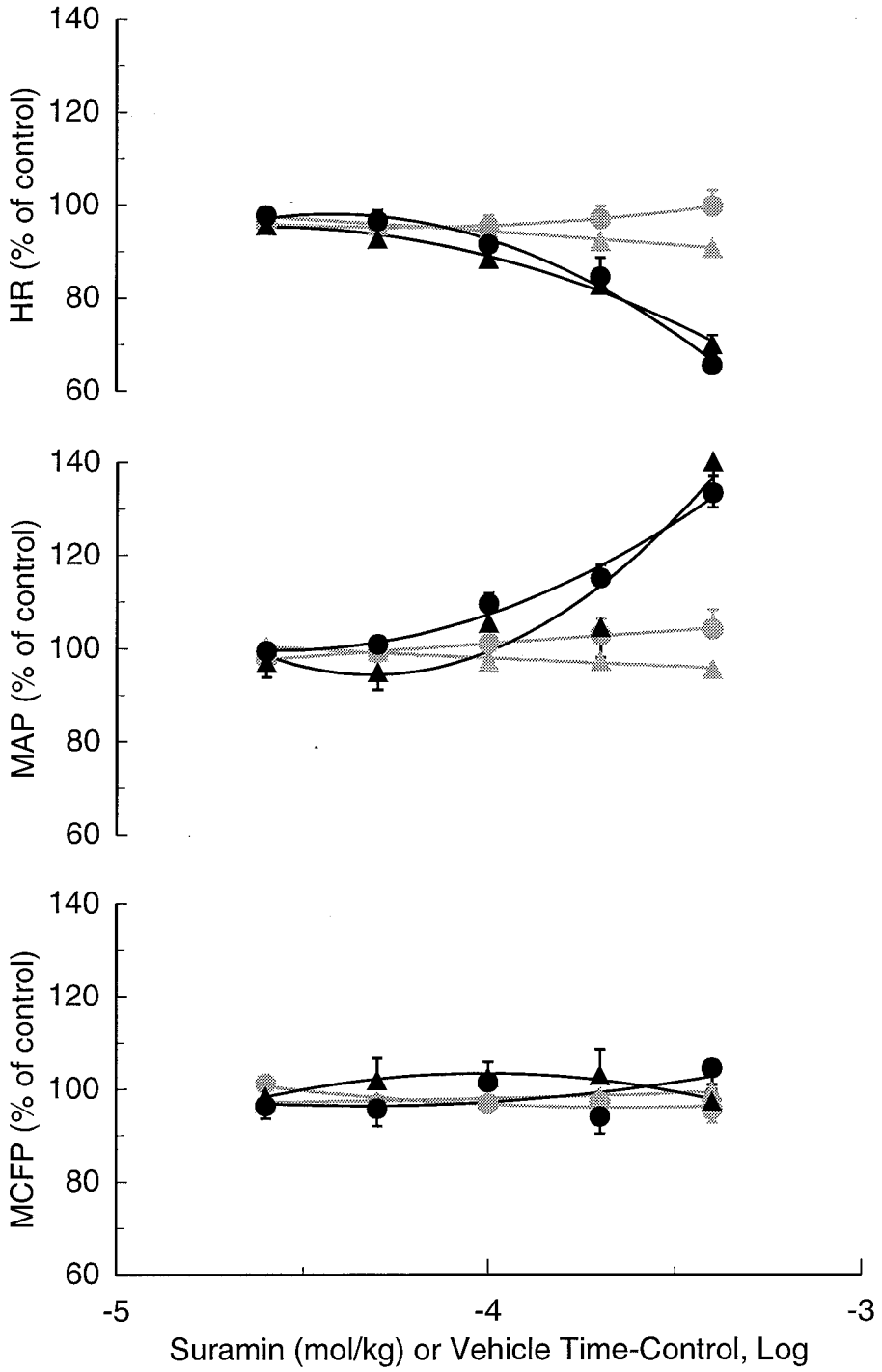


Figure 3.1. Dose-response curves of the effects of suramin (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either saline (circles) or hydralazine (0.3 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

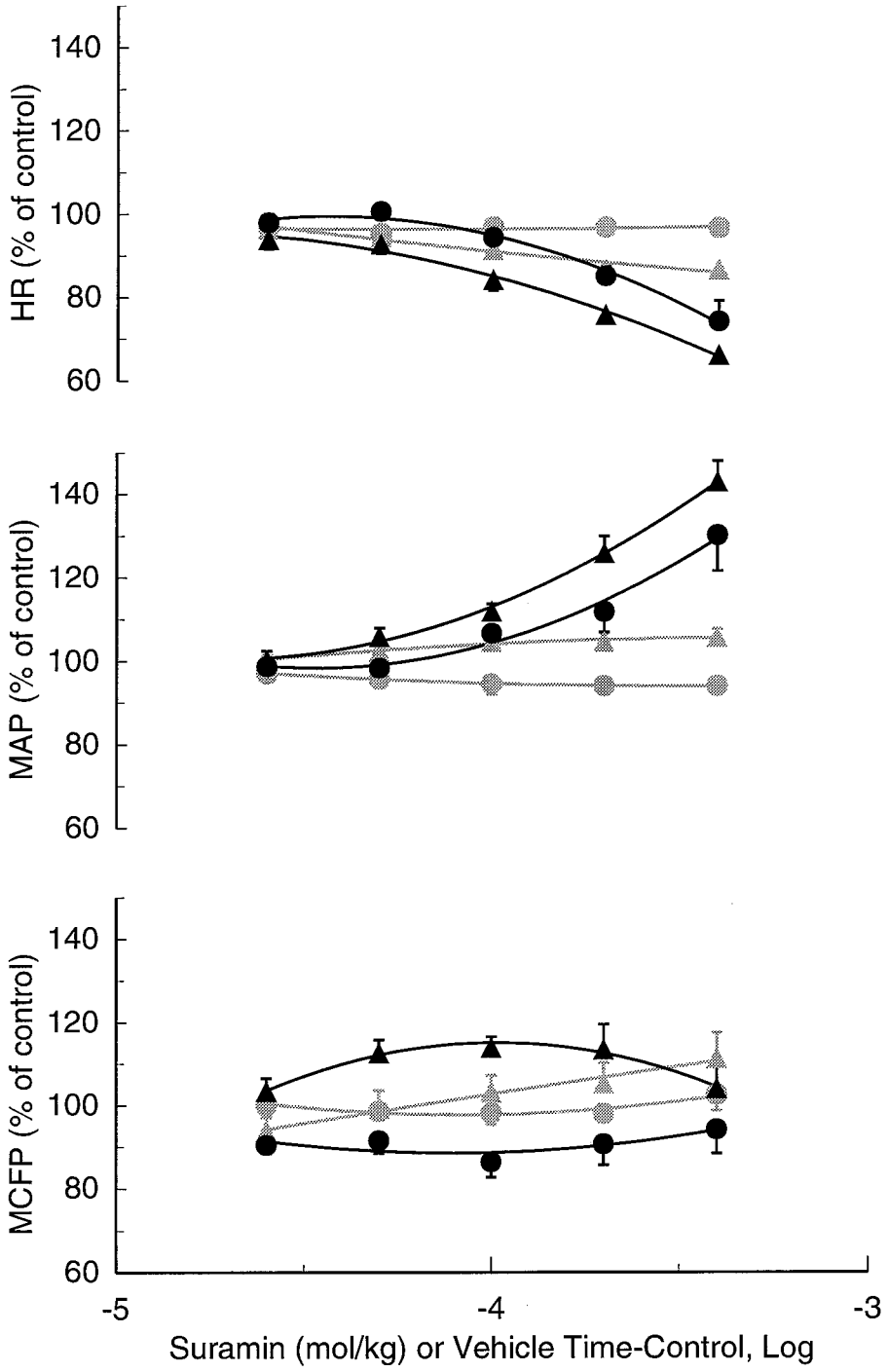


Figure 3.2. Dose-response curves of the effects of suramin (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either vehicle (30% ethanol in double-distilled water) (circles) or nifedipine (0.3 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

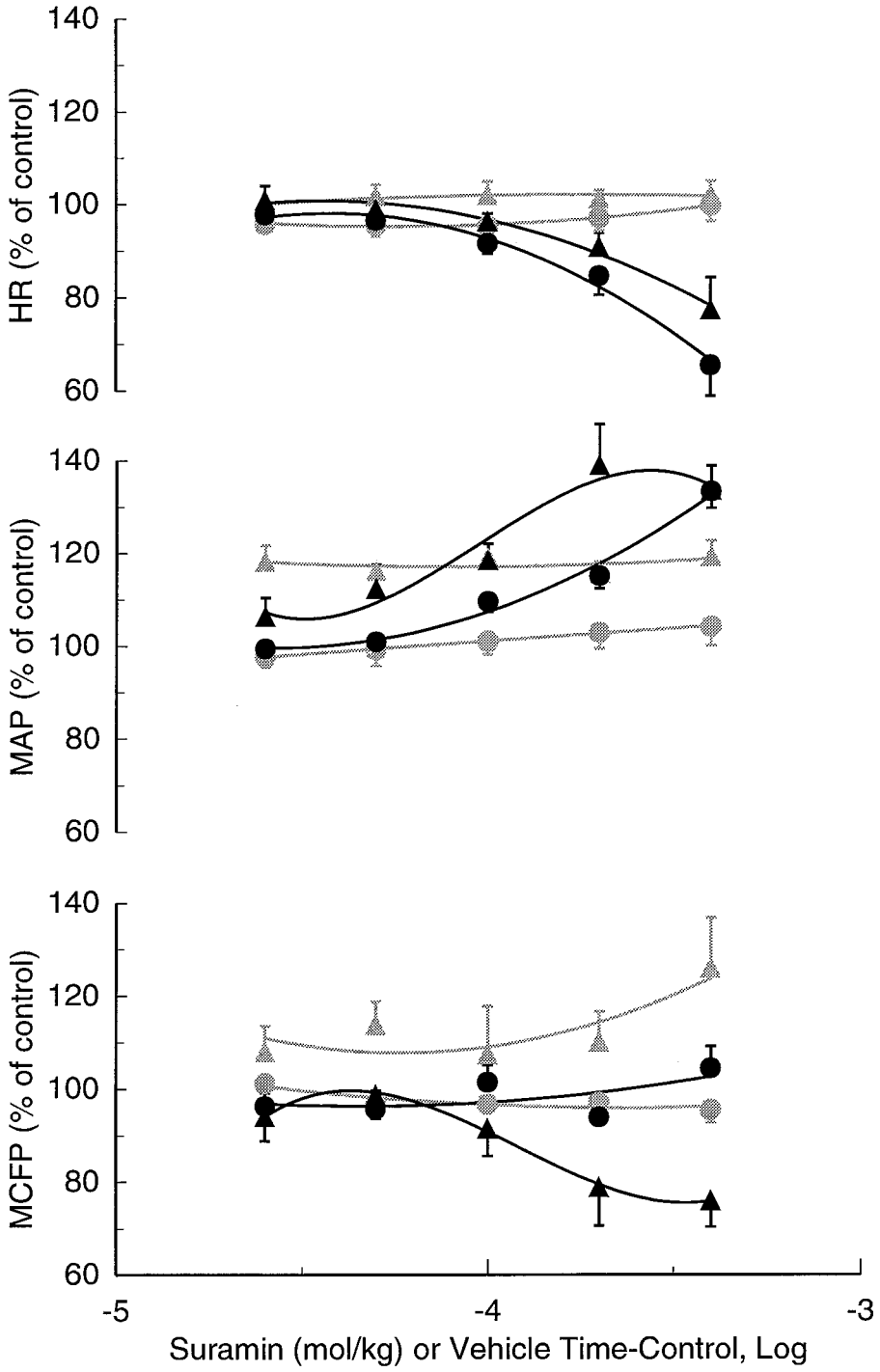


Figure 3.3. Dose-response curves of the effects of suramin (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or mecamylamine (18 μmol kg^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

3.4. *Does Suramin Reveal an α-Adrenoceptor Antagonist-Sensitive Component of Venous Tone?*

3.4.1. Effect of phentolamine in the absence and presence of suramin (Figure 4.1)

The control values for haemodynamic variables are given in Table 4. While saline did not alter MAP, HR, or MCFP, suramin treatment significantly decreased HR (8 ± 2%) and increased both MAP (15 ± 2%) and MCFP (12 ± 2%) (n = 12). Over time, there was no change in MAP, HR, or MCFP, in either the saline- or suramin-treated group although MCFP slightly decreased in the suramin-treated group. A preliminary experiment demonstrated that suramin treatment virtually abolished the pressor response to i.v. injection of α,β-methylene-ATP (P_{2X}-purinoceptor agonist) for at least 3 hours (data not shown).

In the absence of suramin, phentolamine significantly and dose-dependently decreased MAP and increased HR, but had no effect on MCFP. Suramin treatment did not significantly affect either the phentolamine-induced tachycardia or MCFP. The phentolamine-induced depressor effect, however, was significantly greater in the presence than in the absence of suramin.

Table 4. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and following treatment (Post-treatment Control) with either saline or suramin (200 μmol kg^{-1}) in conscious, unrestrained Sprague-Dawley rats.

Figure Ref.	Treatment Group		Pretreatment Controls HR (beats/min)	Pretreatment Controls MAP (mmHg)	Pretreatment Controls MCFP (mmHg)	Post-treatment Controls HR (beats/min)	Post-treatment Controls MAP (mmHg)	Post-treatment Controls MCFP (mmHg)	n
4.1	Phentolamine	Saline[1.1]	404 ± 20	101 ± 1	5.4 ± 0.1	396 ± 18	100 ± 2	5.2 ± 0.1	7
		Suramin[1.1]	412 ± 10	105 ± 3	5.6 ± 0.1	355 ± 11	114 ± 2	5.9 ± 0.2	6
		Saline	401 ± 6	102 ± 2	5.3 ± 0.1	395 ± 18	101 ± 2	5.2 ± 0.1	6
		Suramin	418 ± 18	104 ± 1	5.3 ± 0.3	376 ± 4	117 ± 1	5.7 ± 0.2	6
	Saline Pooled		403 ± 11	101 ± 1	5.4 ± 0.1	396 ± 10	101 ± 1	5.2 ± 0.1	13
	Suramin Pooled		415 ± 10	104 ± 1	5.5 ± 0.1	365 ± 6 [a]	116 ± 1 [a]	5.8 ± 0.1 [a]	12

Values are mean ± s.e.mean. Figures referenced show the dose-response curves as a percentage of the Post-treatment Control for each group. Treatments followed by superscript numbers are the respective vehicle-treated time controls and are also referenced to the figures showing the time-control curves.
[a] significant difference from saline control, $p < 0.05$

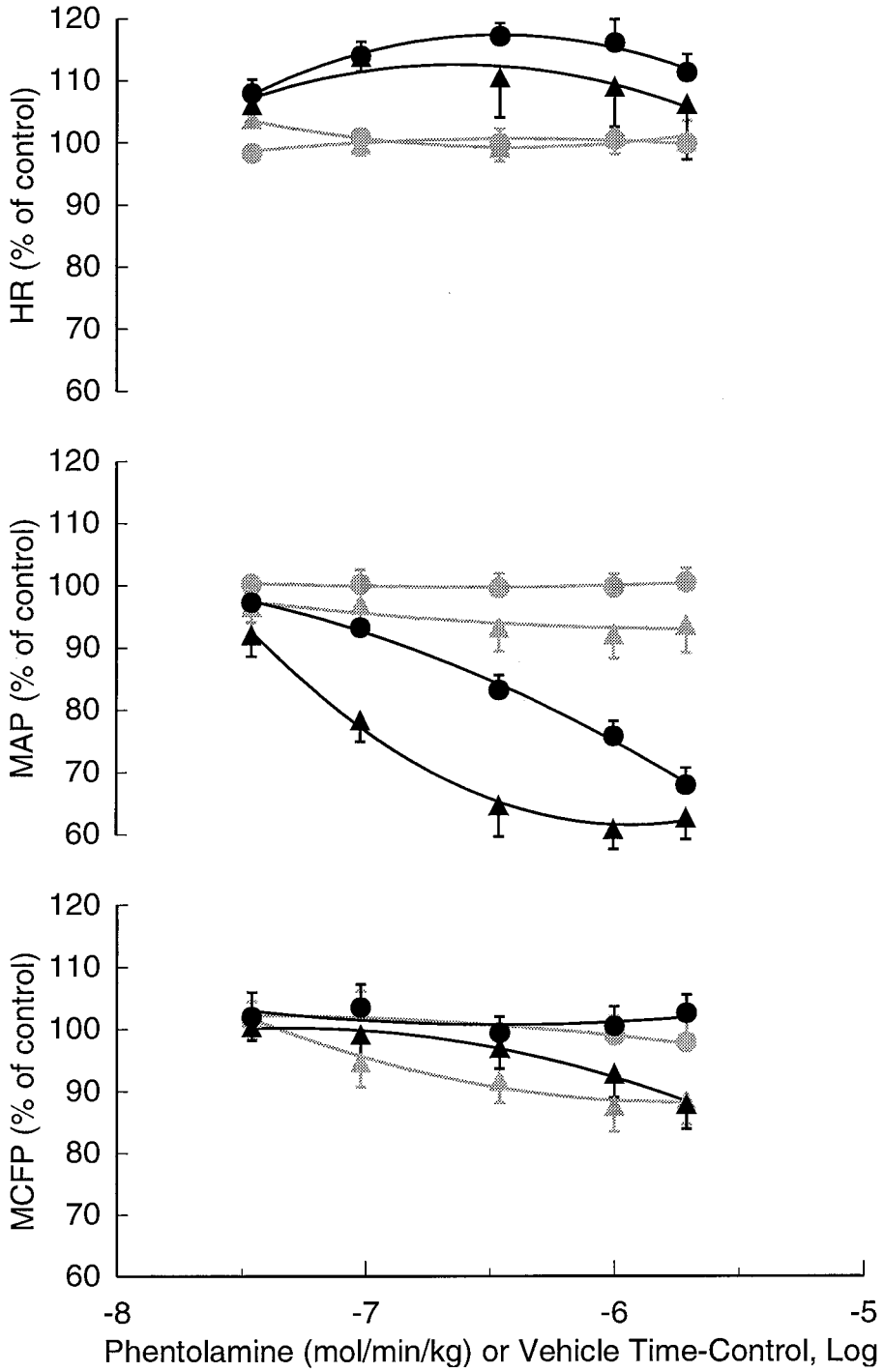


Figure 4.1. Dose-response curves of the effects of phentolamine (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or suramin (200 μmol kg^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

3.5. *Does Sympathetic Cotransmission Contribute to Venous Tone?*

3.5.1. Effect of suramin in the absence and presence of reserpine (Figure 5.1)

The control values for haemodynamic variables are given in Table 5. Reserpine treatment had no effect on MCFP, but significantly decreased both MAP and HR by 21 ± 2% and 11 ± 3%, respectively, of the post-treatment control values for the vehicle (6% citrate in double-distilled water)-treated groups (n = 12). MAP, HR, and MCFP did not change significantly over time in the reserpine-treated control group.

In the vehicle-treated group, suramin significantly and dose-dependently decreased HR and increased MAP, but had no effect on MCFP. Neither the bradycardia nor the pressor effect of suramin was significantly affected by reserpine. In contrast, reserpine revealed a modest suramin-induced decrease in MCFP which was significantly different from the slight increase that was produced by suramin in the absence of reserpine.

3.5.2. Effect of suramin in the absence and presence of guanethidine (Figure 5.2)

The control values for haemodynamic variables are given in Table 5. Saline did not alter MAP, HR, or MCFP. Guanethidine treatment significantly decreased the post-treatment control value for MAP (22 ± 2%) compared to the saline-treated groups, but had no significant effect on either MCFP or HR (n = 13). During continuous guanethidine infusion, MAP, HR, and MCFP were stable over time, none of the measurements differing significantly from the saline-treated control group.

In the absence of guanethidine, suramin dose-dependently and significantly decreased HR and increased MAP, while MCFP was unchanged by suramin. In the presence of guanethidine, neither the suramin-induced bradycardia nor MCFP differed significantly from the saline-treated suramin group. In contrast, the suramin-induced pressor effect was significantly greater in the presence than in the absence of guanethidine. Furthermore, it appeared that this increased pressor response was the result of a guanethidine-suramin interaction rather than of any time-dependent effect of guanethidine on MAP.

Table 5. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and following treatment (Post-treatment Control) with either vehicle, reserpine (3 mg kg^{-1} 24 h prior to study), or guanethidine (3 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}) in conscious, unrestrained Sprague-Dawley rats.

Figure Ref.	Treatment Group		Pretreatment Controls HR (beats/min)	Pretreatment Controls MAP (mmHg)	Pretreatment Controls MCFP (mmHg)	Post-treatment Controls HR (beats/min)	Post-treatment Controls MAP (mmHg)	Post-treatment Controls MCFP (mmHg)	n
5.1	Suramin	DDW (6% Citrate)[1.5]	–	–	–	403 ± 15	103 ± 3	5.2 ± 0.1	6
		Reserpine[1.5]	–	–	–	357 ± 17	83 ± 3	5.4 ± 0.2	6
		DDW (6% Citrate)	–	–	–	381 ± 14	111 ± 3	5.4 ± 0.1	7
		Reserpine	–	–	–	342 ± 20	87 ± 2	5.2 ± 0.1	6
		DDW (6% Citrate) Pooled	–	–	–	392 ± 10	108 ± 2	5.3 ± 0.1	13
		Reserpine Pooled	–	–	–	349 ± 13 [a]	85 ± 2 [a]	5.3 ± 0.1	12
5.2	Suramin	Saline[1.4]	408 ± 11	101 ± 2	5.3 ± 0.2	418 ± 15	102 ± 2	5.1 ± 0.3	6
		Guanethidine[1.4]	425 ± 17	104 ± 3	5.6 ± 0.2	393 ± 10	79 ± 2	5.4 ± 1.1	5
		Saline	396 ± 11	111 ± 3	5.3 ± 0.1	390 ± 10	111 ± 2	5.3 ± 0.1	13
		Guanethidine	401 ± 7	111 ± 2	5.4 ± 0.1	411 ± 7	87 ± 3	5.4 ± 0.2	8
		Saline Pooled	400 ± 8	108 ± 2	5.2 ± 0.1	399 ± 9	108 ± 2	5.3 ± 0.1	19
		Guanethidine Pooled	410 ± 6	108 ± 2	5.4 ± 0.1	404 ± 6	84 ± 2 [a]	5.4 ± 0.1	13

Values are mean ± s.e.mean. DDW = double distilled water. Figures referenced show the dose-response curves as a percentage of the Post-treatment Control for each group. Treatments followed by superscript numbers are the respective vehicle-treated time controls and are also referenced to the figures showing the time-control curves.
[a] significant difference from saline control, $p < 0.05$

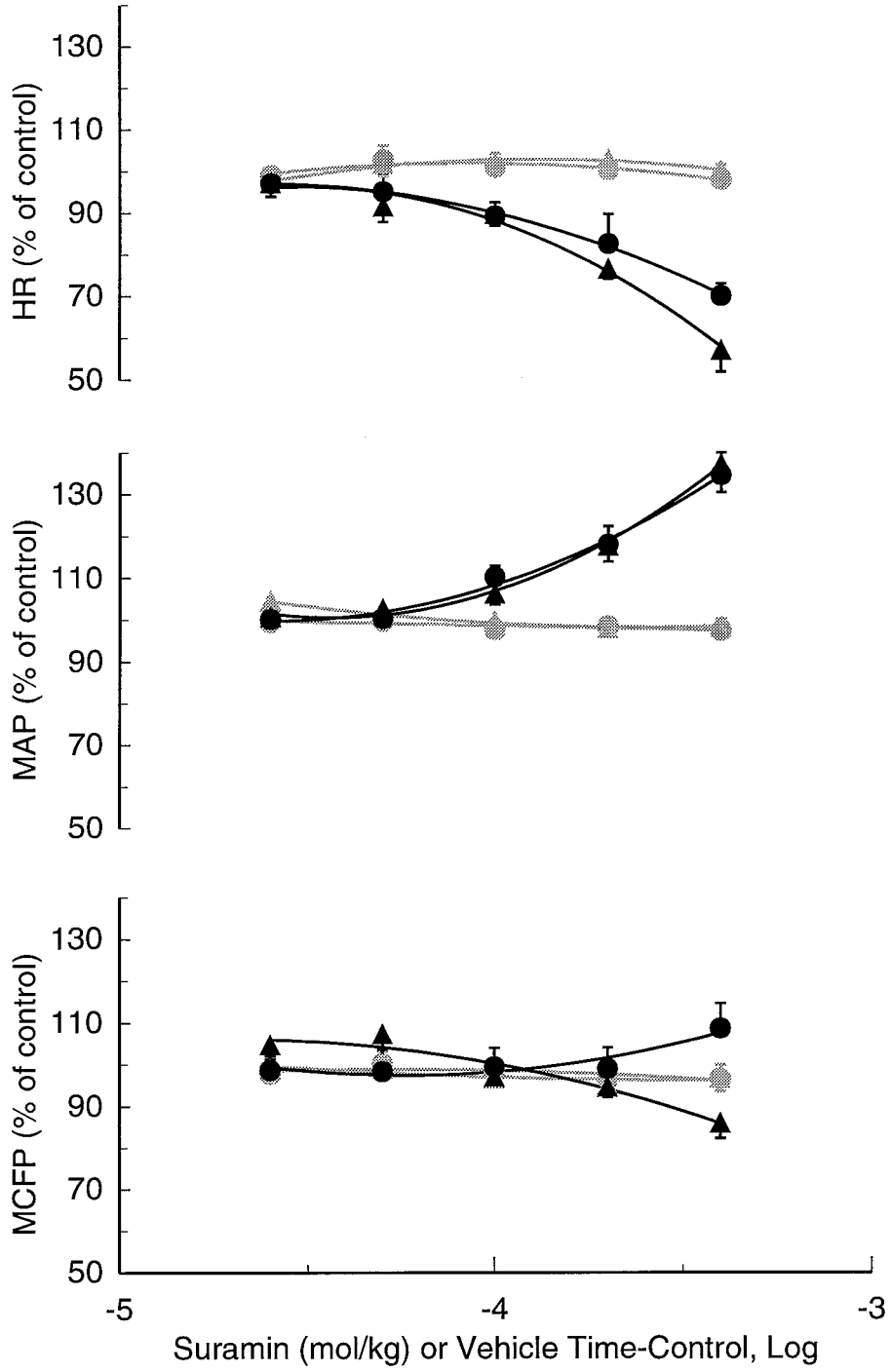


Figure 5.1. Dose-response curves of the effects of suramin (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either vehicle (6% citrate in double-distilled water) (circles) or reserpine (3 mg kg^{-1} 24 h prior to study) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

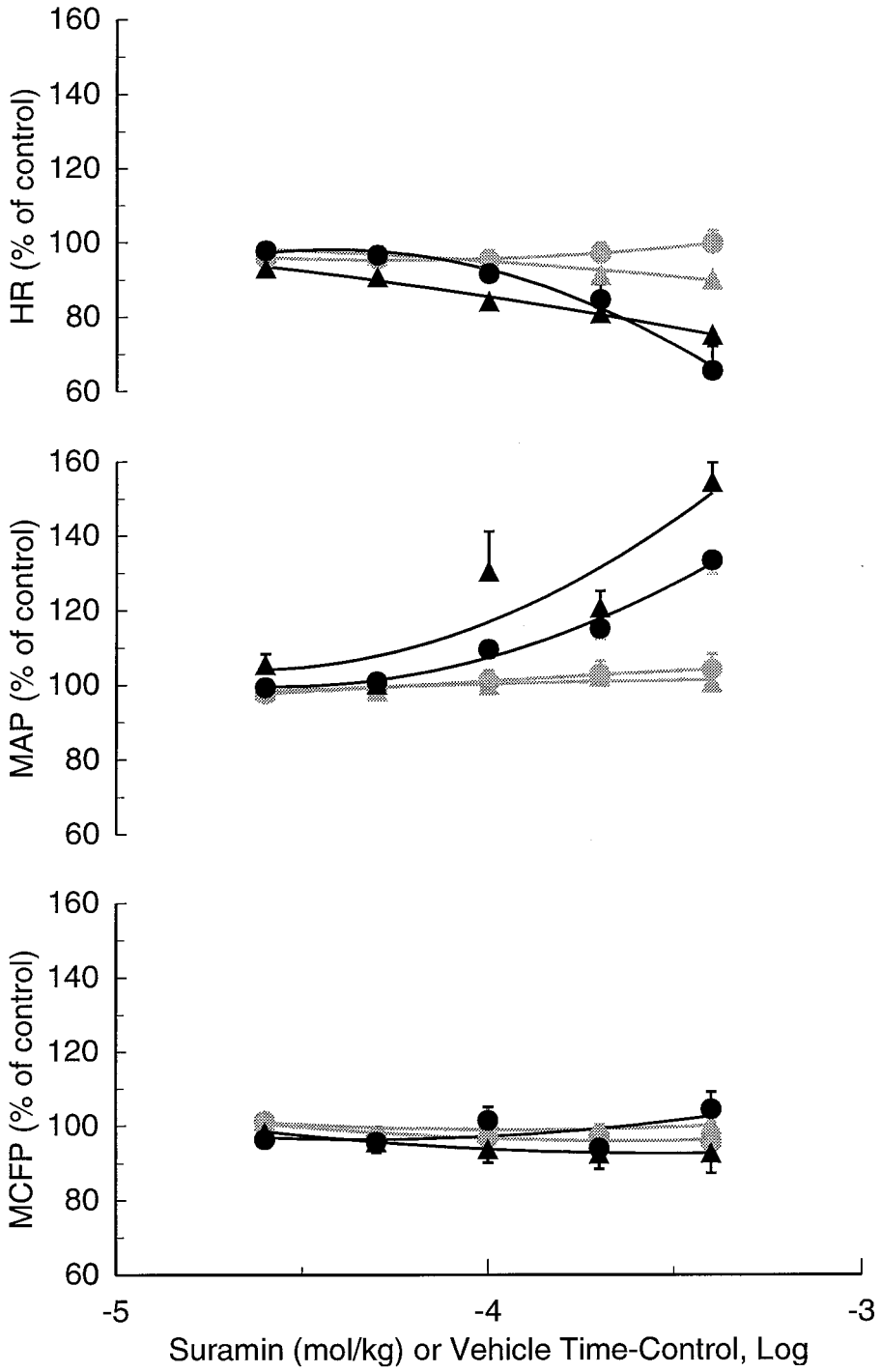


Figure 5.2. Dose-response curves of the effects of suramin (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or guanethidine (3 μmol kg^{-1} min^{-1} for 10 min followed by 0.5 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 5$.

3.6. *Characterization of the Cardiovascular Effects of Exogenous ATP*

3.6.1. Effect of ATP in the absence and presence of mecamylamine (Figure 6.1)

The control values for haemodynamic variables are given in Table 6. While saline had no effect on MAP, HR, or MCFP in any of the groups, mecamylamine treatment significantly decreased MAP (25 ± 2%), HR (9 ± 2%), and MCFP (30 ± 3%) compared to post-treatment control values from saline-treated groups ($n = 12$). The dose of mecamylamine used in these studies inhibited acetylcholine-induced tachycardia by 86 ± 11% (n = 12). In the presence of mecamylamine, neither HR nor MCFP changed significantly with time; however, MAP gradually and significantly increased with time.

In the absence of mecamylamine, infusion of ATP significantly and dose-dependently decreased both HR and MAP. Curve analysis indicated that MCFP was significantly lower in the ATP than the saline control group and that this downward trend was slightly, but significantly, more pronounced in the presence than in the absence of mecamylamine. The ATP-induced bradycardia was not significantly affected by mecamylamine; however, a significant mecamylamine-ATP interaction suggested that the ATP-induced depressor effect was potentiated by mecamylamine.

3.6.2. Effect of ATP in the absence and presence of suramin (Figure 6.2)

The control values for haemodynamic variables are given in Table 6. Suramin treatment produced an increase in MAP (13 ± 2%) and decreases in both MCFP (13 ± 3%) and HR (10 ± 3%), all of which were significantly different from post-treatment control values in saline-treated groups ($n = 12$). Over time, HR, MAP, or MCFP did not change in either the saline- or suramin-treated control groups.

Effect of ATP in the absence of treatment (suramin) – as in section 3.5.1. Neither the ATP-induced depressor effect, bradycardia, nor decline in MCFP was significantly affected by suramin. With respect to the bradycardia, however, curve analysis indicated a significant difference between the ATP dose-response curves in the absence and presence of

suramin, suggesting that the ATP-induced decrease in HR was slightly potentiated by suramin.

3.6.3. Effect of ATP in the absence and presence of cibacron blue (Figure 6.3)

The control values for haemodynamic variables are given in Table 6. Cibacron blue treatment had no significant effect on post-treatment control values for haemodynamic variables compared to saline-treated groups (n = 13), nor did it have a significant effect over time.

Effect of ATP in the absence of treatment (cibacron blue) – as in section 3.5.1. Cibacron blue did not alter the dose-dependent bradycardic effect of ATP. In contrast, the ATP-induced depressor effect was moderately attenuated by cibacron blue as suggested by a significant cibacron blue-ATP interaction and a significant difference in between ATP dose-response curves constructed in the absence and presence of cibacron blue. Cibacron blue also revealed a slight but significant ATP-induced increase in MCFP according to curve analysis.

3.6.4. Effect of ATP in the absence and presence of 8-phenyltheophylline (Figure 6.4)

The control values for haemodynamic variables are given in Table 6. Treatment with 8-phenyltheophylline failed to exert any significant effect on control values for MAP, HR, or MCFP compared to the saline-treated groups (n = 14), nor did it have any significant effect over time.

Effect of ATP in the absence of treatment (8-phenyltheophylline) – as in section 3.6.1. In the presence of 8-phenyltheophylline, the ATP-induced depressor effect was clearly and significantly attenuated, as confirmed by a significant 8-phenyltheophylline-ATP interaction and a significant difference in profile between the ATP dose-response curves in the absence and presence of 8-phenyltheophylline. Similarly, 8-phenyltheophylline abolished the ATP-induced bradycardia and, according to curve analysis, revealed a slight increase in HR. The ATP-induced decline in MCFP persisted in the presence 8-phenyltheophylline and was slightly but significantly more pronounced acccording to curve analysis.

Table 6. Summary of heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) both before (Pretreatment Control) and following treatment (Post-treatment Control) with either saline, mecamylamine (18 μmol kg^{-1} min^{-1}), suramin (200 μmol kg^{-1}), cibacron blue (13 μmol kg^{-1}) or 8-phenyltheophylline (27 μmol kg^{-1}) in conscious, unrestrained Sprague-Dawley rats.

Figure Ref.	*Treatment Group*	*Pretreatment Controls* HR (beats/min)	MAP (mmHg)	MCFP (mmHg)	*Post-treatment Controls* HR (beats/min)	MAP (mmHg)	MCFP (mmHg)	*n*
6.1-6.4 ATP	Saline[1.6]	383 ± 18	98 ± 2	5.3 ± 0.1	377 ± 16	102 ± 2	5.1 ± 0.2	6
	Saline	426 ± 10	107 ± 3	5.7 ± 0.2	420 ± 10	104 ± 3	5.5 ± 0.2	6
	Saline Pooled	404 ± 12	102 ± 2	5.5 ± 0.1	398 ± 11	103 ± 2	5.3 ± 0.1	12
6.1 ATP	Mecamylamine[1.6]	406 ± 12	106 ± 3	5.7 ± 0.3	362 ± 6	72 ± 2	4.4 ± 0.3	5
	Mecamylamine	408 ± 10	107 ± 2	5.4 ± 0.3	362 ± 6	80 ± 2	3.7 ± 0.2	7
	Mecamylamine Pooled	407 ± 7	107 ± 2	5.5 ± 0.2	362 ± 0.4 [a]	77 ± 2 [a]	4.0 ± 0.2 [a]	12
6.2 ATP	Suramin[1.6]	432 ± 17	103 ± 3	5.3 ± 0.1	351 ± 13	112 ± 5	4.6 ± 0.2	6
	Suramin	422 ± 11	105 ± 3	5.4 ± 0.3	367 ± 14	119 ± 3	4.6 ± 0.3	6
	Suramin Pooled	427 ± 10	104 ± 2	5.3 ± 0.2	359 ± 10 [a]	116 ± 3 [a]	4.6 ± 0.2 [a]	12
6.3 ATP	Cibacron Blue[1.6]	375 ± 8	100 ± 2	5.6 ± 0.1	383 ± 15	103 ± 2	5.7 ± 0.2	7
	Cibacron Blue	393 ± 23	102 ± 2	5.2 ± 0.1	360 ± 13	102 ± 3	5.2 ± 0.2	6
	Cibacron Blue Pooled	389 ± 10	100 ± 2	5.4 ± 0.1	373 ± 10	102 ± 2	5.4 ± 0.2	13
6.4 ATP	8-Phenyltheophylline[1.6]	396 ± 19	102 ± 2	5.5 ± 0.1	391 ± 14	105 ± 3	5.5 ± 0.2	7
	8-Phenyltheophylline	392 ± 11	106 ± 2	5.0 ± 0.2	404 ± 16	107 ± 3	5.3 ± 0.2	7
	8-Phenyltheophylline Pooled	392 ± 12	104 ± 2	5.2 ± 0.1	397 ± 10	106 ± 2	5.4 ± 0.1	14

Values are mean ± s.e.mean. Figures referenced show the dose-response curves as a percentage of the Post-treatment Control for each group. Treatments followed by superscript numbers are the respective vehicle-treated time controls and are also referenced to the figures showing the time-control curves. Treatment groups referenced 6.1-6.4 apply to all other ATP-treated groups.

[a] significant difference from saline control, $p < 0.05$

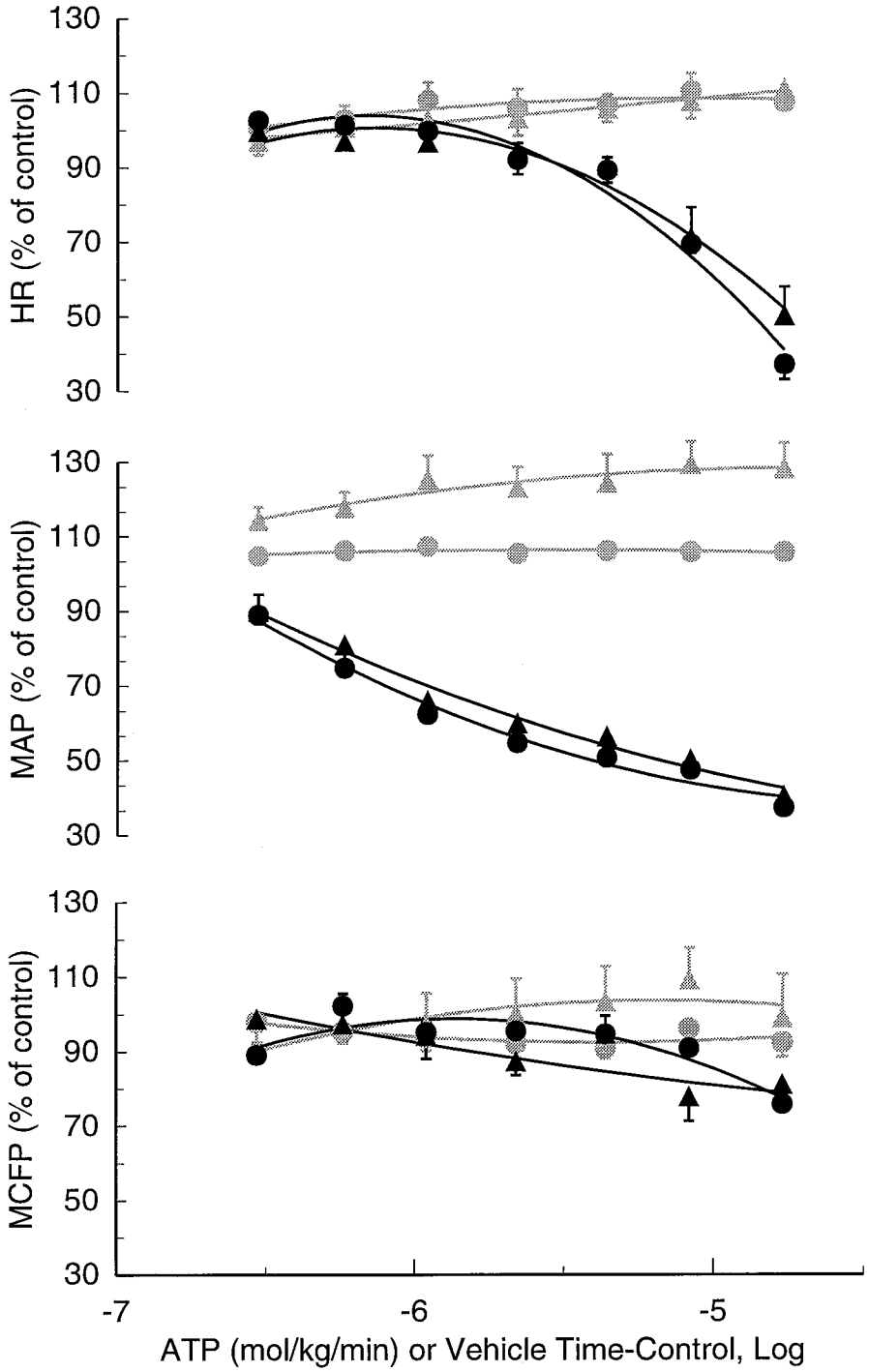


Figure 6.1. Dose-response curves of the effects of ATP (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats continuously infused with either saline (circles) or mecamylamine (18 μmol kg^{-1} min^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 5$.

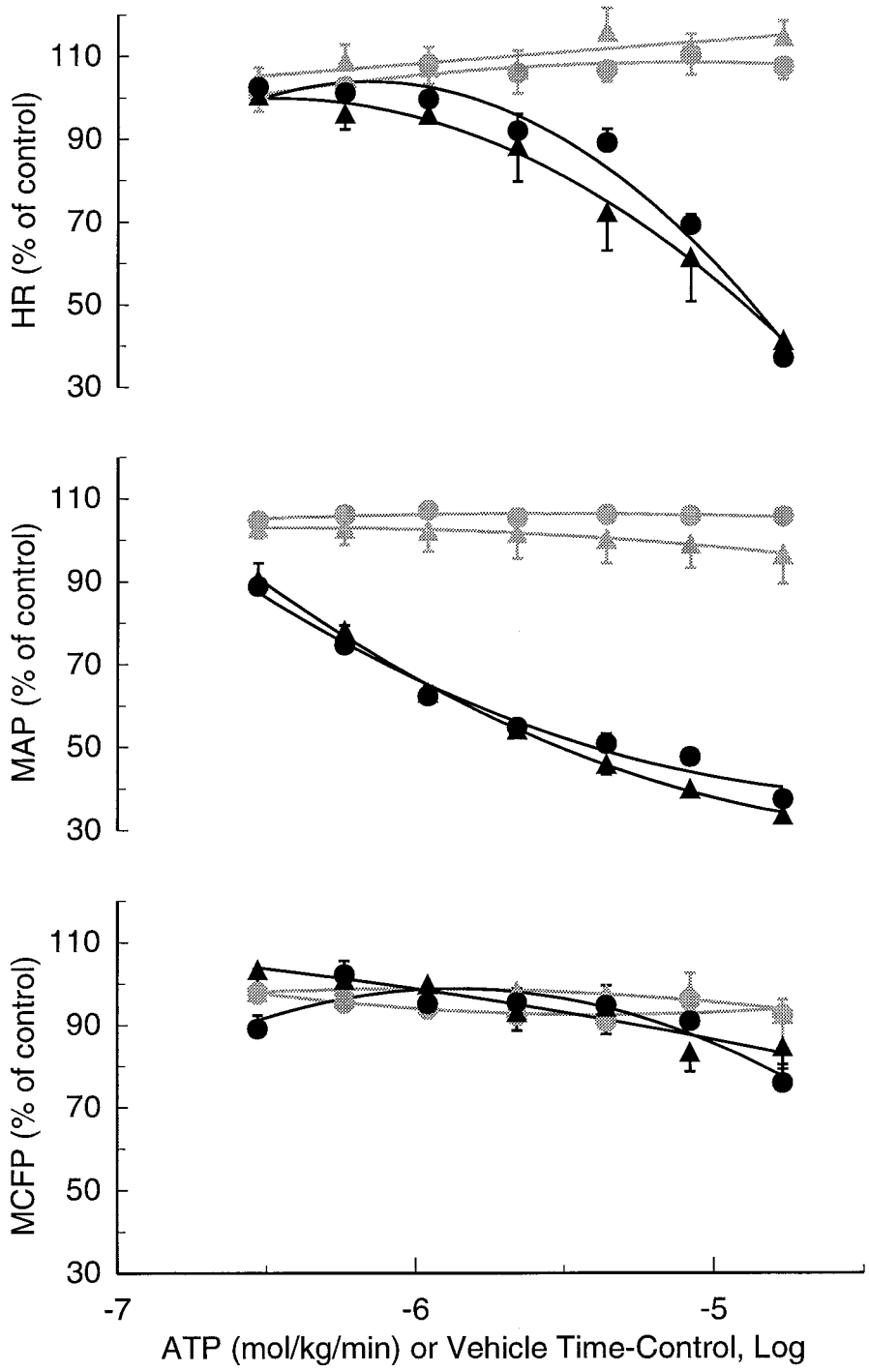


Figure 6.2. Dose-response curves of the effects of ATP (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or suramin (200 μmol kg^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

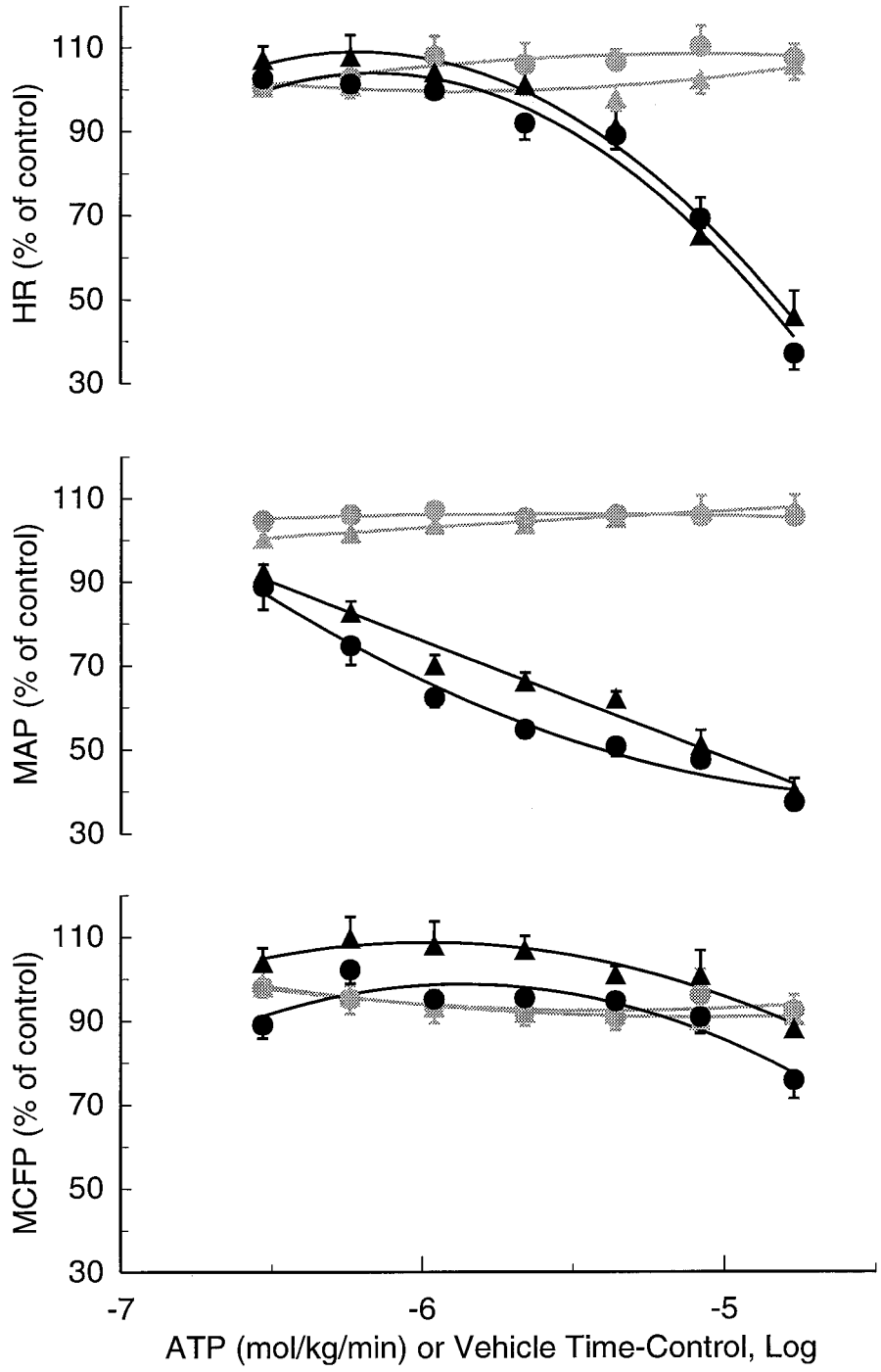


Figure 6.3. Dose-response curves of the effects of ATP (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or cibacron blue (13 μmol kg^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

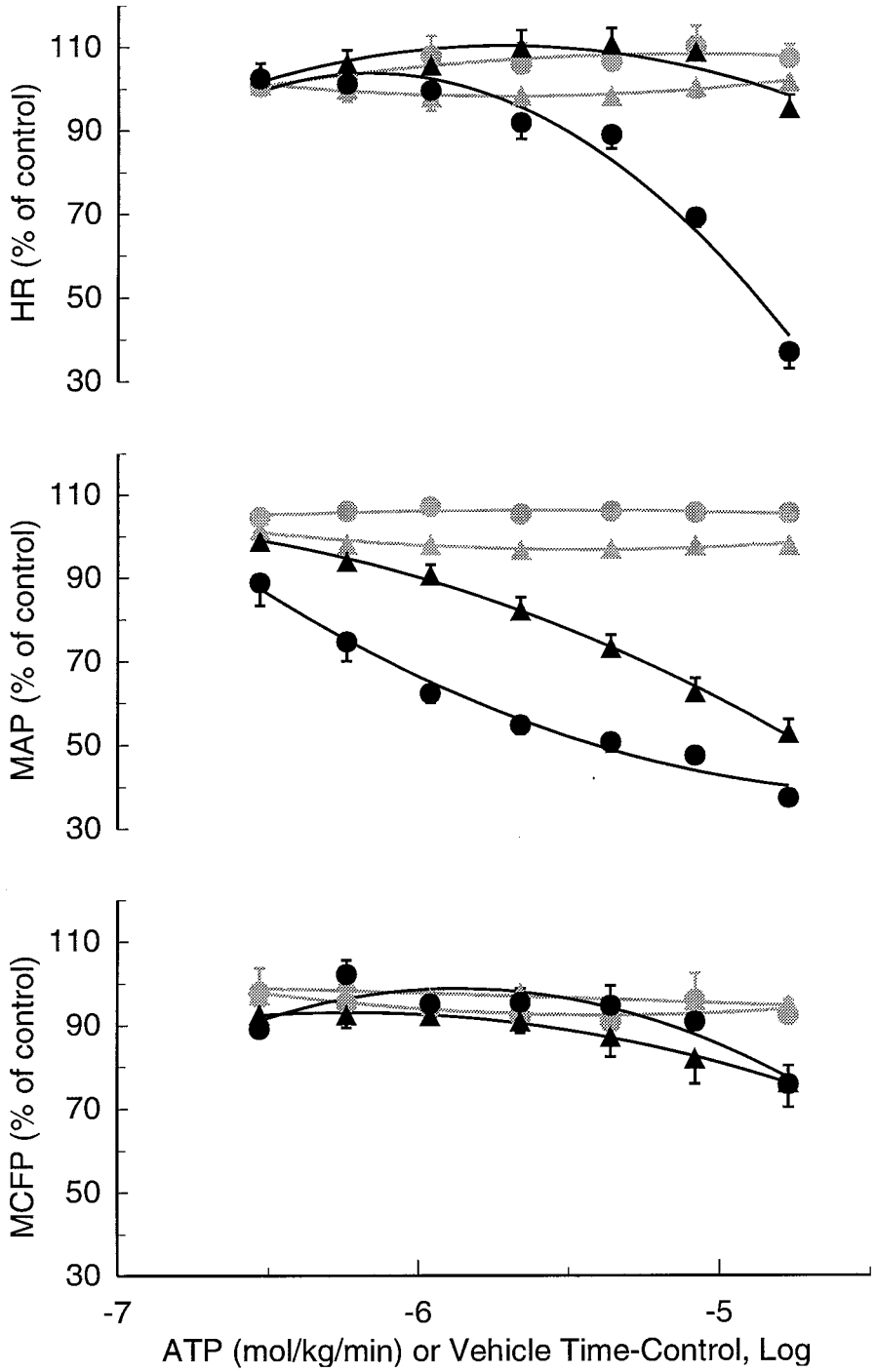


Figure 6.4. Dose-response curves of the effects of ATP (black symbols) or vehicle (shaded symbols) on heart rate (HR), mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats treated with either saline (circles) or 8-phenyltheophylline (27 μmol kg^{-1}) (triangles); each point represents mean ± s.e.mean, $n \geq 6$.

4. DISCUSSION

It has been demonstrated that ATP plays an important role as a cotransmitter of NA in numerous sympathetically innervated blood vessels and vascular beds (see Burnstock 1990d; Westfall *et al.* 1990; Olsson & Pearson 1990). In comparison, very few studies have demonstrated such a role for ATP in whole animals (Flavahan *et al.* 1985; Bulloch & McGrath 1988a,b; Schlicker *et al.* 1989; Daziel *et al.* 1990; Tarasova & Rodionov 1992) and, apparently, in none of these have conscious animals been used. In an attempt to correlate observations made *in vitro* with those made *in vivo*, this study assessed the role of ATP in the control of MAP, HR, and MCFP in conscious, unrestrained rats by examining the effects of receptor antagonists (of α-adrenoceptors, P_1- and P_2-purinoceptors, and autonomic ganglia), chemical sympathectomy (by reserpine or guanethidine), and ATP *per se*. Furthermore, we compared the contribution of endogenous ATP and NA in maintaining basal vascular tone with that during drug-induced vasodilatation and concomittant elevation of sympathetic nerve activity.

The role of ATP in the venous system, estimated by the effects of pharmacological manipulations on MCFP, was of particular interest in this study. MCFP was defined by Guyton as "the pressure that would be measured at all points in the entire circulatory system if the heart were stopped suddenly and the blood were redistributed instantaneously in such a manner that all pressures were equal" (Guyton *et al.* 1973). It has been demonstrated that MCFP is a measure of total body vascular capacitance and is dependent on blood volume, unstressed volumes, and arterial and venous compliances (reviewed in Tabrizchi & Pang 1992; Rothe 1993). However, since venous compliance is much greater than arterial compliance (Guyton *et al.* 1973; Yamamoto *et al.* 1980), MCFP largely reflects overall venous smooth muscle tone. It has been demonstrated that MCFP is a measure of the ratio of blood volume to the overall compliance of the circulatory system; therefore, in order for MCFP to provide an accurate measure of venous tone, blood vol-

ume must remain constant. The method of measuring MCFP used in this study was introduced by Yamamoto *et al.* (1980) and is especially advantageous since it does not require extensive surgery and an accompanying prolonged recovery period. More importantly, since experiments can be conducted in conscious, unanaesthetized rats with intact cardiovascular reflex mechanisms, more physiologically relevant conclusions can be drawn. Indeed, it has been demonstrated that MCFP measured in pentobarbitone-anaesthetized rats may not accurately reflect total body venous tone since, under these conditions, central venous pressure failed to equilibrate with portal venous pressure during circulatory arrest (Tabrizchi *et al.* 1993).

4.1. *Resistance of MCFP to α-Adrenoceptor Antagonism in Rats with Normal and Reflexly-Increased Venous Tone*

This set of experiments was undertaken to determine the role of α-adrenoceptors and the autonomic nervous system in the maintenance of vascular tone (see Results 3.2). Blockade of α-adrenoceptors by phentolamine produced vasodilatation in both normal and vasodilator (hydralazine or nifedipine)-treated rats. These results indicate that basal arterial tone, and that remaining following administration of vasodilator drugs, is maintained via α-adrenoceptor activation, presumably by NA released from perivascular sympathetic nerves. In addition, it was observed that hydralazine, but not nifedipine, potentiated the vasodilatatory effect at low phentolamine doses, suggesting that hydralazine may increase the contribution of α-adrenoceptors to the maintenance of MAP. The significance of this finding is unclear.

In rats treated with vehicle, phentolamine produced tachycardia whereas in both hydralazine- and nifedipine-treated rats, phentolamine caused modest but insignificant bradycardia. Reflex activation of the sympathetic nervous system is presumably responsible not only for the phentolamine-induced tachycardia, but also for the tachycardia observed following treatment with either hydralazine or nifedipine. Indeed, D'Oyley *et al.*

(1989) demonstrated attenuation of hydralazine-induced tachycardia following ganglion blockade with hexamethonium in the conscious rat, while nifedipine has been found to induce reflex tachycardia in anaesthetized and conscious rats (Nordlander 1985; Waite *et al.* 1988). It is possible, though inconclusive according to the present results, that the moderate phentolamine-induced bradycardia observed in the presence of both hydralazine and nifedipine was the result of blockade of cardiac α-adrenoceptors.

Under basal conditions phentolamine did not alter MCFP, while hydralazine treatment revealed only a negligible, insignificant decline in MCFP in response to blockade of α-adrenoceptors. In contrast, nifedipine revealed a significant phentolamine-induced decrease in MCFP. Previous studies have demonstrated that both hydralazine (D'Oyley *et al.* 1989) and nifedipine (Waite *et al.* 1988) have minor direct venodilatatory effects but are capable of very effectively increasing venous tone indirectly via activation of autonomic reflexes. The present results suggest that neither basal nor reflexly-increased venous tone is appreciably maintained by the activation of α-adrenoceptors. This is in accord with previous findings that MCFP is largely resistant to prazosin and rauwolscine (α_1- and α_2-adrenoceptor-selective antagonists, respectively); however, the same study found that in the presence of a reflexly-induced increase in MCFP, both α-adrenoceptor antagonists significantly decreased MCFP (D'Oyley and Pang 1989) which does not agree with the present findings. Similarly, Ito and Hirakawa (1984) were able to demonstrate a phentolamine-induced decrease in MCFP in pentobarbitone-anaesthetized open chest dogs. In this same preparation, however, prasozin did not affect MCFP unless venous tone had been elevated by infusion of NA (Ito & Hirakawa 1984). In another study, phenoxybenzamine (non-selective α-adrenoceptor antagonist) failed to affect MCFP in pentobarbitone-anaesthetized dogs either in the absence or presence of infused adrenaline (Hirakawa *et al.* 1984). Clearly, there are considerable discrepancies between the effects of α-adrenoceptor antagonism on MCFP and several studies have failed to show that α-adrenoceptor blockade lowers MCFP.

Although this study showed that the phentolamine-induced decline in MCFP in the presence of hydralazine was negligible, that observed in the presence of nifedipine was small but significant. The electrophysiological mechanism of ATP-induced vasoconstriction is well-characterized and can be described as "electromechanical coupling" since ATP acts by opening the intrinsic ion channel of the P_{2X}-purinoceptor which subsequently initiates the EJP and voltage-gated Ca^{2+} channels, ultimately leading to activation of the Ca^{2+}-dependent contractile machinery of vascular smooth muscle (see Introduction 1.2.3 and 1.5.3.2). The finding that removal of extracellular Ca^{2+} completely abolished ATP-mediated contractions in dog saphenous vein (Saïag *et al.* 1990) supports the proposal that ATP produces venoconstriction via electromechanical coupling. Moreover, nifedipine has been shown to selectively block purinergic rather than adrenergic nerve-mediated vasopressor responses in pithed rats (Bulloch & McGrath 1988b). In comparison, the mechanism of NA-induced contraction can be described as "pharmacomechanical coupling" since contraction is evoked either without membrane potential changes or accompanied by a slow depolarization. In the case of NA-induced contraction, the source of contractile Ca^{2+} may be intracellular stores or nifedipine-resistant receptor-operated Ca^{2+} channels. Therefore, the results of the present experiments may suggest that prevention of Ca^{2+} influx through nifedipine-sensitive Ca^{2+} channels removes ATP-mediated reflex venomotor tone thereby unmasking the venodilator action of phentolamine. In the presence of hydralazine, ATP-mediated venoconstriction may obscure phentolamine-induced venodilatation.

The failure of phentolamine to appreciably reduce MCFP, either under basal conditions or in the presence of hydralazine, was not due to an absence of sufficient basal tone since mecamylamine-induced ganglion blockade markedly and dose-dependently reduced MCFP in both the absence and presence of hydralazine. This is in keeping with the previous observation that hexamethonium-induced ganglion blockade produces a similar dose-dependent depression of MCFP (D'Oyley & Pang 1990). In light of these observa-

tions, it appears that the weak venodilatatory activity of phentolamine in the presence of raised venomotor tone is indicative of a small role for α-adrenoceptors in the maintenance of neurogenic venous tone. Mecamylamine also produced marked decreases in MAP and HR in the absence of hydralazine. However, while the HR response to mecamylamine was unaltered by hydralazine, the mecamylamine-induced reduction in MAP was virtually abolished. This latter effect was unexpected since hydralazine failed to prevent a phentolamine-induced depressor effect. It is possible to speculate that the mecamylamine-induced decrease in venomotor tone (MCFP) and therefore venous return stimulated an increase in vasopressin release which served to obscure the mecamylamine-mediated depressor effect since it is well-known that decreased stretch of the atria promotes the release of vasoactive peptides such as angiotensin II and vasopressin (Share 1988; Keeton & Campbell 1981) and, moreover, that vasopressin exerts negligible effects on MCFP while producing potent arterial vasoconstriction (Pang & Tabrizchi 1986).

4.2. *Possible Role of Purinergic Neurotransmission in Basal and Reflexly-Increased Venous Tone*

In order to determine whether purinergic tone was responsible for phentolamine-, but not mecamylamine-, resistant normal and reflexly-increased venous tone, experiments were performed using suramin, a non-selective P_2-purinoceptor antagonist (see Results 3.3). The finding that suramin increased MAP and decreased HR similarly in hydralazine- and nifedipine-treated rats indicates that the mechanisms responsible for the suramin-induced effects on MAP or HR involve neither nifedipine-sensitive voltage-gated Ca^{2+} channels nor hydralazine-sensitive mechanisms (perhaps involving intracellular Ca^{2+}). Although the effects of suramin on MAP and HR were not systematically investigated, results from several different experiments in this study implicate direct excitatory vasomotor and cardiodepressant activities, as will become apparent later in this discussion.

MCFP was unchanged by suramin in the both the absence and presence of hydralazine treatment. In the presence of nifedipine, however, suramin produced a very modest but insignificant increase in MCFP. When designing this experiment, it was expected that if suramin decreased MCFP via antagonism of P_{2X}-purinergic tone (in the absence or presence of hydralazine), then nifedipine treatment should eliminate any suramin-induced decrease in MCFP by interfering with P_{2X}-purinoceptor-mediated vasoconstriction at a site beyond the receptor. This, however, was obviously not the case. It therefore appears that endogenous ATP is unimportant under basal conditions and after elevation of venomotor tone. The selectivity of suramin *in vivo* has previously been established in the pithed rat (Schlicker *et al.* 1989; Urbanek *et al.* 1990). However, in light of reports of unselective action *in vitro* (Nally & Muir 1992) it is possible that in the conscious rat preparation used in this study, the effect of suramin may have involved action(s) apart from antagonism of P_2-purinoceptors. If suramin was, indeed, selective for P_2-purinoceptors, it is possible to speculate that any antagonism of postjunctional P_2-purinoceptors was obscured by a prejunctional action of suramin which would have enhanced release of sympathetic transmitters (primarily of NA). A presynaptic action of suramin would explain the discrepancy between these results and those from experiments involving phentolamine in the presence of nifedipine from which it was concluded that inhibition of a nifedipine-sensitive purinergic component unmasked phentolamine-sensitive venomotor tone. Indeed, a prejunctional action of suramin was postulated for its effect in the pithed rat (Schlicker *et al.* 1989). In addition, it is possible that suramin directly increased venomotor activity which is consistent with suramin's direct excitatory vasomotor activity for the maintenance of MAP as proposed earlier.

Ganglion blockade by mecamylamine did not alter either the suramin-induced bradycardia or pressor effect which suggests that neither requires intact autonomic ganglia. Therefore, it appears that the bradycardia is predominantly the result of a direct cardiodepressant action of suramin rather than an indirect reflex-induced withdrawal of

sympathetic tone to the heart. Furthermore, it can be concluded that suramin did not increase MAP by interfering with neurogenic vascular tone. This observation is in accordance with the ability of suramin to elicit a sustained increase in MAP in the pithed rat (Schlicker *et al.* 1989; Urbanek *et al.* 1990).

Interestingly, mecamylamine treatment revealed an appreciable depressant effect of suramin on MCFP. According to the results of the acetylcholine test for blockade, near complete ganglion blockade was obtained in these experiments (79 ± 9%). It follows, therefore, that the suramin-induced decrease in MCFP was a direct effect of suramin rather than an indirect effect involving antagonism of sympathetic or purinergic tone, or a reflex response to the suramin-induced pressor effect. Although the possibility of such indirect actions is disputable considering the high degree of ganglion blockade, it is not an untenable hypothesis. Despite a significant reduction in the acetylcholine-induced tachycardia in the presence of ganglion blockade, found by other investigators (Waite *et al.* 1988; Glick *et al.* 1992), it cannot be definitively stated that this was an accurate measure of the degree of ganglion blockade since there are no thorough studies examining the adequacy of this criterion. Thus, the blunting of cardiac responses to acetylcholine-induced reflex may not accurately represent the overall degree of bockade of autonomic ganglia and, in particular, blockade of ganglia responsible for maintaining vascular tone. In light of the proposed mechanism of action of ATP-induced vasoconstriction (*i.e.* electromechanical coupling), it is possible that only minimal innervation is sufficient for cotransmitter ATP to exert a constrictor effect since the ATP signal (*i.e.* receptor-ligand binding) will be greatly amplified by voltage-dependent mechanisms. If this is the case, then even the very small fraction of neurally-released ATP that presumably remains in the presence of mecamylamine should be sufficient to produce venoconstriction. In addition, the pattern of nerve activity during ganglion blockade may have been altered such that purinergic transmission was favoured over adrenergic transmission. The stimulation parameter-dependence of the ratio of cotransmitter NA and ATP is well-characterized (see Introduction

1.5.3.3). Furthermore, the results from these experiments do not rule out the possibility that perivascular purinergic nerves may exist separately from sympathetic nerves and may be differentially affected by mecamylamine.

4.3. *Does Suramin Reveal an α-Adrenoceptor Antagonist-Sensitive Component of Venous Tone?*

Since neither phentolamine nor suramin above was successful in depressing MCFP, experiments were conducted to determine if concurrent administration of both antagonists revealed such an effect (see Results 3.4). Thus, phentolamine dose-response curves were constructed in the absence and presence of a high dose of suramin. The phentolamine-induced tachycardia was not affected by suramin and was probably a reflex response to the accompanying depressor effect. Interestingly, this depressor effect was significantly greater in the presence of suramin which suggests that antagonism of arterial postsynaptic P_{2X}-purinoceptors may have been involved. In other words, there may exist an α-adrenoceptor antagonist-resistant MAP response which is due to excitatory purinergic tone and is inhibited by suramin. In contrast, MCFP remained unaffected by phentolamine in the pressence of suramin. Assuming suramin antagonized postsynaptic P_{2X}-purinoceptors in the venous system, these results suggest that purinergic venomotor tone was not responsible for opposing any venodilatatory activity of phentolamine. It is unlikely that the dose of suramin was not sufficient to produce blockade of venous P_{2X}-purinoceptors since a preliminary experiment demonstrated that the pressor response to i.v. injection of α,β-methylene-ATP was almost completely attenuated for at least 3 h. In addition, it has been shown previously in the pithed rat that suramin at a dose of 100 μmol kg^{-1} (half that used in the present experiments) produced a parallel shift to the right (by a factor of 6) of the dose-response curve for α,β-methylene-ATP, but not NA or neuropeptide Y (Urbanek *et al.* 1990). In addition, Schlicker *et al.* (1989) reported that suramin blockade persisted for at least 30 min in the pithed rat.

One might speculate that in the venous system suramin acts primarily by antagonizing P_2-purinoceptors on sympathetic nerve terminals, thereby enhancing release of NA and, possibly, ATP. Such an action for suramin has recently been demonstrated, for the first time, by Allgaier *et al.* (1994). These authors found that suramin, but not 8-(*p*-sulphophenyl)-theophylline (P_1-purinoceptor antagonist), completely prevented ATP-induced inhibition of NA release from chick sympathetic neurons. Interestingly, the same study also demonstrated that α,β-methylene-ATP had no effect on NA release, while 2-methylthio-ATP (P_{2Y}-purinoceptor agonist) produced a facilitation of NA release which was prevented by cibacron blue (P_{2Y}-purinoceptor antagonist). Similarly, phentolamine is known to block not only postsynaptic α-adrenoceptors but also presynaptic α_2-autoreceptors (Starke *et al.* 1989), following which release of both NA and ATP are enhanced (Bulloch & Starke 1990; MacDonald *et al.* 1992; Msghina *et al.* 1992; Bao 1993). Thus, concurrent administration of suramin and phentolamine may have resulted in a substantial enhancement of NA and ATP release such that blockade of postsynaptic receptors was overcome and venoconstriction was elicited (see Bevan *et al.* 1987). However, the ability of endogenously-released agonists to overcome receptor antagonism is highly unlikely on the basis of relatively very slow on-off rates for exogenous antagonists in addition to very effective physiological mechanisms for clearance of endogenous neurotransmitters such as ATP and NA. Another possible explanation for the inability of concurrently administered suramin and phentolamine to depress MCFP is an inaccessibility to antagonists of excitatory adrenergic and/or purinergic receptors in the venous system. These results might also be interpreted as evidence for the putative existence of phentolamine- and suramin-insensitive γ-adrenoceptors; however, studies have previously demonstrated an absence of such effects in veins (Laher *et al.* 1986; Hirst & Jobling 1989).

Earlier experiments were described in which phentolamine produced a significant depression of MCFP in the presence of nifedipine which, presumably, blocked purinergic tone at a site beyond the P_{2X}-purinoceptor. If suramin exerted primarily postsynaptic

inhbition of P_{2X}-purinoceptors, one could argue that phentolamine, in the presence of suramin, should have produced a decrease in MCFP similar to that produced in the presence of nifedipine. However, this was not so. The cause of this apparent discrepancy may simply be that the NA to ATP ratio is higher in reflexly-increased versus basal sympathetic venomotor tone. There is a good deal of evidence which suggests that the pattern of nerve stimulation influences the ratio of released NA and ATP from sympathetic nerves (Kennedy *et al.* 1986b; Sjöblom-Widfeldt *et al.* 1990, Sjöblom-Widfeldt & Nilsson 1990; Evans & Cunnane 1992). It is therefore possible that the pattern of sympathetic nerve activity is altered following reflex activation of venomotor tone, as following infusion of nifedipine, such as that the proportion of ATP to NA release is increased.

4.4. *Sympathetic Cotransmission and Venous Tone*

The use of chemical sympathectomy (*e.g.* by reserpine, guanethidine, or 6-hydroxydopamine) in the study of sympathetic NA-ATP cotransmission has provided key evidence in support of cotransmission in the cardiovascular system (see Introduction 1.5.3.1). This set of experiments was therefore designed to approach the question of sympathetic cotransmission *in vivo* in a manner analogous to that used to demonstrate cotransmission *in vitro*. It is important to recognize that reserpine interferes with the NA but not the ATP uptake mechanism of chromaffin granules (Winkler *et al.* 1981) while guanethidine selectively renders the membrane of catecholaminergic terminal axons inexcitable (Hausler & Haefely 1979). In the present experiments, reserpine failed to alter control values for MCFP even though both MAP and HR were significantly decreased. Furthermore, the dose of reserpine used in these experiments was previously shown to abolish catecholamine stores by at least 98% (Gillespie & McGrath 1974; Brizzolara & Burnstock 1990) without affecting the release of ATP or the α-adrenoceptor antagonist-resistant component in several vessels and tissues (Kügelgen & Starke 1985; Warland & Burnstock 1987; Kirkpatrick & Burnstock 1987). Thus, the unaltered MCFP in reserpinized

rats might be interpreted as supporting the existence of a non-catecholamine transmitter in the maintenance of venous tone. Indeed, this hypothesis is supported by the observation that suramin produced a slight but significant decrease in MCPF following reserpine, but not guanethidine, treatment. Guanethidine has previously been shown to block not only the adrenergic but also the purinergic component of perivascular sympathetic nerve stimulation in several arteries (Kennedy *et al.* 1986; Evans & Cunnane 1992). Taken together, the present results suggest that sympathetically-released ATP, acting at P_{2X}-purinoceptors, may participate in maintaining venous tone, though perhaps only to a small extent, following depletion of NA from sympathetic nerve terminals.

The finding that there was no significant difference in post-treatment control MCFP values between reserpine- and guanethidine-treated groups, however, casts doubt on proposals concerning NA-ATP cotransmission in the venous system since these results point to the possible involvement of neurotransmitters other than ATP and NA in the maintenance of venous tone. That suramin failed to lower MCFP in guanethidine-treated rats further suggests that ATP is *not* involved, leaving the possibility that a "non-adrenergic, non-purinergic" element may be responsible for maintaining venous tone. Indeed, non-adrenergic, non-cholinergic (NANC) neurotransmission is well-characterized in the portal vein of several species (Burnstock *et al.* 1984; Kennedy & Burnstock 1985a; Brizzolara *et al.* 1993) and has been shown to be resistant to guanethidine (Burnstock *et al.* 1979) – it is possible, though highly speculative, that analogous nerves serve the venous system and release transmitters whose actions are resistant to blockade of the conventional α-adrenoceptor or P_{2X}-purinoceptor. It is important to recognize, however, that guanethidine posseses local anaesthetic activity and consequently may have paralyzed all neurotransmission, in which case non-neurogenic mechanisms would be responsible for sustaining venous tone. It is also possible to speculate that had reflexes been stimulated, the contribution of ATP in reserpinized rats might have been increased and a larger suramin-sensitive component might have become apparent since, as discussed previously, the

pattern of nerve stimulation may be altered during increased sympathetic nerve activity such that the ATP to NA ratio of released transmitter is increased.

P_{2X}-purinoceptor-mediated vasoconstriction to exogenous ATP has been demonstrated *in vitro* in the human and dog saphenous vein (Saïag *et al.* 1990; Rump & von Kügelgen 1994), in the dog maxillary internal vein (Saïag *et al.* 1992), and in the dog cutaneous vein (Flavahan & Vanhoutte 1986). In addition, it has been demonstrated that α,β-methylene-ATP is particularly active on capacitance vessels of the cat intestinal circulation *in vivo*, producing greater venoconstriction than either injected noradrenaline or high frequency stimulation of sympathetic nerves (Taylor & Parsons 1989). Nevertheless, as in this study, it has proved difficult to demonstrate a purinergic component of neurogenic venoconstriction (Rump & von Kügelgen 1994). The explanation for this could be that exogenous ATP and its analogues stimulate primarily extrajunctional P_{2X}-purinoceptors. Consistent with a relatively minor role for ATP in the maintenance of venous tone is the demonstration that the densities of [^{3}H]α,β-methylene-ATP (P_{2X}-purinoceptor radioligand) binding sites in veins, such as the rabbit mesenteric vein and inferior vena cava (but not the portal vein) are relatively very low compared to those in muscular arteries such as the rat mesenteric, tail, and central ear arteries (Bo & Burnstock 1993).

Interestingly, suramin-induced bradycardia was not affected by either reserpine or guanethidine which indicates that the response is dependent on neither sympathetically-released NA nor functional sympathetic nerves. These results are in accordance with the inability of mecamylamine-induced ganglion blockade to affect suramin-induced decrease in HR. Therefore, it appears that suramin possesses direct cardiodepressant activity. Similarly, the suramin-induced increase in MAP was not altered by reserpine and was even potentiated by guanethidine. These results suggest that the pressor effect of suramin does not require a functioning sympathetic nervous system.

4.5. *Cardiovascular Effects of Exogenous ATP*

Since there have been no previous investigations into the effects of exogenous ATP on venous tone as reflected by MCFP, experiments were conducted in our conscious rat model to determine the effects of infused ATP on MCFP in the absence and presence of various receptor antagonists. In vehicle-treated rats, ATP produced a profound decrease in MAP which persisted, apparently unaffected, in the presence of mecamylamine and suramin. In contrast, both cibacron blue (P_{2Y}-purinoceptor antagonist) and 8-phenyltheophylline (P_1-purinoceptor antagonist) attenuated the ATP-induced decrease in MAP; however, only the effect of the latter reached statistical significance. Although neither antagonist has been characterized extensively *in vivo*, the same dose of 8-phenyltheophylline and one-half the dose of cibacron blue used in this study have previously been shown to significantly attenuate vasodilatation due to adenosine and ATP, respectively, in fetal lambs (Konduri *et al.* 1992, 1993). The present results suggest that i.v. infusion of ATP produces vasodilatation primarily via adenosine acting at P_1-purinoceptors which would require ATP to be extensively metabolized (presumably by ectonucleotidases in the cardiac and pulmonary vascular beds) before reaching the arterial circulation. This proposal is consistent with the finding that, in anaesthetized dogs, arterial levels of ATP during i.v. infusion are negligible while venous ATP levels are approximately 90% of the expected plasma concentration for any given infusion rate (Sollevi *et al.* 1984).

ATP infusion also produced a marked bradycardia which was unaltered in the presence of mecamylamine or cibacron blue but completely abolished by 8-phenyltheophylline. 8-phenyltheophylline even revealed a modest but statistically insignificant ATP-induced tachycardia while suramin very slightly enhanced the ATP-induced decline in HR. It therefore appears that the ATP-induced bradycardia is primarily (if not totally) the result of adenosine acting at cardiac P_1-purinoceptors, probably of the A_1-subtype (see Pelleg *et al.* 1990). The very slight ATP-induced tachycardia observed in the presence of 8-

phenyltheophylline may have been a reflex response to ATP-induced vasodilatation (*i.e.* mediated cardiac β_1-adrenoceptors) or, possibly, ATP may have increased HR via stimulation of cardiac P_{2Y}-purinoceptors. Indeed, Mantelli *et al.* (1993) demonstrated that blockade of inhibitory A_1-purinoceptors converted an ATP-induced negative inotropic effect to a positive inotropic effect that could be antagonized by suramin or cibacron blue. This explanation is consistent not only with the slight enhancement of the ATP-induced bradycardia by suramin (presumably via blockade of P_{2Y}-purinoceptors), but also with the failure of mecamylamine to alter the ATP-induced bradycardia. It is possible that the inability of ganglion blockade to modify the cardiac effects of ATP is indicative of a presynaptic action of exogenous ATP, and/or adenosine, as an inhibitor of neurotransmitter release from sympathetic nerve terminals (see Westfall *et al.* 1990b). Based on the data presented, however, the involvement of stimulatory cardiac P_{2Y}-purinoceptors in the response to ATP is highly speculative since the decrease in HR produced by ATP was unaffected by cibacron blue.

The response of MCFP to ATP infusion, although insignificant, consisted of a very small increase followed by a decline. The decrease in MCFP was slightly more pronounced in the presence of either mecamylamine or 8-phenyltheophylline, while suramin had no effect on the response of MCFP to ATP. The presence of cibacron blue, in contrast, revealed a slight but insignificant ATP-induced increase in MCFP. The slight ATP-induced decline in MCFP was most likely the result of ATP, and not adenosine since (i) during i.v. ATP infusion only 10% of venous ATP is degraded to adenosine (Sollevi *et al.* 1984) and (ii) Glick *et al.* (1992) demonstrated that i.v. infusion of adenosine *per se* produces relatively much larger decreases in MCFP than did ATP in the present study. Presumably the venous response to ATP is complex and may involve some of the following factors in addition to others: (i) reflex increase in sympathetic (or possibly other) tone, (ii) stimulation of presynaptic purinoceptors by ATP and adenosine, thereby opposing the hypotension-induced reflex by decreasing release of excitatory transmitters (*i.e.* NA and ATP),

(iii) endothelium-dependent and/or -independent venodilatation by ATP (and, possibly, adenosine), and (iv) ATP-induced venoconstriction. Consequently, the response of MCFP to ATP in the absence of any pretreatment was presumably the *net* effect of a number of different ATP-dependent mechanisms which potentially may act synergistically or antagonistically. It is probably safe to assume that if ATP has no venous dilator effect its profound depressor effect should have evoked a substantial increase in reflex venous tone similar to hydralazine, which has virtually no venous effects and increased MCFP markedly (D'Oyley *et al.* 1989). If this was not the case, then ATP must have exerted venodilatatory actions which obscured this reflex tone such that the net effect was not very different from the control.

The finding that mecamylamine revealed only a slightly more pronounced ATP-induced decline in MCFP supports the idea that ATP (and/or its metabolite adenosine), when infused alone, stimulates sympathetic presynaptic purinoceptors, thereby decreasing excitatory transmitter release (see Westfall e*t al.* 1990b). Although, this proposal is questionable in light of the possibility that ATP was unable to produce further venodilatation under conditions in which venomotor tone had already been significantly depressed by mecamylamine, the observation that 8-phenyltheophylline produced an effect on the ATP-induced decline in MCFP similar to that of mecamylamine not only supports the notion of a presynaptic site of action of ATP, but also implicates ATP, and not adenosine, as the mediator of the slight decrease in MCFP.

The observation that cibacron blue revealed a slight but insignificant increase in MCFP in response to ATP might be the result of (i) inhibition of presynaptic autoinhibitory purinoceptors, thus enhancing reflex venoconstrictor tone, and/or (ii) inhibition of postsynpatic P_{2Y}-purinoceptors responsible for venodilatation. If, in fact, cibacron blue acted presynaptically then this action would be in keeping with the notion of a measureable neuromodulatory role of i.v.-infused ATP in the venous system. A postynaptic site of action for cibacron blue, on the other hand, is also a reasonable hypothesis since this action,

too, would facilitate reflex venoconstriction. However, the failure of suramin to produce effects similar to cibacron blue, either presynaptically or postsynaptically, casts serious doubt on such an action of cibacron blue or even on the *in vivo* selectivity of these antagonists. Still, it could be argued that this apparent discrepancy originates from the additional P_{2X}-antagonistic property of suramin but not cibacron blue. It should be recognized, however, that suramin is a competitive antagonist with low affinity for vascular P_2-purinoceptors (Leff *et al.* 1990) – this might have enabled ATP to easily displace suramin, thus accounting for suramin's inability to alter responses to ATP.

4.6. *Summary and Conclusions*

1. In the conscious rat, phentolamine (a non-selective α-adrenoceptor antagonist) was found to be a more effective arterial than venous vasodilator in both basal conditions and during drug (hydralazine or nifedipine)-induced vasodilatation and reflex venoconstriction. While MCFP was not significantly decreased by phentolamine either under basal conditions or during hydralazine treatment, phentolamine did significantly decrease MCFP in the presence of nifedipine. Following suramin treatment, the phentolamine-induced depressor effect was significantly greater whereas MCFP remained unchanged. Under basal conditions, mecamylamine very effectively reduced both MAP and MCFP whereas in the presence of hydralazine-induced vasodilatation and elevated venomotor tone, ganglion blockade reduced MCFP but not MAP.

CONCLUSIONS: Arterial tone is primarily maintained by α-adrenoceptor activation in both basal conditions and during increased sympathetic nerve activity, whereas venous tone, although dependent on autonomic nerve activity, is completely resistant to blockade of α-adrenoceptors under basal conditions and partially resistant in the presence of elevated venomotor tone. The finding that phentolamine produced a greater decrease in MCFP in

the presence of nifedipine than in the presence of hydralazine suggests that the α-adrenoceptor antagonist-resistance of MCFP may be purinergic in origin. Blockade of purinergic mechanisms at receptor level by suramin may not have revealed an α-antagonist-sensitive component as did nifedipine (via blockade at site beyond the purinoceptor) because of differences in the ratio of NA to ATP released from sympathetic nerve terminals as a result of different patterns of nervous activity under basal conditions and during elevated sympathetic nerve activity. It is unclear why phentolamine but not blockade of autonomic reflexes by mecamylamine reduced MAP in the presence of hydralazine.

2. Blockade of P_2-purinoceptors by suramin produced a dose-dependent increase in MAP and decrease in HR neither of which was affected by hydralazine, nifedipine, mecamylamine, reserpine, or guanethidine. Suramin failed to reduce MCFP in the absence or presence of hydralazine, nifedipine, or guanethidine. In contrast, mecamylamine treatment revealed a significant dose-dependent decrease in MCFP, while reserpine treatment revealed a slight but significant decline in MCFP.

CONCLUSIONS: The mechanisms responsible for the suramin-induced pressor effect and bradycardia involve neither influx of Ca^{2+} through nifedipine-sensitive voltage-gated Ca^{2+} channels, nor hydralazine-sensitive mechanisms possibly involving intracellular Ca^{2+}. Furthermore, neither the suramin-induced MAP nor HR effect is dependent on the integrity of the sympathetic or autonomic nervous system. Suramin may antagonize presynaptic purinoceptors *in vivo*, thus accounting for its inability to reduce basal or reflexly-increased MCFP. The ability of suramin to decrease MCFP in reserpinized rats may indicate a role for sympathetic NA-ATP cotransmission in the venous system, while the appreciable suramin-induced decrease in MCFP during near-complete ganglion blockade may reflect the role of "electromechanical coupling" and the existence of purinergic mechanisms in the venous system.

3. I.v. infusion of ATP produced profound depressor and bradycardic effects. The ATP-induced depressor effect was unaffected by mecamylamine and suramin whereas blockade of P_1-purinoceptors by 8-phenyltheophylline clearly and significantly attenuated this response. Blockade of P_{2Y}-purinoceptors by cibacron blue only slightly and insignificantly attenuated the depressor effect of ATP. ATP-induced bradycardia was not affected by mecamylamine or cibacron blue whereas 8-phenyltheophylline completely abolished this response and even revealed a slight, but insignificant, increase in HR in response to ATP. Suramin slightly but insignificantly enhanced the ATP-induced bradycardia. ATP produced a slight but insignificant depression of MCFP which was unaltered in the presence of suramin, and slightly but insignificantly enhanced both during mecamylamine-induced ganglion blockade and following 8-phenyltheophylline treatment. Cibacron blue, in constrast, revealed a slight but insignificant ATP-induced increase in MCFP.

CONCLUSIONS: I.v. infusion of ATP produces a profound depressor effect predominantly via activation of P_1-purinoceptors following breakdown to adenosine. ATP *per se* may also contribute to the depressor effect, though only minimally, presumably by activation of endothelial P_{2Y}-purinoceptors. The ATP-induced bradycardia is apparently mediated exclusively by adenosine while ATP *per se* may exert a direct stimulatory effect on the heart via P_{2Y}-purinoceptor activation. The effects of ATP on venous tone are not clear and because ATP has the potential to act at various sites, it is very difficult to extrapolate mechanisms of action from measured responses which are, at best, ambiguous. It is not surprising, therefore, that the present results probably reflect the complexity of the actions of exogenous ATP.

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