

THE ACTIONS OF CALCIUM ANTAGONISTS ON SYSTEMIC HEMODYNAMICS,
BLOOD FLOW DISTRIBUTION AND VENOUS TONE OF THE RAT

By

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ABSTRACT

The purpose of my study was to determine and compare the effects of three calcium antagonists on systemic hemodynamics, ECG, blood flow distribution, tissue conductance and venous tone of the rat.

The effects of a representative drug from Spedding's (1985) three subclasses of calcium antagonists on systemic hemodynamics, ECG, cardiac output and the distribution of blood flow were investigated by the microsphere technique in pentobarbital-anesthetized rats. The representative drugs were: I, nifedipine (12 and 36 $\mu\text{g/kg/min}$); II, verapamil (43 and 83 $\mu\text{g/kg/min}$) and III, flunarizine (174 and 275 $\mu\text{g/kg/min}$). Low and high doses were selected to give a decrease in mean arterial pressure of 10 and 20 mmHg, respectively, compared with control rats. At equal depressor levels, all the drugs similarly decreased total peripheral resistance while slightly but not significantly increasing cardiac output (CO) and stroke volume. Heart rate was decreased by verapamil and flunarizine, but increased by nifedipine. The high dose of nifedipine decreased contractility as measured by dP/dt and had no effect on PR-interval, while verapamil decreased dP/dt and prolonged the PR-interval. The low dose of nifedipine and both doses of flunarizine slightly but not significantly decreased dP/dt and had no effect on PR-interval. All three drugs similarly affected the distribution of blood flow. Blood flow to lungs, liver, and heart was increased while flow to the intestine, kidneys, spleen and skin was decreased. Arterial conductances in lungs, liver, heart and skeletal muscle were increased by the three drugs. These results show that representative drugs from the three

subclasses of calcium antagonists had similar effects on the distribution of blood flow and arterial conductances but different chronotropic, dromotropic and inotropic effects.

A final set of experiments were designed to evaluate calcium antagonist actions on venous tone, as venous tone is a primary determinant of CO and the calcium antagonists generally increase CO. The effects of three calcium antagonists, verapamil, nifedipine and flunarizine on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP), an index of total body venous tone, were investigated in the conscious rat. Infusions of all three drugs caused a dose-dependent decrease in MAP and an increase in MCFP, compared with the corresponding values in control rats. HR was decreased by verapamil and flunarizine and slightly increased by nifedipine. Further experiments investigated whether the increase in MCFP by verapamil was indirectly caused by reflex activation of the autonomic nervous system. Rats were pretreated with a continuous infusion of the ganglionic blocker hexamethonium prior to infusion of verapamil. After treatment with hexamethonium, verapamil did not increase the MCFP. In fact the highest dose of verapamil significantly decreased MCFP. The results suggest that calcium antagonists have greater dilator effects in arterioles compared to veins. It appears that any direct venodilator effects of verapamil in conscious rats are masked due to reflex activation of the autonomic nervous system.

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ABBREVIATIONS

Mean Arterial Pressure = MAP

Heart Rate = HR

Left Ventricular Pressure = LVP

Cardiac Output = CO

Final Arterial Pressure = FAP

Mean Circulatory Filling Pressure = MCFP

Total Peripheral Resistance = TPR

Venous Plateau Pressure = VPP

Counts Per Minute = cpm

Contractility = dP/dt

Blood Flow = BF

Stroke Volume = SV

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1. INTRODUCTION

1.1 Historical aspects

The development of the coronary vasodilators prenylamine and verapamil (iproveratril, or isoptin) (Haas and Hartfelder 1962) and the subsequent discovery of their efficacy in patients with coronary insufficiency (Tschirdewhaz and Klepzig 1963) and angina pectoris (Knoch et al. 1963) led to the search for their mechanism of action. Initial pharmacological and electrophysiological experiments indicated that these drugs decreased contractility in isolated mammalian myocardium and intact in situ hearts without producing major changes in action potential configuration. These effects were reversed by addition of calcium, β -adrenergic catecholamines or cardiac glycosides (Fleckenstein 1964). Initially it was thought that such effects were mediated via blockade of β -adrenoreceptors (Melville and Benfey 1965) but further experiments disproved this hypothesis and indicated that verapamil's mechanism of action was either inhibition of calcium movement into the cell, or competition for an intracellular binding site of calcium (Fleckenstein 1967; Nayler 1968). The evidence that such compounds interfered with calcium dependent excitation-contraction coupling led Fleckenstein to give them the name "calcium antagonists" (Fleckenstein 1969).

Though the concept of calcium antagonism was first proposed by Fleckenstein, Godfraind and colleagues reached similar conclusions as a result of their work on isolated smooth muscle. In vitro experiments with vascular smooth muscle and two piperazine derivatives, cinnarizine and lido-flazine, indicated that these agents interfered with the excitation-contraction coupling in vascular smooth muscle (Godfraind et al. 1969; Godfraind and Kaba 1969a). The contractile response of isolated arteries to K^+ depolarization and adrenaline stimulation was blocked by cinnarizine, or

removal of calcium (Godfraind and Kaba 1969b). The contraction of aorta to adrenaline contained a tonic and phasic component. The phasic component was calcium insensitive while the tonic component was abolished in the absence of calcium or presence of cinnarizine (Godfraind and Kaba 1969a).

Though the concept of calcium antagonism, or calcium entry blockade, as a mechanism of drug action is a relatively recent idea the clinical benefit of such a drug action has been utilized for centuries. Tanshinone, the active ingredient of a traditional Chinese remedy for coronary disorders has been shown to exhibit the same effects on isolated guinea pig papillary muscle as verapamil, nifedipine and diltiazem (Patmore and Whiting 1982).

In recent years, many more drugs with calcium antagonistic properties have been synthesized and tested in both experimental and clinical settings. These compounds are heterogeneous in chemical structure and differ in their tissue selectivity, and possibly in their exact site of action. Table 1 gives the name and chemical classification of a number of calcium antagonists as well as the newly developed calcium channel agonists. Development of a valid classification scheme for the calcium antagonists, based on their chemical nature and pharmacological actions, would be useful for both their therapeutic use and further future development.

1.2 Classification of calcium antagonists

Calcium antagonists were originally classified into two subgroups based on their potency and specificity for inhibiting contractions of isolated cardiac and smooth muscle (Fleckenstein 1983). Group A consists of the more potent and specific drugs, such as verapamil, nifedipine, diltiazem and D-600 while group B consists of less potent and specific agents such as prenylamine, fendiline and caroverine. Unfortunately this classification does not take into account the heterogeneous chemical nature of calcium antagonists or their different pharmacological specificity.

TABLE 1. Chemical Identity of Calcium Antagonists

Chemical Group	Calcium Antagonist
Dihydropyridines	Nifedipine Nimodipine Nitrendipine Niludipine Darodipine (PY 108-068) Nisoldipine Isradipine (PN 200-110) Nicardipine Felodipine
Dihydropyridine calcium agonists	Bay K 8644 CGP 28392 YC 170
Phenethylamines	Verapamil Gallopamil (D-600) Anipamil Desmethoxyverapamil Ronipamil Tiapamil Bepridil
Benzothiazepines	Diltiazem Fostedil (KB-944)
Diphenylalkylamines	Cinnarizine Flunarizine Lidoflazine Perhexiline Prenylamine

Godfraind proposed a modified Fleckenstein classification of two groups, each with an A and B subgroup, to take into account the relative specificities of the different drugs (Godfraind 1987). Group I compounds are the selective calcium antagonists; group IA being selective for myocardial calcium channels (dihydropyridines, phenylalkylamines, benzothiazepines), and group IB having no detectable effect on myocardial calcium channels (cinnarizine, flunarizine). Group II compounds are less specific agents; group IIA drugs act on both the slow calcium channel and the fast sodium channel at similar concentrations (bepridil, fendiline, prenylamine, lidoflazine) while group IIB includes drugs which have their primary site of action at a different locus (phenothiazines, loperamide etc.). Glossman and colleagues (Glossman et al. 1982) characterized the calcium antagonists into four groups based on binding studies. Group 1A are the dihydropyridines which bind with high affinity and displace other dihydropyridines in a competitive manner. Diphenylalkylamines, such as cinnarizine, displace dihydropyridine binding in a competitive manner but with low affinity and are classed as group 1B. Group 2 drugs such as verapamil displace dihydropyridine binding in a negative allosteric fashion while group 3 drugs (diltiazem) cause dihydropyridine binding to increase in a positive allosteric manner. Similar to Glossman's scheme, Murphy proposed that the calcium antagonists could be divided into two groups, I being the dihydropyridines and II being the drugs which allosterically regulate this site (Murphy et al. 1983). Rodenkirchen and colleagues (1983) proposed a three group ranking based on the cardiodepressive actions of these compounds; I, the cardiodepressive drugs which are frequency dependent (verapamil, diltiazem), II, the cardiodepressive drugs which are not frequency dependent (dihydropyridines) and III, the drugs which are non specific and affect calcium fluxes at only high concentrations (flurazepam, phenobarbital).

Based on the inotropic, chronotropic and dromotropic effects of calcium antagonists, Taira also proposed a three group classification scheme (Taira 1987). Dihydropyridines being relatively more specific for the coronary vasculature (group I), verapamil and diltiazem produce equipotent decreases of chronotropy and dromotropy (group II), while bepridil and MCI-176 are more potent at producing negative dromotropy than negative chronotropy (group III). All such classifications are based on limited effects of calcium antagonists and do not take into account all levels of action (in vitro, in vivo, biochemical).

A classification of calcium antagonists has been proposed by Spedding (1985), based on lipophilicity, chemical structure and pharmacological activity. Group 1 calcium antagonists are the 1, 4-dihydropyridines which include nifedipine and nimodipine. Group 2 includes structurally diverse agents such as verapamil and diltiazem which are basic compounds with similar lipophilicities. Group 3 contains diphenylalkylamines such as cinnarizine and flunarizine. In vitro experiments indicate that representative drugs from the three classes bind to different sites on the calcium channel and display different pharmacological actions (Spedding 1982, 1983, 1984; Spedding and Berg 1984). Furthermore, this classification holds for the action of various calcium antagonists on the ECG of the pithed rat preparation (Spedding 1982). However, few comparative studies of drugs from these classes have been done in vivo.

The aim of my research was to examine the action of a representative drug from each of Spedding's three classes on hemodynamics, blood flow distribution and venous tone in the rat. Before discussing these experiments, some of the more recent and relevant calcium antagonist literature, with emphasis on mechanisms and sites of action, will be discussed. It is appropriate to first briefly review the role calcium plays in muscular

contraction in order to better understand why calcium antagonists are effective myocardial and vascular smooth muscle relaxants.

1.3 The role of calcium in muscular contraction

In the typical skeletal muscle fiber, an action potential leads to release of a small amount of trigger calcium from triad junctions of the muscle cell and this in turn induces a release of sequestered calcium from the sarcoplasmic reticulum. Calcium released from the sarcoplasmic reticulum then interacts with the actin-troponin-tropomyosin complex of the myofilament causing a conformational shift which allows interaction of the myosin globular head with the actin filament and so initiate contraction. With relaxation, calcium is actively sequestered into the sarcoplasmic reticulum and stored (Endo 1977). Cardiac muscle contracts in the same manner as skeletal muscle but the source of activator calcium is different in that it depends on intracellular entry of extracellular calcium. Contractions in heart cells are thus very susceptible to alterations in extracellular calcium concentration, or inhibition of calcium entry into the cell (Fleckenstein 1977).

Contraction of smooth muscle is different from that in heart or skeletal muscle. Calcium entry from the extracellular space is necessary for contraction but the contractile apparatus differs in that there is a actin-tropomyosin-calmodulin complex producing a calcium sensitive actomyosin ATPase system (Perry and Grand 1979). Calcium entering the cell is bound by calmodulin, and this complex activates myosin light chain kinase which in turn phosphorylates the myosin light chain allowing actin to activate the MgATPase and induce muscle contraction (Adelstein 1987). Thus, in both myocardial and smooth muscle, contractile activity is dependent on the entry of extracellular calcium. It is for this reason that the calcium antagonists are predominantly cardiac and vascular smooth muscle relaxants.

Calcium antagonist actions in vitro and in vivo demonstrate this relative specificity as well as the heterogeneous actions of different calcium antagonists.

1.4 The effects of calcium antagonists on cardiovascular tissue

1.4.1 In Vitro actions on:

1.4.1.1 Cardiac muscle. Compounds Bay a 1040 (nifedipine, Fleckenstein 1972), verapamil (Singh and Vaughn-Williams 1972) and diltiazem (Nakajima et al. 1975) were shown to inhibit the contractile ability of isolated guinea pig papillary muscle while having little effect on the myocardial action potential. Studies on isolated cat myocardium indicated that the inhibition of contraction by verapamil was frequency dependent while that of nifedipine was not (Bayer and Ehara 1978). Diltiazem was shown to have intermediate frequency dependence (Fleckenstein 1983). Verapamil's action was demonstrated to be stereospecific using isolated right atria from the cat (Bayer and Ehara 1978) and isolated cat papillary muscle (Bayer et al. 1975) with (-)-verapamil being more potent than the (+)-isomer. Furthermore, the (+)-isomer of verapamil had a significant effect on the rise rate of the action potential (a sodium dependent action) at concentrations comparable to that which inhibited contractile activity (Bayer et al. 1975).

Isolated Purkinje fibers of the dog showed no change in membrane potential, maximum upstroke velocity, or action potential amplitude upon administration of nifedipine. Nifedipine shortened the plateau of the action potential and reduced automaticity when abnormal pacemaker activity was induced with barium (Dandman and Hoffman 1980). Abnormal induction of pacemaker activity in rabbit papillary muscle was inhibited by the calcium antagonists in the order nifedipine > diltiazem \geq verapamil (Roy and Pruneau 1986). Furthermore, all three drugs produced negative chronotropy in isolated guinea pig hearts (Millard et al. 1984). Calcium antagonists also

inhibit the conduction of action potentials across the AV-node thus they demonstrate a negative dromotropic action (Taira 1987). In conclusion, calcium antagonists affect the myocardium by: 1) inhibiting entry of calcium into the myocardial cell producing negative inotropy, 2) inhibiting the generation of calcium dependent action potentials in pacemaker regions of the heart producing negative chronotropy and 3) inhibiting conduction of action potentials through the AV-node producing negative dromotropy.

1.4.1.2 Vascular smooth muscle. Calcium antagonists differ in their ability to inhibit cardiac muscle and vascular smooth muscle contractility. The dihydropyridines are more potent in inhibiting vascular smooth muscle contraction than in inhibiting cardiac muscle contraction. For example, nifedipine was 15x less potent in inhibiting contraction of human trabecular strips than in relaxing human coronary arteries (Godfraind et al. 1984). Another dihydropyridine, felodipine, was 100x more potent on isolated vascular smooth muscle than on isolated myocardium (Ljung 1985). The diphenylalkylamines (cinnarizine and flunarizine) have little effect on contractile activity of isolated myocardium (van Neuten and Janssen 1973; van Neuten et al. 1978) but are potent inhibitors of smooth muscle contraction. These drugs also have a much longer onset and duration of action than other calcium antagonists (Van Neuten 1969; van Neuten and Janssen 1973; van Neuten et al. 1978). Verapamil has a similar potency on both cardiac and vascular muscle while diltiazem is intermediate between nifedipine and verapamil (Fleckenstein 1983). Potency of cinnarizine, flunarizine, and nifedipine was shown to be different with regard to stimulus (depolarization, NA, $\text{PGF}_{2\alpha}$) and vessel (Godfraind and Miller 1983). Therefore, while calcium antagonist actions appear to be due to inhibiting calcium entry into the cell, each drug does this in a characteristic manner. This is exemplified by structure activity relationships for the different calcium antagonist groups.

The activity of dihydropyridine analogues has been shown by Hansch analysis to depend only on steric factors (Loev 1974; Rodenkirchen 1979) whereas verapamil analogues display both steric and electronic influences for maximal activity (Mannhold 1978; Goll et al. 1986). Unfortunately the diphenylalkylamines and benzothiazepines have not undergone extensive structure activity analysis. Recently the development of novel dihydropyridines which stimulate the entry of calcium into the cell, and thus stimulate contraction, has provided an additional tool for investigating calcium entry and calcium antagonist effects. Bay K 8644 has been shown to increase the contractile activity of isolated guinea pig myocardium and rabbit aorta in a manner which is inhibited by nifedipine (Schramm et al. 1983). Furthermore, enantiomers of Bay K 8644 (Franckowiak 1985) as well as a new dihydropyridine analogue 202-791 (Hof et al. 1985), were found to block, or enhance, calcium uptake and K^+ depolarized smooth muscle contraction.

The heterogeneous effects of the calcium antagonists seen in vitro are further complicated in vivo by the presence of reflex mechanisms. Thus, in vivo calcium antagonist actions are not necessarily the same as in vitro calcium antagonist actions.

1.4.2 In Vivo actions on:

1.4.2.1 Cardiac muscle. The effects of dihydropyridine calcium antagonists on the myocardium in vivo are very different from those seen in vitro. In normotensive animals nicardipine (Hof 1983), nisoldipine (Drexler et al. 1985a), nifedipine (Gross et al. 1979; Kanda and Flaim 1984) and felodipine (Ljung 1985, Nordlander 1985) all increased heart rate while nimodipine (Duncker et al. 1986), darodipine (Hof 1983, 1984, 1985) and isradipine (Hof 1987, Hof et al. 1987) all slightly decreased heart rate. In anesthetized dogs (Gross et al. 1979) the increased heart rate caused by nifedipine disappeared long before hypotensive effects disappeared.

Nifedipine, nisoldipine, nimodipine and nicardipine increased the heart rate in renal hypertensive dogs (Takata and Kato 1986) while felodipine caused an increase in heart rate in renal hypertensive rabbits (Bolt and Saxena 1984) and spontaneously hypertensive rats (Nordlander 1985). Chronic administration of felodipine to SHR rats (Nordlander 1985) resulted in a slight lowering of heart rate. Nifedipine was also shown to have no effect on the ECG of the pithed rat (Spedding 1982). This lack of depressant effect, for dihydropyridines, on the chronotropy and dromotropy of the heart in vivo is accompanied by a lack of negative inotropy. In all studies in which the contractility of the myocardium was measured (Hof 1983; Hof 1984; Kanda and Flaim 1984; Hof 1985; Hof 1987; Hof et al. 1987) the dihydropyridines increased or maintained contractility rather than depressed it. Presumably the lack of dihydropyridine depressant actions on the myocardium in vivo is due to: 1) reflex increase in sympathetic tone concomittant upon hypotension and 2) their relative specificity for vascular smooth muscle.

Verapamil and diltiazem in anesthetized cats (Hof 1983, 1984), SHR rats (Flaim et al. 1986), rats with myocardial infarction (Drexler et al. 1985c), normal Sprague-Dawley rats (Flaim and Zelis 1982) and anesthetized cats (Hof 1983) caused a decrease in heart rate and contractility. Verapamil generally reduced contractility to a greater extent than diltiazem, the latter even increased contractility in the anesthetized cat (Hof 1983) though not to the same extent as nicardipine. Both verapamil and diltiazem decreased heart rate and prolonged PR-interval of pithed rats (Spedding 1982).

Little work has been done with diphenylalkylamine calcium antagonists on the intact animal but they have been shown to decrease heart rate (Kato et al. 1981) with no effect on the PR-interval (Spedding 1982).

1.4.2.2 Vascular smooth muscle. Though calcium antagonists differ

in their ability to affect the heart in vivo all agents tested were shown to lower peripheral vascular resistance while increasing or maintaining cardiac output and stroke volume in conscious rats (Flaim and Zelis 1982; Kanda and Flaim 1984; Drexler et al. 1985), open-chest anesthetized cats (Hof et al. 1982; Hof 1983, 1984), anesthetized rabbits (Hof 1985, 1987, Hof et al. 1987), conscious dogs (Gross et al. 1979; Ljung 1985), renal hypertensive rabbits (Bolt and Saxena 1984), SHR rats (Nordlander 1985, Flaim et al. 1986), and infarcted rats (Drexler et al. 1985c). This indicates that the calcium antagonists are arterial dilators but have minimal effects on veins. The effect of calcium antagonists on the peripheral circulation will be discussed in greater detail in the discussion section. Differences in the actions of calcium antagonists on the myocardium and vascular smooth muscle has implications in the treatment of disease.

1.4.3 Actions in experimental and clinical cardiovascular disease

1.4.3.1 Actions in experimental cardiovascular disease. Induction of arrhythmias in rats by occlusion of the left coronary artery induces ventricular arrhythmias which are susceptible to calcium antagonist treatment. The incidence of ectopic beats, ventricular tachycardia, and ventricular fibrillation were reduced by the calcium antagonists verapamil, cinnarizine, flunarizine and prenylamine (Fagbemi 1984). Studies in conscious rats indicated that verapamil, anipamil, D-888 all protect the ischemic myocardium from ventricular fibrillation but the dihydropyridines nifedipine and felodipine only protect at doses much higher than those producing a maximum hypotensive effect (Walker 1987).

In congestive heart failure the administration of a calcium antagonist may reduce the afterload of the heart and thus relieve failure. In rat models of heart failure the calcium antagonist diltiazem was found to cause a favorable profile of blood flow distribution (Drexler et al. 1985b;

Drexler et al. 1985c) while increasing cardiac output. Effective doses of diltiazem were lower than that needed to produce a significant negative inotropic response.

Treatment of spontaneously hypertensive rats with diltiazem (Flaim et al. 1986) and felodipine (Nordlander 1985) lowered MAP by reducing the peripheral vascular resistance. Furthermore, long term felodipine administration (Nordlander 1985) was effective in maintaining a reduction of MAP and TPR. Felodipine, nicardipine, nimodipine, nifedipine and nisoldipine were effective in lowering the MAP of renal hypertensive animals (Bolt and Saxena 1984; Takato and Kato 1986). Therefore, experiments with hypertensive animals indicate that the vascular selectivity of the dihydropyridines can be utilized in the treatment of hypertension of different origins. Differences in tissue selectivity of the calcium antagonists has implications in the clinical as well as the experimental setting.

1.4.3.2 Actions in clinical cardiovascular disease. Only three calcium antagonists, verapamil, nifedipine and diltiazem, are approved for clinical use in the USA. These compounds have found use in the treatment of a number of cardiovascular disorders such as hypertension, supraventricular arrhythmias, angina pectoris and Prinzmetal's angina, hypertrophic cardiomyopathy and congestive heart failure. In addition, a number of newer calcium antagonists are undergoing clinical trials.

In the treatment of hypertension the dihydropyridine calcium antagonists are most used, presumably because of their relative specificity for the vasculature and resulting lack of negative chronotropic and inotropic effects (Toggart and Zelis 1983). Nifedipine was found to be effective in the lowering of blood pressure in essential hypertension (Blau et al. 1986) with little effect on the myocardium unless administered directly into the coronary artery (Terris et al. 1986). The lack of negative cardiac effects

allows the potential combination therapy of dihydropyridines with β -blockers. Nitrendipine and propranolol together had an additive effect on blood pressure lowering with no development of tolerance over a one year period of study (McMahon 1986). Similar additivity was found with nitrendipine and hydrochlorthiazide (Massie et al. 1986). Newer dihydropyridines such as isradipine (Hamilton 1987) and felodipine (Muir et al. 1985) were shown to be efficacious in the management of essential hypertension. The use of verapamil in patients with hypertension showed that it effectively lowered blood pressure without raising plasma noradrenaline levels as did nifedipine (Agabiti-Rosei et al. 1986; Elliott 1987). It appears that verapamil and nifedipine are equally effective in the management of hypertension (Elliott 1987).

All three of the clinically available calcium antagonists have been found to be effective in the treatment of Prinzmetal's variant angina and angina pectoris (Stone et al. 1980; Schroeder 1982; Stone 1987; Krikler 1987). These agents are especially effective in preventing the vasospasm which occurs in Prinzmetal's angina. Newer dihydropyridines such as isradipine were recently shown to be effective in reducing anginal attack rate and nitrate consumption (Taylor et al. 1987).

In treating arrhythmias, the drug of choice is the more cardioselective agent verapamil. Verapamil is effective in alleviating paroxysmal supraventricular tachycardia, supraventricular tachycardias associated with Wolff-Parkinson-White syndrome and AV junctional tachycardias (Krikler and Spurrell 1974). The effect of verapamil on ventricular arrhythmias is more questionable, but some studies indicate that verapamil is of benefit in treating ventricular arrhythmias (Singh et al. 1983).

In congestive heart failure, administration of the calcium antagonist nifedipine lowered peripheral vascular resistance while increasing cardiac

output, thus improving circulation while not greatly affecting the damaged myocardium (Matsui et al. 1979, Matsumoto et al. 1980). Nitrendipine reduced preload and increased stroke volume in patients with congestive heart failure (Cohn 1986).

Calcium antagonists have been used in a number of other disorders such as treatment of hypertrophic cardiomyopathy (Chatterjee 1987), prophylaxis of migraine and treatment of vertigo (Vanhoutte 1987). Additionally, there are a number of disease states in which calcium antagonist treatment is being considered. The calcium antagonist nimodipine was found to be somewhat beneficial in cerebral ischemia due to stroke (Gelmers 1987). Nifedipine was shown to relieve pulmonary edema (Polese 1979) but similar decreases of pulmonary vascular resistance did not occur upon administration of diltiazem (Klein et al. 1983). Antiatherogenic effects of calcium antagonists have been reported (Weinstein 1987) but only at high doses and the relevance in the treatment of this disorder has not been assessed.

There are a number of calcium dependent processes which are not affected by presently available calcium antagonists. The study of these processes, and how calcium is involved at the molecular level, may contribute specific and therapeutically useful calcium antagonists.

1.5 Calcium antagonist actions on calcium dependent processes other than cardiac and smooth muscle contraction

The ability of calcium antagonists to affect other calcium dependent processes appears to be minimal. Calcium dependent neurotransmitter release from nerve terminals is not susceptible to calcium antagonist blockade. Furthermore, calcium antagonists, at pharmacologically relevant concentrations, have little effect on calcium dependent production and release of hormones from the anterior and posterior pituitary, endocrine pancreas, and steroidegenic organs (Veldhuis 1982).

Aggregation of platelets by calcium is well documented but platelets can aggregate without calcium and antiplatelet activity of the currently available calcium antagonists occurs only at concentrations 100-1000x that needed for inhibition of smooth or myocardial muscle contractility (Rink 1987). Release of vasoactive substances by mast cells in allergic reactions is critically dependent on the entry of calcium into the cell. However, calcium antagonist drugs do not affect histamine release at concentrations which block calcium channels (Pearce 1987). Calcium antagonists appear to inhibit the permeability and shape changes of endothelial cells in an acute inflammatory reaction but there is no evidence to suggest that these drugs modify the calcium controlled regulation of the invading immunogens (Northover 1987).

In recent years, it has become apparent that the endothelium of vascular smooth muscle releases relaxant factors, endothelium derived relaxant factors (EDRF), in response to chemical stimuli. Relaxation of isolated arterial preparations induced by a number of vasoactive substances including acetylcholine, substance P, calcium ionophore A 23187, ATP, histamine, thrombin and serotonin is dependent on the presence of endothelium and calcium ions (Furchgott 1984; Rubanyi 1987). The relative insensitivity of EDRF release to nifedipine and verapamil indicates that the release of EDRF may be analogous to other calcium dependent release processes (Peach et al. 1987; Rubanyi 1987).

Inhibition of the calcium-calmodulin interaction is not a major effect of calcium antagonists. Calmodulin sensitive phosphodiesterase activity of brain isolates was not affected by verapamil, nifedipine, flunarizine, bepridil or diltiazem up to concentrations of 10^{-6} M (Daly et al. 1983, Lugnier et al. 1984). Similarly, felodipine did not affect the myosin light chain phosphorylating activity in rabbit aorta and atria at concentrations

below 10^{-5} M, approximately 1000x greater than that necessary for inhibition of contraction (Silver et al. 1984). Though the effect of calcium antagonists on calmodulin appear to be minimal it demonstrates that the specificity of these drugs is relative and that they have effects not related to calcium entry into the cell.

1.6 Calcium antagonist actions on non-calcium dependent processes

Other pharmacological effects of the calcium antagonists have been investigated such as the blockade of α -adrenoreceptors and muscarinic receptors. D-600 in concentrations from 10^{-6} to 10^{-5} M inhibited the binding of radioligands to α -adrenoceptors and muscarinic receptors in rat brain homogenates (Fairhurst et al. 1980) and isolated rat myocardium (Karliner et al. 1982; Nayler et al. 1982). Furthermore D-600 was shown to bind to opiate receptors (Fairhurst et al. 1980). Verapamil was shown to block α -receptors (Karliner et al. 1982; Nayler et al. 1982; Psychoyos et al. 1986), regardless of subtype (Motulsky 1983), and muscarinic receptors (Karliner et al. 1982; Nayler et al. 1982). Diltiazem and nifedipine produced little α -adrenoceptor blockade (Nayler et al. 1982; Motulsky 1983) indicating that the interaction of these drugs with membrane receptors is not common for all calcium antagonists.

Inhibition of the fast sodium current, which carries the action potential in ventricular muscle, is generally seen at concentrations greater than 10^{-6} M by verapamil and D-600 (Bayer et al. 1975). In general, the concentration of calcium antagonist necessary for blocking calcium entry into cells is much lower than that needed for receptor blockade or fast channel inhibition.

From the preceeding discussion it is apparent that calcium antagonists act by blocking calcium entry through specific plasma membrane channels. Study of the structure and function of these channels may give indications

as to why calcium antagonists are relatively tissue specific.

1.7 Calcium entry into the cell via membrane channels: molecular site of calcium antagonist action

Entry of calcium into the cytoplasm of cells is known to occur in three ways: 1) via a leak current, 2) through voltage operated channels or 3) through receptor operated channels. Entry of calcium via the leak current is partially inhibited by inorganic calcium antagonists such as lanthanum, but not by organic calcium antagonists (verapamil, nifedipine etc., Cauvin and Malik 1984). The amount of calcium which can enter the cell via the leak current is sufficient to produce a contraction only if the sequestering ability of the sarcoplasmic reticulum is compromised (Johns et al. 1987).

Separation of calcium influxes into those involving voltage dependent channels and receptor operated channels was demonstrated by the measurement of ^{45}Ca calcium influx into vascular and visceral smooth muscle cells. Inhibition of both ^{45}Ca uptake and K^+ -induced vascular smooth muscle contraction by calcium antagonists was closely correlated (Cauvin et al. 1984; Cauvin and Malik 1984). Noradrenaline-induced contraction of the aorta was not accompanied by a change in membrane potential and the increase in calcium flux caused by noradrenaline and K^+ were additive. The calcium channel agonist Bay K 8644 was able to increase the ^{45}Ca flux in vessels contracted with noradrenaline but not in K^+ depolarized vessels (Cauvin et al. 1984; Cauvin and Malik 1984; Yamamoto et al. 1984). Flunarizine inhibited both noradrenaline-induced contraction and ^{45}Ca influx in rat aorta and mesenteric resistance vessels (Godfraind and Dieu 1981). In recent years, electrophysiological and biochemical study of the voltage dependent calcium channels has yielded much information about their nature, structure and pharmacology. Knowledge of receptor operated calcium channels

is limited to findings from pharmacological experiments.

1.7.1 Voltage dependent calcium channel

1.7.1.1 Types of voltage dependent calcium channels. Both biochemical and electrophysiological studies have indicated that voltage dependent calcium channels are not a homogenous population.

Electrophysiological studies on calcium conductance of various tissues revealed that there were three calcium channel types (L, T and N) and that certain tissues have more than one channel type. Voltage clamp analysis of calcium currents in heart cells (Lee and Tsien 1983; Bean 1985) and vascular smooth muscle cells (Sturek and Hermsmeyer 1986), and patch clamp analysis of heart cells (Nilius et al. 1985) and vascular smooth muscle cells (Worley et al. 1986; Bean et al. 1986) indicated the presence of two calcium channels (L and T). The L channel requires strong depolarizations for activation and inactivates slowly while the T channel is activated by small depolarizations and inactivates quickly. The T channel is inactivated at membrane potentials at or above -30mV while the conductance of the L channel begins at this membrane potential. It is likely that the contribution to intracellular calcium from the T channel is minimal but may play a role in the automaticity of the SA node and the conduction of the action potential at the AV node as inward currents at relatively negative membrane potentials are needed. The third calcium channel, the N channel, was discovered in chick dorsal root ganglion (Nowycky et al. 1985). This channel requires strong depolarizations for activation and strongly negative potentials for inactivation. It's presence in nerve cells may explain the lack of calcium antagonist effect on neurotransmitter release even though this is a calcium dependent process.

Isolation of calcium channels from a number of sources has been achieved using radiolabelling or photoaffinity labelling with specific 1-4

dihydropyridine analogues. Treatment of channel preparations with various agents indicated that channels from heart, brain and skeletal muscle were different in a tissue, but not species, specific manner (Glossmann et al. 1984a, 1985a). Skeletal muscle channels exhibit increased dihydropyridine binding with increased pH but brain channels maximally bind dihydropyridines at physiological pH. Heparin decreased $^3\text{[H]}$ -nimodipine binding in the order skeletal muscle > heart = brain and chelators decreased dihydropyridine binding in brain > heart > muscle (Glossmann et al. 1984a, Glossmann et al. 1985a). It is likely that the crude method of calcium channel isolation and testing is not able to detect subtle differences in channel type but is instead separating channels based on their interactions with other membrane components. Therefore, it is simplest to assume that there are three types of calcium channels with similar structures, but not conductance characteristics.

1.7.1.2 Structure of the voltage dependent calcium channel.

Structure of the voltage dependent calcium channel appears to be similar for all channel preparations tested. The protein nature of calcium channels was demonstrated by heat and trypsin inactivation of dihydropyridine specific binding (Glossmann et al. 1982; Glossmann and Ferry 1983). Furthermore, the integrity of voltage dependent calcium channels appears to be critically dependent on the presence of certain phospholipids as addition of phospholipases A and C abolished radiolabelled dihydropyridine binding (Glossmann et al. 1982; Glossmann and Ferry 1983; Glossmann et al. 1985a). The glycoprotein nature of dihydropyridine binding sites (calcium channels) was demonstrated by their affinity for wheat germ agglutinin sepharose columns (Curtis and Catterall 1983; Glossmann and Ferry 1983).

Determination of molecular weight of calcium channels by radiation inactivation indicated that the calcium channel is a protein of molecular

weight 180,000 to 210,000 kDa for rabbit skeletal muscle (Norman et al. 1983), guinea pig brain (Ferry et al. 1983; Glossmann et al. 1985a) and guinea pig heart (Glossmann et al. 1985a). Determination of molecular weight for the dihydropyridine binding site in smooth muscle yielded a value of 278,000kDa (Venter et al. 1983). Photoaffinity labelling of rabbit skeletal muscle T-tubule membranes with either radiolabelled dihydropyridines or diltiazem resulted in isolation of a polypeptide of molecular weight 170,00kDa (Galizzi 1986). The presence of d-cis diltiazem reduced the molecular weight determination of the dihydropyridine binding site to approximately 107,00kDa (Norman et al. 1983; Ferry et al. 1983; Glossmann 1985a) while binding of radiolabelled desmethoxyverapamil or diltiazem to guinea pig skeletal muscle membranes reduced the molecular weight of the isolate to 107 and 131kDa respectively (Goll et al. 1984). When guinea pig skeletal muscle T-tubule membranes are purified and solubilized in digitonin, polypeptides of 155, 65, and 32kDa were identifiable while photoaffinity labelling of the same preparation with subsequent isolation of labelled proteins under non-reducing conditions in SDS polyacrylamide gel electrophoresis resulted in isolation of one protein of molecular weight 155kDa (Striessnig et al. 1986). Added to the fact that treatment of membrane preparations with sulfhydryl reagents results in a decrease in dihydropyridine binding (Glossmann et al. 1984a, Glossmann et al. 1985a) it would seem that the calcium channel is an oligomeric structure which is held together, to a certain degree, by disulfide linkages. Interruption of the structure of the channel by binding of calcium antagonists or perturbation by excessive reducing agents can disrupt this structure and result in isolation of various subunits of the channel.

1.7.1.3 Calcium antagonist actions on the voltage dependent calcium channel. Studies of calcium channel conductance in the presence of

calcium antagonists and binding of radiolabelled calcium antagonists to channel preparations demonstrates the heterogeneous nature of calcium antagonist-calcium channel interaction.

Administration of dihydropyridines block the L channel current but has no effect on the T channel current or the N channel current while the calcium channel agonist Bay K 8644 enhances conductance of the L but not the T or N channel (Nowicky et al. 1985; Bean 1985; Lee and Tsien 1983; Bean et al. 1986). Dihydropyridine calcium blockade was shown to be voltage dependent using voltage clamp analysis on cardiac Purkinje fibers (Sanguinetti and Kass 1984). Furthermore, it was postulated that the use dependence of verapamil and the voltage dependence of the dihydropyridines is related to the hydrophilicities of the drugs. Verapamil exists mainly in the charged form at physiological pH while the dihydropyridines are neutral. It is postulated that verapamil must enter the channel in the open state to stabilize it in the inactivated state, analogous to blockade of the fast sodium channel by lidocaine. Dihydropyridines, on the other hand, bind to the inactivated state of the channel therefore the block is larger at more positive membrane potentials as more channels are in the inactivated state. Further support for this hypothesis was obtained when it was demonstrated that the partially ionized dihydropyridine, nicardipine, elicited some use dependent block (Sanguinetti and Kass 1984) and verapamil was ineffective at increased membrane potentials. Voltage clamp confirmed the use dependence of D-600 and showed that diltiazem was use dependent in an intermediate way to D-600 and nitrendipine (Lee and Tsien 1983). This may explain the relative specificity of the dihydropyridines for vascular smooth muscle as this muscle is more depolarized and therefore has a larger proportion of its L channels in the inactivated state.

Binding studies using both crude homogenates and solubilized purified

calcium channels indicate that functional calcium channels contain three binding sites for calcium antagonists which interact in an allosteric fashion with a divalent cation requirement. Binding of radiolabelled dihydropyridines to guinea pig brain (Glossmann et al. 1982), bovine heart (Glossmann et al. 1983a), guinea pig heart, skeletal muscle T-tubules and brain (Glossmann et al. 1984a), calf aorta, rabbit heart and guinea pig ileum (Luchowski 1984) was decreased by calcium chelators. Furthermore, dihydropyridine binding can be reconstituted by addition of any one of several divalent cations (Glossmann et al. 1984a, Luchowski et al. 1984).

Binding of radiolabelled dihydropyridines is competitively displaced by other dihydropyridines. Verapamil and its congeners displace dihydropyridines in a negative allosteric fashion and diltiazem increases dihydropyridine binding in a positive allosteric fashion in all membrane preparations tested (Glossmann et al. 1982, Murphy and Snyder 1982, Glossmann et al. 1984a, Towart and Schramm 1984, Glossmann et al. 1985a, Triggle 1986, Vaghy et al. 1987). Binding of the phenethylalkylamine site with the radioligand [^3H]-desmethoxyverapamil is allosterically inhibited by the addition of d-cis-diltiazem (Glossmann et al. 1985b). These allosteric interactions appear to be pharmacologically significant as contractions of isolated rat mesenteric arteries and taenia coli induced by depolarization were inhibited by nifedipine and diltiazem together in more than an additive manner while nifedipine and D-600 inhibited in an additive manner (Yousif and Triggle 1985). Furthermore, binding of the radioligands is stereospecific which is in agreement with in vitro and in vivo pharmacological experiments. (-)-Verapamil has a higher affinity of binding than (+)-verapamil (Ferry and Glossmann 1982), the eudismic ratio of the (+) to the (-) isomer of the dihydropyridine isradipine is approximately 100 (Glossmann et al. 1983b, Vaghy et al. 1987) and the (-) isomer of nifedipine is more potent than the

(+) (Towart et al. 1981). The d-cis enantiomer of diltiazem increases dihydropyridine binding while the l-enantiomer decreases DHP binding (Glossmann et al. 1983b).

Therefore, it appears calcium antagonists bind to the voltage dependent calcium channel at stereospecific sites that are connected in an allosteric manner. Access to their specific site of action depends on the frequency of channel use, chemical nature of the drug, membrane potential and location of the binding site on the channel. Use of the diphenylalkylamines, such as cinnarizine and flunarizine, has not been attempted in isolated channel preparations or patch clamped cells but smooth muscle experiments indicate that these compounds displace dihydropyridine binding in a competitive manner with low affinity (Spedding 1983). The future of calcium channel research would appear to be purification and characterization of the different channels to deduce the interrelationships of the various subunits and how they produce their conductance characteristics. Recently solubilized isolated channels from skeletal muscle have been reconstituted into a phospholipid bilayer and been shown to retain L channel characteristics including calcium conductance which is blocked by D-600 and stimulated by Bay K 8644 (Flockerzi et al. 1986).

1.7.2 Receptor operated calcium channels

Receptor activation of smooth muscle induces contractions which have a phasic and a tonic component. Serotonin and noradrenaline induced tonic, but not phasic, contractions of isolated vascular smooth muscle are susceptible to calcium antagonist blockade (Towart 1981; Cauvin and Malik 1984; Cauvin et al. 1984, Wong et al. 1986). Lidoflazine decreased the contractile response of guinea pig ileum to angiotensin II in a noncompetitive manner (Godfraind et al. 1966). Cinnarizine and its derivative flunarizine inhibited noradrenaline induced vasoconstriction of the perfused hindlimb of

the dog (van Neuten and Janssen 1971; van Neuten and Janssen 1973) as well as the contraction of isolated ileum by histamine, bradykinin, angiotensin II and methacholine (Van Neutin and Janssen 1973) in a noncompetitive manner.

In the intact animal, calcium antagonists have been shown to modify the vasoconstricting activities of several agonists. Nitrendipine blocked the vasoconstrictor influence of noradrenaline, angiotensin II and vasopressin in anaesthetized rats (Pedrinelli and Tarazi 1985). Constriction of various vascular beds in the anesthetized cat by noradrenaline (Hof et al. 1985), and the anesthetized cat and rabbit by angiotensin II and vasopressin (Hof 1984, 1985) was attenuated by darodipine. Ouabain induced vasoconstriction in the anesthetized cat was also attenuated by the calcium antagonist darodipine (Hof and Hof 1985).

It appears that the agonist receptor interaction in some way stimulates the entry of calcium into the cell (tonic component) as well as the release of sarcoplasmic reticulum calcium (phasic component). Receptor stimulated entry of calcium into the cell is susceptible to calcium antagonist blockade. Voltage clamp analysis of isolated myocardial cells has indicated that the conductance of the voltage operated calcium channel can be modulated by cyclic-AMP. Injection of cAMP analogues increases the calcium conductance (Cachelin et al. 1982) as does injection of the catalytic subunit of cAMP dependent protein kinase (Osterreider 1982) or application of the β -adrenoreceptor agonist isoprenaline (Bean et al. 1984) an agent known to increase intracellular cAMP. This raises the question, does receptor operated modulation of the voltage dependent calcium channel account for the increase in calcium influx upon agonist-receptor interaction or is there a separate calcium channel which is opened by the receptor activation? At the present time it is not possible to distinguish between these possibilities. Recently it has been suggested that the agonist

receptor interaction may cause release of sarcoplasmic reticulum calcium by way of the second messenger inositol triphosphate (IP_3) (Johns 1987). Furthermore the agonist receptor interaction would appear to sensitize the contractile apparatus such that less calcium is needed to sustain contraction (Morgan 1987). Differences in second messenger production or differences of cellular machinery for interpreting the second messenger may explain why some tissues stimulated by an agonist are susceptible to calcium antagonist blockade and some are not.

In recent years there has been a growing controversy whether α_1 adrenoceptors, α_2 adrenoceptors, or both, stimulate calcium entry into vascular smooth muscle cells and are thus susceptible to calcium antagonism. It has been hypothesized that α_2 -adrenoceptor stimulation causes an influx of calcium and is therefore susceptible to blockade by calcium antagonists while α_1 -adrenoceptor stimulation does not cause an influx of calcium. Evidence indicates that the susceptibility of α -adrenoceptor stimulated effects to calcium antagonists is dependent on species (van Meel et al. 1981a, 1981b; Timmermans et al. 1983a, 1983b; Saeed et al. 1983; Llenas and Massingham 1983; Kalkman 1984; Morita et al. 1985), vessel type (Muller-Schweinitzer 1983; Caverio et al. 1983; Medgett and Rajanayagam 1984; Toda 1986; van Brummelen et al. 1987), presence of cardiovascular reflexes (DeJonge et al. 1981), receptor reserve (Bou and Massingham 1984; Jim et al. 1986; Pedrinelli and Taraza 1986; Bou and Massingham 1986) and type of α_1 -agonist (Beckerlingh 1984; Matthews et al. 1985; Bou and Massingham 1986). In general, effects caused by α_2 -adrenoceptor stimulation are susceptible to inhibition by calcium antagonists while α_1 -adrenoceptor stimulated effects vary in their susceptibility to calcium antagonists. Obviously a greater understanding of agonist stimulated calcium channel opening is needed at the biochemical and electrophysiological level.

The above introduction has outlined the effects of calcium antagonists on intact animals, isolated tissues and calcium channels. Though a large amount of research has been done on the calcium antagonists, few comparative studies of their actions on intact animals have been performed. Thus experiments were designed to evaluate the validity of Spedding's classification of calcium antagonists with regard to their effect on the intact rat.

1.8 Experimental Aims

The purpose of the experiments conducted were to 1) determine and compare the effect of representative drugs from Spedding's calcium antagonist subgroups on systemic hemodynamics and blood flow distribution and 2) investigate the effects of these drugs on capacitance vessels.

1.8.1 Calcium antagonist effects on systemic hemodynamics and blood flow distribution of the pentobarbital-anesthetized rat

The initial series of experiments were designed to determine the effects of three calcium antagonists, verapamil, nifedipine and flunarizine, on systemic hemodynamics, ECG and blood flow distribution of the pentobarbital-anesthetized rat using the radiolabelled microsphere technique. Though several studies have been undertaken to determine the effects of various calcium antagonists on systemic hemodynamics and blood flow distribution in conscious rats (Flaim and Zelis 1982; Kanda and Flaim 1984; Drexler et al. 1985; Flaim et al. 1986), anesthetized rats (Gulati et al. 1983), open-chest anesthetized cats (Hof et al. 1982; Hof 1983; Hof 1984; Hof et al. 1985) and anesthetized rabbits (Hof 1985), no study has yet compared the cardiovascular effects of representative drugs from the three subclasses in the same preparation. Spedding (1982) used representative drugs from the three subclasses to determine their electrocardiographic effects in the pithed rat preparation. The effects of a high and a low dose of nifedipine (class I), verapamil (class II) and flunarizine (class III) on

mean arterial pressure (MAP), heart rate (HR), left ventricular systolic pressure (LVP), cardiac contractility (dP/dt), ECG, cardiac output (CO) and blood flow distribution were investigated in pentobarbital-anesthetized rats. Cardiac output and the distribution of blood flow were determined by the microsphere technique (Pang 1983a; Pang 1983b).

1.8.2 Calcium antagonist actions on venous tone of the conscious rat

Cardiac output is controlled by cardiac and vascular factors including heart rate, cardiac contractility, blood volume, vascular compliances and vascular resistances (Greenway 1982). An increase of venous tone alone can increase venous return and thereby increase cardiac output. Various investigators have determined that cardiac output is increased or maintained following calcium antagonist administration in patients with hypertension (Elliott 1987), congestive heart failure (Matsumoto et al. 1980) and angina pectoris (Soward et al. 1986). In conscious rats (Flaim and Zelis 1982; Kanda and Flaim 1984; Drexler et al. 1985a), open chest anesthetized cats (Hof et al. 1982; Hof 1983; Hof 1984), anesthetized rabbits (Hof 1985) and conscious dogs (Gross et al. 1979) calcium antagonists induce similar increases in cardiac output. Furthermore, calcium antagonists with in vivo cardiodepressive actions, e.g. verapamil and diltiazem, have been shown to increase cardiac output (Hof 1983). The purpose of this study was therefore to determine the effect of nifedipine, verapamil and flunarizine on total body venous tone in the conscious rat. Venous tone was measured using mean circulatory filling pressure (MCFP) (Yamamoto et al. 1980; Pang and Tabrizchi 1986). MCFP is the pressure that would occur throughout the circulation if the circulation was instantaneously arrested and all pressures were brought to an equilibrium (Guyton 1973). This measure was shown to be related to cardiac output (Guyton 1955; Guyton 1973) and providing that the blood volume remains constant an increase in MCFP indicates an

increase in the total body venous tone (Grodins 1959).

The role of the autonomic nervous system in the effect of verapamil on MCFP was also determined by use of the ganglion blocker hexamethonium.

2. MATERIALS AND METHODS

The actions of the calcium antagonists on systemic hemodynamics, ECG and blood flow distribution were investigated in the pentobarbital-anesthetized rat preparation using radioactively labelled microspheres while their effect on venous tone was determined in the conscious rat preparation. The surgical preparations and experimental designs used in the two studies were as follows.

2.1 Surgical preparations

2.1.1 Microsphere studies

Male Sprague-Dawley rats (260-410 g, Charles River Canada) were anesthetized with sodium pentobarbital (60 mg/kg) and subjected to cannulations (PE 50) of the right iliac artery, for the measurement of MAP (Grass Polygraph, Model 79D, Mass.) by a pressure transducer (P231D, Gould Statham, Calif.), the right femoral vein for the infusion of drug and the left iliac artery for the removal of the reference blood sample. Cannulae (PE 50) filled with heparinized saline (25 IU/ml) were inserted into the left ventricle via the right common carotid artery for the injection of microspheres and measurement of LVP. HR was determined electronically from the upstroke of the arterial pulse pressure using a tachograph (Grass, Model 7DAG). DP/dt was determined electronically from the LVP using a polygraph differentiator (Grass, Model 7P20C). An ECG trace was obtained using Grass EB2 subdermal electrodes placed on the right and left forelimbs and the right hindleg (Lead I) and recorded on a Grass polygraph (Model 79D). From the ECG recordings, P-R and QRS intervals were measured according to the method of Budden et al. (1980).

2.1.2 Mean circulatory filling pressure (MCFP) studies

MCFP in conscious rats was determined by the method of Yamamoto et al.

(1980). Male Sprague-Dawley rats (300–400 g, Charles River, Canada) were anesthetized with halothane (5% for induction, 1.5% for maintenance) and subjected to cannulations of the iliac artery for the measurement of arterial pressure by a pressure transducer (P23D8, Gould Statham, CA, USA), the femoral vein for the infusion of drugs and the inferior vena cava via the femoral vein for the measurement of central venous pressure by a pressure transducer (P23DB, Gould Statham, CA). A saline-filled balloon-tipped catheter was inserted into the right atrium through the right external jugular vein. The proper position of the balloon was tested by the inflation of the balloon to stop the circulation completely, which caused a simultaneous decrease in mean arterial pressure (MAP), to less than 25 mmHg, and an increase in venous pressure. All cannulae were filled with heparinized saline (25 IU/ml) and tunneled subcutaneously to the back of the neck, exteriorized and secured. The rats were allowed at least 12 hr to recover from surgery before further use. Heart rate (HR) was determined electronically from the upstroke of the arterial pulse pressure using a tachograph (Grass, Model 7P20C).

2.2 Microspheres used in the blood flow distribution studies

CO and the distribution of blood flow were determined by the reference sample method (Malik et al. 1976) using radioactive microspheres, labelled with either ^{57}Co or ^{113}Sn (15 μm diameter, New England Nuclear). Blood was withdrawn at 0.35 ml/min with a withdrawal pump (Harvard Apparatus) from the iliac arterial cannula into a heparinized syringe for 1.5 min. Ten sec after the start of blood withdrawal, a 150 μl sample of a vigorously-vortexed precounted microsphere suspension [containing 20,000–30,000 microspheres in Ficoll 70 (10%) and Tween 80 (0.05%)] was injected and flushed (150 μl saline) over 10 sec into the left ventricle. To avoid variations in the

distribution of the microspheres labelled with either ^{57}Co or ^{113}Sn half of the experiments in each group were first injected with ^{57}Co -labelled followed by ^{113}Sn -labelled microspheres and the order of the injection was reversed in the second half of the experiment. Where measurement of blood flow in the left and right kidneys differed by more than 20%, it was assumed that the microspheres were inadequately mixed and the experiment was rejected. Experiments were also rejected if the calculated cardiac outputs for the two microsphere administrations were very different, as it was assumed that clot formation in the iliac arterial cannula had impaired the withdrawal of the reference sample. Whole organs, except for muscle and skin (30 g each), were excised, weighed and loaded into vials for counting. Blood samples, tissue samples, test tubes and syringes used for the injection of microspheres and the collection of blood were counted for radioactivity by a Searle 1185 series dual channel automatic gamma counter with a 3 inch NaI crystal at energy settings of 80-160 kev and 330-480 kev for ^{57}Co and ^{113}Sn , respectively. At these energy settings the spillover of ^{57}Co into the ^{113}Sn channel was negligible (0.03%) and therefore no spillover correction was made. The spillover of ^{113}Sn into the ^{57}Co channel was 16%. Correction of ^{57}Co counts was done by subtracting ^{113}Sn spillover from ^{57}Co counts.

2.3 Experimental protocol

2.3.1 Effect of verapamil, nifedipine and flunarizine on hemodynamics and blood flow distribution of the pentobarbital-anesthetized rat

Experiments were performed according to double-blind and random designs. Preliminary experiments were conducted to select a low and high dose of each drug capable of causing decreases in MAP by 10 and 20 mm Hg, as compared to control animals treated with the vehicle. All drugs were dissolved in the same vehicle (30% ethanol, 0.014% tartaric acid; in distilled

water) so that only one control group was necessary and to simplify the blind and random design. Drug groups were designated, A = vehicle, B = verapamil 43 ug/kg/min, C = verapamil 83 ug/kg/min, D = nifedipine 12 ug/kg/min, E = nifedipine 36 ug/kg/min, F = flunarizine 174 ug/kg/min, G = flunarizine 275 ug/kg/min, and randomized by line in an eight by seven block which was concealed from the person performing the experiments. Stock solutions of drugs, S1 = vehicle, S2 = verapamil 1 mg/ml, S3 = nifedipine 0.3 mg/ml, S4 = flunarizine 2 mg/ml, were made up and protected from light at all times. Solutions were drawn up into syringes protected from the light by aluminum foil. All cannulae through which the drug was to pass were painted black to protect the drug from the light. After surgical preparations, the rats were allowed 30 min to equilibrate before drug infusion was commenced. After injections of the first set of microspheres, drugs or vehicle were infused at 0.07 ml/min for 12 min. At the end of the infusions, the second set of microspheres was given. Afterwards, the rats were killed by an injection of KCL i.v.

2.3.2 Effect of verapamil, nifedipine and flunarizine on MCFP of the conscious rat

MCFP measurements were made after temporarily stopping the circulation by means of inflating the balloon previously inserted into the right atrium. Within 5 s following inflation of the balloon with saline, MAP decreased and central venous pressure increased simultaneously to a plateau. Central venous pressure measured within 5 s of circulation arrest was referred to as venous plateau pressure (VPP). MAP, HR and VPP were measured at the onset of the experiment and at the plateau phase of response to each dose of drug. Individual dose-response curves (MAP, HR, MCFP) for nifedipine, verapamil and flunarizine were constructed and compared to their respective vehicle controls. Rats were divided into seven groups with $n = 6$

in each group: ethanol, nifedipine, saline, verapamil, tartaric acid, flunarizine and a time control group. Verapamil (5.8×10^{-8} to 8.5×10^{-7} mol/kg/min) and saline (2×10^{-3} to 3×10^{-2} ml/min) were infused for 10 minutes, while nifedipine (1.1×10^{-8} to 4.6×10^{-7} mol/kg/min), ethanol (30% ethanol in distilled water, 8×10^{-4} to 3×10^{-2} ml/min), flunarizine (1.6×10^{-7} to 1.3×10^{-6} mol/kg/min) and tartaric acid (0.02% in distilled water, 9×10^{-3} to 7×10^{-2} ml/min) were infused for 15 minutes. Nifedipine was protected from the light at all times during dissolution and was drawn up into a syringe wrapped in aluminum foil. All cannulae used for the infusion of nifedipine were painted black. MCFP measurements were made at the end of each infusion period. Infusions were stopped for 15 min between doses to allow MAP to recover to predrug levels. In the time control group, MCFP measurements were made at time intervals corresponding with those made for the drug groups (onset of experiment, 15 min after onset and every 30min thereafter for 2.5 hr) to assess the affect of time on the MCFP of the conscious rat.

2.3.3 Role of the autonomic nervous system on MCFP during verapamil administration

The role of the autonomic nervous system in the maintenance of MCFP in rats treated with verapamil was ascertained by the use of two additional groups of rats ($n = 6$ in each group): hexamethonium; hexamethonium + verapamil. In both groups, hexamethonium was continuously infused via the central venous cannula at 0.15 mg/kg/min for the duration of the experiment at an infusion rate of 0.05 ml/hr. The infusion of hexamethonium was temporarily stopped during the measurement of central venous pressure. Verapamil was infused at the same dose-regimen as previously described, after sufficient blockade of ganglionic transmission had been obtained, one hr following the start of the hexamethonium infusion. MCFP was determined

prior to hexamethonium administration, after 1 hr equilibration with hexamethonium and at time intervals during the infusion of verapamil or hexamethonium as described. In each rat of the hexamethonium control group, reflex HR responses to the administration of a bolus i.v. dose of acetylcholine (2 μ g) was used to indicate the effectiveness of ganglionic blockade. In all cases reflex tachycardia was reduced by at least 50% throughout the course of the experiment.

2.4 Drugs

All stock drug solutions for the microsphere studies were made up fresh weekly. On the day of the experiment, appropriate drug dilutions from the stock solutions were made up by a person that wasn't performing the experiments. Verapamil HCl (Knoll Ag., Ludwigshafen, Germany), nifedipine (Bayer, Leverkusen, Germany) and flunarizine diHCl (Sigma Chemical Co., St. Louis) were dissolved in 0.014% tartaric acid and 30% ethanol. Microspheres were suspended in Ficoll (Pharmacia Fine Chemicals AB, Sweden) and Tween 80.

For the MCFP experiments, drug solutions were prepared daily. Verapamil HCl (Knoll Ag., Ludwigshafen, Germany), hexamethonium (K K Laboratories Inc., Plainview N.Y.) and acetylcholine (Sigma Chemical Co., St. Louis) were dissolved in normal saline, flunarizine diHCl (Sigma Chemical Co., St. Louis) was dissolved in tartaric acid (0.02% in distilled water) and nifedipine (Bayer, Leverkusen, Germany) was dissolved in ethanol (30% in distilled water).

2.5 Calculations

Total peripheral resistance (TPR) was calculated by dividing MAP (mmHg) by CO (ml/min). CO and BF were calculated as follows:

$$\text{CO (ml/min)} = \frac{\text{Rate of withdrawal of blood (ml/min)} \times \text{total injected cpm}}{\text{cpm in withdrawn blood}}$$

$$\text{Tissue BF (ml/min)} = \frac{\text{Rate of withdrawal of blood (ml/min)} \times \text{tissue cpm}}{\text{cpm in withdrawn blood}}$$

Total amount of radioactivity (cpm) injected was obtained by subtracting the amount of radioactivity left in the tube, injecting syringe and flushing syringe from the amount of radioactivity originally present in the tube. Radioactivity (cpm) in blood was obtained by adding the amount of radioactivity in the blood sample to that in the cannula and syringe used for collecting blood. Tissue conductance was calculated by dividing blood flow by MAP. Stroke volume (SV) was determined by dividing CO by HR.

MCFP was calculated using the equation of Samar and Coleman (1978) and a value of 1/60 for the arterial-to-venous compliance ratio (18).

$$\text{MCFP} = \text{VPP} + 1/60 (\text{FAP} - \text{VPP})$$

FAP represents the final arterial pressure (mmHg) obtained within 5 s following circulatory arrest.

2.6 Statistical analysis

2.6.1 Microsphere Studies

Analysis of variance (ANOVA) with repeated measures was used to compare hemodynamic, ECG and blood flow data obtained during the first and second injections of the microspheres while ANOVA, complete random design, was used to compare data between different groups of rats. To obtain homogeneity of variances, data of blood flow and conductance changes were logarithmically-transformed prior to analysis. Duncan's multiple range test was used to compare group means. In all cases, a probability of error of less than 0.05 was preselected as the criterion for statistical significance. Results are presented as means \pm SEM.

2.6.2 MCFP Studies

Data were analyzed by analysis of variance/covariance. For multiple comparisons of data, Duncan's multiple range test was used to compare group means. In all cases a probability of error of less than 0.05 was preselected as the criterion for statistical significance.

3. RESULTS

Results obtained from the microsphere studies include the effects of calcium antagonists on systemic hemodynamics and ECG, blood flow distribution and tissue conductance. MCFP studies determined the effect of verapamil, nifedipine and flunarizine on venous tone of the rat as well as the role of the autonomic nervous system in the venous response to verapamil.

3.1 Calcium antagonist effects on systemic hemodynamics and ECG

Table 2 gives the pretreatment values for systemic hemodynamic and ECG measurements. Effects of the vehicle and calcium antagonists on these variables are presented as percent change from pretreatment values in Fig. 1. Infusions of the vehicle and all doses of the calcium antagonists caused significant decreases of MAP compared with pretreatment values. The decreases in MAP produced by both doses of verapamil and the high doses of nifedipine and flunarizine were significantly greater than that by the vehicle. CO was slightly increased by the vehicle and all doses of the calcium antagonists but the increase was only statistically significant for the low doses of nifedipine and flunarizine compared with pretreatment values. The increase in CO by the low doses of nifedipine and flunarizine were not different from that of vehicle-treated rats. TPR was significantly decreased by all doses of calcium antagonists but not the vehicle. However, the decreases of TPR of rats treated with all doses of the calcium antagonists were not statistically significant when compared with the vehicle-treated rats. The vehicle and all doses of calcium antagonists significantly decreased LVP. The decrease in LVP induced by both doses of verapamil and the high dose of nifedipine were significantly greater than that induced by vehicle. DP/dt was significantly decreased by all doses of the calcium antagonists compared with pretreatment values, but not the vehicle.

TABLE 2. Pretreatment values of systemic hemodynamic and ECG variables
for all rat groups

	A	B	C	D	E	F	G
MAP	116±6	113±6	110±3	118±5	121±4	124±6	114±2
CO	79±4	77±6	90±5	81±5	102±14	92±10	89±8
TPR	1.5±0.1	1.5±0.1	1.3±0.1	1.5±0.1	1.3±.02	1.4±0.1	1.4±0.1
LVP	149±6	138±7	144±7	148±6	153±4	154±7	146±4
dP/dt	8530±580	7560±480	8000±400	7880±280	8530±390	8470±610	7880±340
HR	371±17	344±17	366±11	351±17	389±22	401±21	388±12
SV	0.22±0.02	0.23±0.02	0.25±0.01	0.23±0.01	0.27±0.04	0.23±0.02	0.23±0.02
PR int.	49±2	49±2	46±2	51±2	46±1	47±2	49±1
QRS int.	34±1	34±1	36±1	38±1	33±1	34±1	33±.9

MAP = mean arterial pressure (mmHg); CO = cardiac output (ml/min.);
 TPR = total peripheral resistance (mmHg/ml/min); LVP = left ventricular
 pressure (mmHg); dP/dt = contractility (mmHg/sec.); HR = heart rate
 (beats/min.); SV = stroke volume (ml/beat); PR interval (msec); QRS
 interval (msec); A = vehicle control; B = verapamil, 43 µg/kg/min;
 C = verapamil, 83 µg/kg/min; D = nifedipine, 12 µg/kg/min; E = nifedipine,
 36 µg/kg/min; F = flunarizine, 174 µg/kg/min; G = flunarizine, 275 µg/kg/min.

All values represent the mean ± SEM; n = 8.

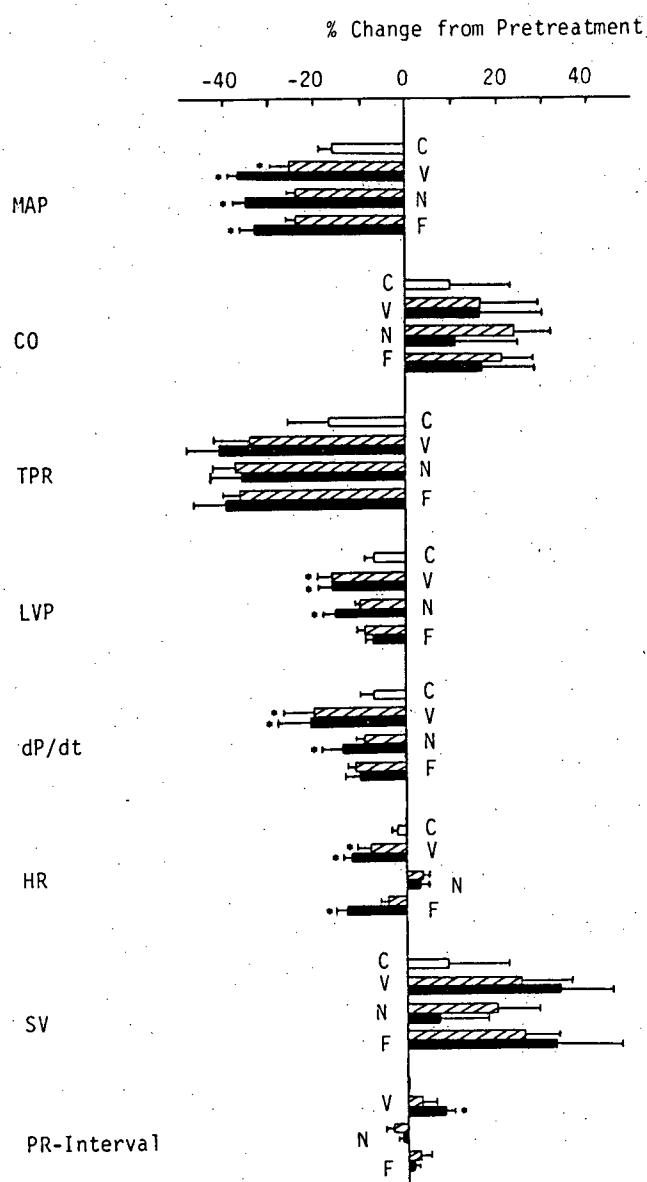


FIG 1. Effects of a low dose (crossed column) and a high dose (solid column) of verapamil (V), nifedipine (N), flunarizine (F) and vehicle (C, open column) on hemodynamics and ECG in pentobarbital-anesthetized rats. Each column represents the mean \pm SEM; $n = 8$. See Table 1 for abbreviations and units. * Denotes drug effects which are statistically significant from vehicle.

Both doses of verapamil and the high dose of nifedipine caused significantly greater decreases of dP/dt than did the vehicle.

HR was decreased in a dose-dependent manner by both verapamil and flunarizine, increased by the low dose of nifedipine, and not changed by vehicle or the high dose of nifedipine compared with pretreatment values. The changes in HR in rats treated with verapamil and flunarizine were significantly different from vehicle-treated rats. SV was increased by the low doses of verapamil and flunarizine and slightly increased by vehicle and other doses of the calcium antagonists. None of the increases of pretreatment SV by the calcium antagonists were significantly different from that in vehicle-treated rats.

The P-R interval was increased by the high dose of verapamil but not by the vehicle or other doses of the calcium antagonists. The increase of P-R interval by the high dose of verapamil was statistically significant compared with vehicle-treated rats. Neither the vehicle nor any doses of the calcium antagonists significantly altered the QRS interval.

3.2 Calcium antagonist effects on blood flow distribution

Table 3 gives pretreatment values of blood flow to various organs in the different groups of rats. The effects of the drugs, and vehicle, on blood flow are shown as percent change from pretreatment blood flow in Figs. 2 and 3. All three antagonists caused similar changes in the distribution of blood flow. Changes in blood flow to the lungs was increased by all doses of the calcium antagonists compared with vehicle-treated rats. The vehicle and all doses of calcium antagonists produced similar increases in blood flow to the heart. All drugs increased blood flow to the liver, however, these changes were statistically significant only for both doses of nifedipine and the low dose of flunarizine. All the drugs tended to decrease blood flow to the intestine, kidneys, skin, spleen and brain. The

TABLE 3. Pretreatment values of organ blood flow (ml/min) for all rat groups

	A	B	C	D	E	F	G
Lungs	1.5±0.3	1.3±0.3	2.1±0.5	1.4±0.3	1.8±0.5	1.5±0.2	1.1±0.2
Heart	4.0±0.4	4.6±0.5	5.2±0.5	4.7±0.5	4.4±0.6	4.7±0.5	4.7±0.3
Liver	1.7±0.4	1.9±0.3	2.4±0.3	1.9±0.5	1.9±0.5	2.5±0.3	2.7±0.5
Stomach	1.0±0.1	1.0±0.1	1.2±0.2	1.2±0.2	1.5±0.2	1.5±0.3	1.3±0.2
Intestine	10.7±1.1	10.1±1.0	10.9±1.0	10.8±0.6	13.8±1.2	13.1±2.8	11.2±0.8
Cae Col	3.9±0.5	4.3±0.9	4.1±0.4	4.1±0.3	5.1±0.9	4.3±0.5	4.1±0.5
Kidneys	15.6±1.2	15.3±1.2	18.5±1.2	15.3±1.0	20.9±2.3	18.2±2.2	19.0±1.9
Spleen	1.4±0.3	1.3±0.2	1.6±0.3	1.1±0.1	1.6±0.2	1.4±0.2	1.9±0.4
Muscle	1.5±0.3	1.6±0.1	1.9±0.2	1.6±0.1	1.9±0.3	1.9±0.4	1.8±0.2
Skin	2.2±0.5	2.2±0.3	2.6±0.4	2.5±0.3	3.3±0.8	2.6±0.5	2.4±0.4
Testis	1.3±0.1	1.3±0.1	1.3±0.1	1.4±0.1	1.6±0.1	1.3±0.2	1.4±0.1
Brain	1.3±0.2	1.5±0.2	1.7±0.2	1.4±0.1	1.7±0.2	1.3±0.2	1.5±0.2

A = vehicle control; B = verapamil, 43 µg/kg/min; C = verapamil, 83 µg/kg/min; D = nifedipine, 12 µg/kg/min; E = nifedipine, 36 µg/kg/min; F = flunarizine, 174 µg/kg/min; G = flunarizine, 275 µg/kg/min; Cae Col = caecum + colon.

All blood flows are calculated for whole organs, except for skin and muscle which are 30 g each.

All values represent the mean ± SEM; n = 8.

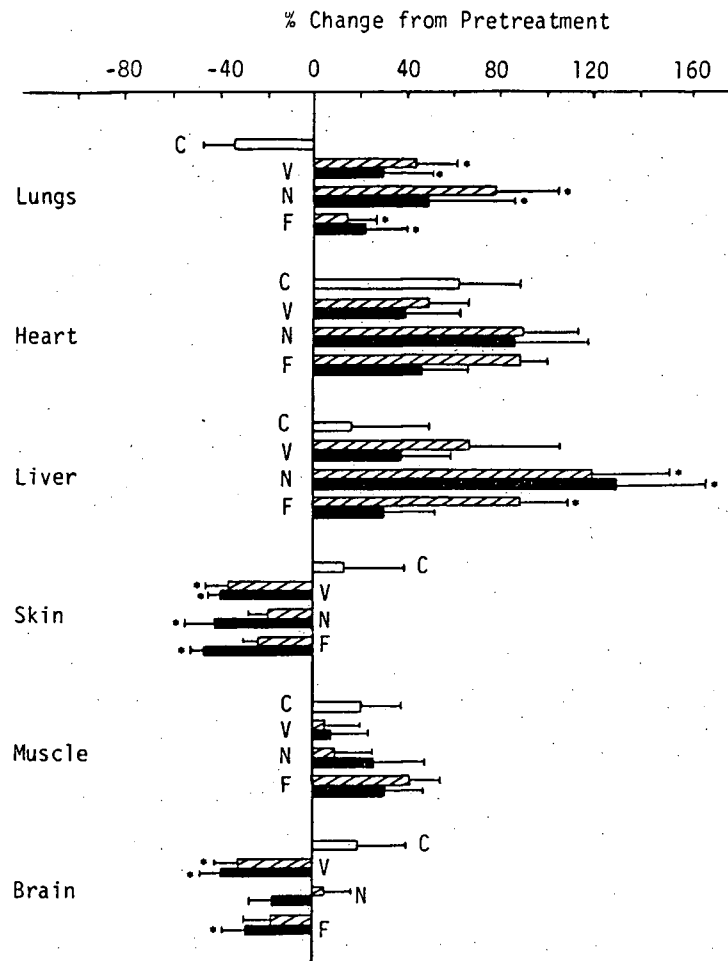


FIG 2. Effects of a low dose (crossed column) and a high dose (solid column) of verapamil (V), nifedipine (N), flunarizine (F) and vehicle (C, open column) on organ blood flow in pentobarbital-anesthetized rats. Each column represents blood flow (mean \pm SEM; $n = 8$) to whole organs, except for skin (30 g) and muscle (30 g). * Denotes drug effects which are statistically significant from vehicle.

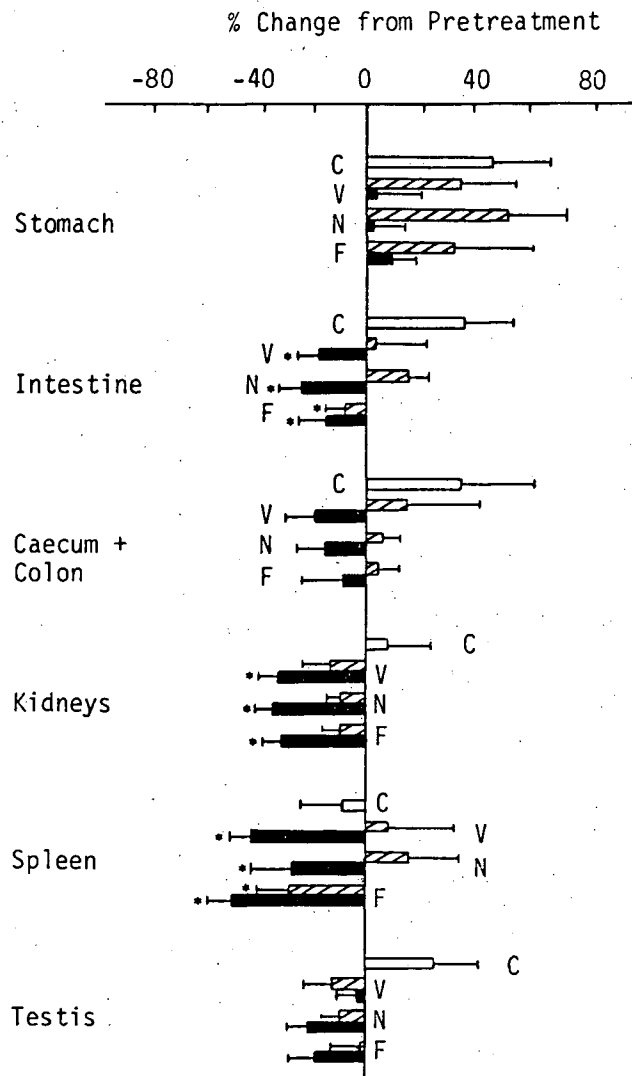


FIG 3. Effects of a low dose (crossed column) and a high dose (solid column) of verapamil (V), nifedipine (N), flunarizine (F) and vehicle (C, open column) on organ blood flow in pentobarbital-anesthetized rats. Each column represents blood flow (mean \pm SEM; $n = 8$) to whole organs. * Denotes drug effects which are statistically significant from vehicle.

decreases in blood flow to the intestine and spleen were statistically significant for the high doses of verapamil and nifedipine, and both doses of flunarizine compared with vehicle-treated rats. Renal blood flow was decreased by high doses of all calcium antagonists, skin blood flow was significantly decreased by both doses of verapamil and the high dose of nifedipine and flunarizine, brain blood flow was decreased by both doses of verapamil and the high dose of flunarizine when compared with the changes in vehicle-treated rats. Change in blood flow to the other organs were not affected by any drug compared with the vehicle.

3.3 Calcium antagonist effects on tissue conductance

Table 4 gives the pretreatment values of conductance for the various organs while effects of drugs and vehicle are shown in Figs. 4 and 5 as percent change from pretreatment conductance. Conductance in the lungs was increased by all calcium antagonists as compared with the vehicle. Conductances in the liver were significantly increased by all drugs compared with the vehicle except for the high dose of flunarizine. Vehicle and calcium antagonists caused similar increases in conductances in the heart, stomach, muscle, caecum and colon. Heart and muscle conductance increases tended to be greater for the high doses of the calcium antagonists than for the vehicle. Conductances in all other organs were not significantly affected by the calcium antagonists, as compared with the vehicle, although conductances in the intestine, kidneys and skin did show a tendency to decrease compared with the vehicle control.

3.4 Calcium antagonist effects on MAP, HR and MCFP

Table 5 gives the control values of MAP, HR and MCFP for the 9 treatment groups. Results of the dose-response curve studies for the calcium antagonists and vehicle are presented as percent of pretreatment values in Figs. 6-8. Nifedipine (Fig. 6) was found to decrease MAP and increase HR in

TABLE 4. Pretreatment values of organ conductances (ml/min/mmHg $\times 10^{-2}$)
for all rat groups

	A	B	C	D	E	F	G
Lungs	1.4 \pm 0.3	1.1 \pm 0.3	1.9 \pm 0.5	1.2 \pm 0.2	1.5 \pm 0.4	1.1 \pm 0.2	0.9 \pm 0.2
Heart	3.4 \pm 0.2	4.2 \pm 0.3	4.8 \pm 0.1	4.0 \pm 0.4	3.6 \pm 0.5	3.7 \pm 0.3	4.2 \pm 0.3
Liver	1.5 \pm 0.3	1.7 \pm 0.3	2.2 \pm 0.4	1.6 \pm 0.3	1.6 \pm 0.4	2.0 \pm 0.2	2.4 \pm 0.5
Stomach	0.8 \pm 0.1	0.9 \pm 0.1	1.1 \pm 0.1	1.0 \pm 0.1	1.2 \pm 0.2	1.2 \pm 0.2	1.1 \pm 0.2
Intestine	9.2 \pm 0.9	9.2 \pm 1.2	10.1 \pm 1.0	9.3 \pm 0.6	11.6 \pm 1.2	10.5 \pm 1.8	9.9 \pm 0.8
Cae Col	3.5 \pm 0.5	3.8 \pm 0.7	3.7 \pm 0.3	3.6 \pm 0.3	4.2 \pm 0.7	3.4 \pm 0.3	3.7 \pm 0.5
Kidneys	13.6 \pm 1.1	13.8 \pm 1.2	16.8 \pm 1.0	13.2 \pm 0.9	17.4 \pm 1.8	14.7 \pm 1.3	16.8 \pm 1.7
Spleen	1.3 \pm 0.3	1.0 \pm 0.2	1.5 \pm 0.3	0.9 \pm 0.1	1.3 \pm 0.2	1.2 \pm 0.1	1.7 \pm 0.4
Muscle	1.3 \pm 0.2	1.5 \pm 0.2	1.8 \pm 0.2	1.3 \pm 0.1	1.6 \pm 0.3	1.5 \pm 0.3	1.6 \pm 0.2
Skin	1.9 \pm 0.4	2.0 \pm 0.3	2.4 \pm 0.3	2.1 \pm 0.2	2.7 \pm 0.6	2.0 \pm 0.3	2.1 \pm 0.2
Testis	1.1 \pm 0.1	1.2 \pm 0.1	1.2 \pm 0.1	1.2 \pm 0.1	1.3 \pm 0.1	1.0 \pm 0.1	1.2 \pm 0.1
Brain	1.2 \pm 0.2	1.3 \pm 0.2	1.6 \pm 0.1	1.2 \pm 0.2	1.4 \pm 0.2	1.1 \pm 0.2	1.4 \pm 0.2

A = vehicle control; B = verapamil, 43 μ g/kg/min; C = verapamil, 83 μ g/kg/min; D = nifedipine, 12 μ g/kg/min; E = nifedipine, 36 μ g/kg/min; F = flunarizine, 174 μ g/kg/min; G = flunarizine, 275 μ g/kg/min; Cae Col. = caecum + colon.

All conductances are calculated for whole organs, except for skin and muscle which are calculated for 30 g of tissue.

All values are the mean \pm SEM; n = 8.

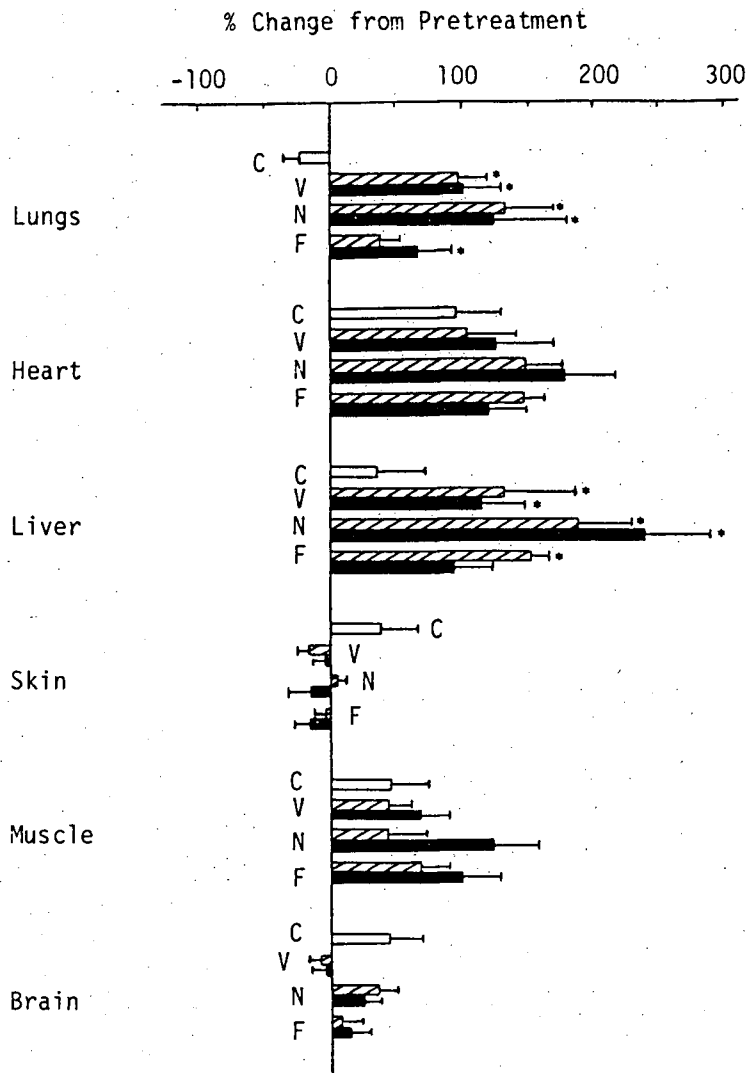


FIG 4. Effects of a low dose (crossed column) and a high dose (solid column) of verapamil (V), nifedipine (N), flunarizine (F) and vehicle (C, open column) on arterial conductance in pentobarbital-anesthetized rats. Each column represents conductance (mean \pm SEM; $n = 8$) to whole organs, except for skin (30 g) and muscle (30 g). *Denotes drug effects which are statistically significant from vehicle.

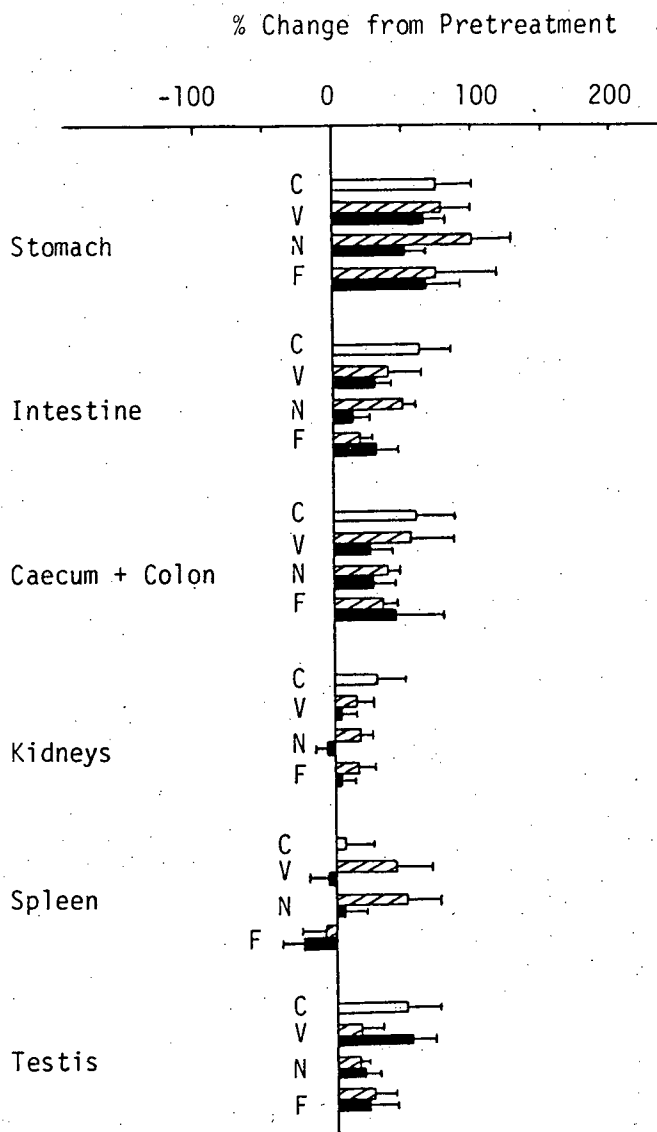


FIG 5. Effects of a low dose (crossed column) and a high dose (solid column) of verapamil (V), nifedipine (N), flunarizine (F) and vehicle (C, open column) on arterial conductance in pentobarbital-anesthetized rats. Each column represents conductance (mean \pm SEM; $n = 8$) to whole organs. * Denotes drug effects which are statistically significant from vehicle.

TABLE 5. Baseline values of MAP, HR and MCFP for all rat groups

Group	Control values		
	MAP	HR	MCFP
I) Ethanol	115 \pm 5	387 \pm 9	6.1 \pm 0.2
II) Nifedipine	109 \pm 4	376 \pm 17	6.1 \pm 0.1
III) Saline	104 \pm 3	373 \pm 20	5.5 \pm 0.2
IV) Verapamil	105 \pm 2	366 \pm 12	5.9 \pm 0.2
V) Tartaric acid	113 \pm 4	383 \pm 13	5.8 \pm 0.2
VI) Flunarizine	108 \pm 6	393 \pm 7	5.9 \pm 0.2
VII) Before hexamethonium	114 \pm 5	378 \pm 13	5.9 \pm 0.1
After hexamethonium	103 \pm 3	374 \pm 11	4.9 \pm 0.1
VIII) Before hexamethonium	113 \pm 5	388 \pm 3	5.7 \pm 0.1
After hexamethonium	104 \pm 2	383 \pm 10	5.1 \pm 0.2

MAP (mmHG) = baseline values for mean arterial pressure; HR (beats/min) = heart rate; MCFP (mmHG) = mean circulatory filling pressure for all rat groups.

Each group represents the mean \pm SEM; n = 6.

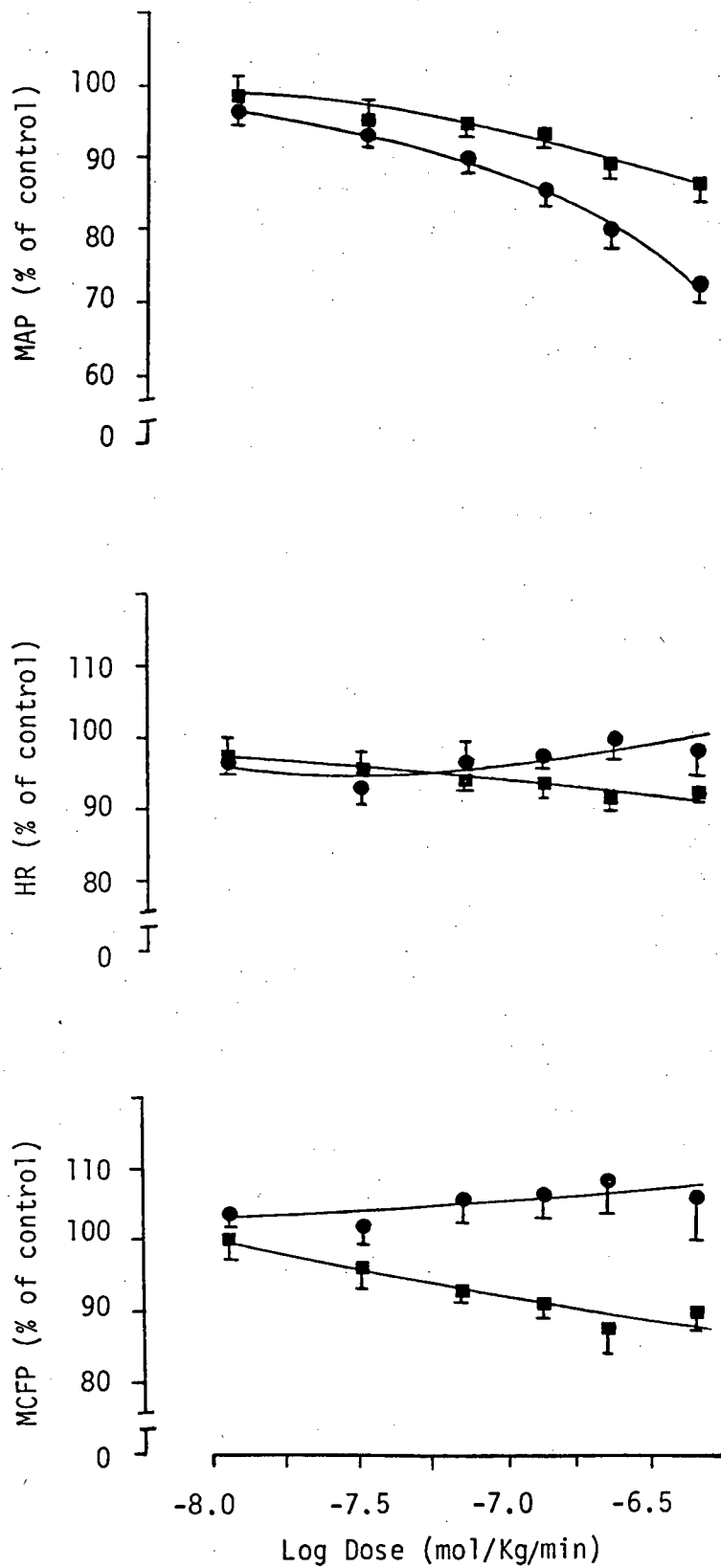


FIG. 6. Dose-response curves for the effect of nifedipine (●) and alcohol vehicle (■) on mean arterial pressure (MAP), Heart rate (HR) and mean circulatory filling pressure (MCFP) represented as % of control values. Each point represents the mean \pm SEM; $n = 6$. Data is plotted to match the dose of drug given with its corresponding vehicle infusion rate.

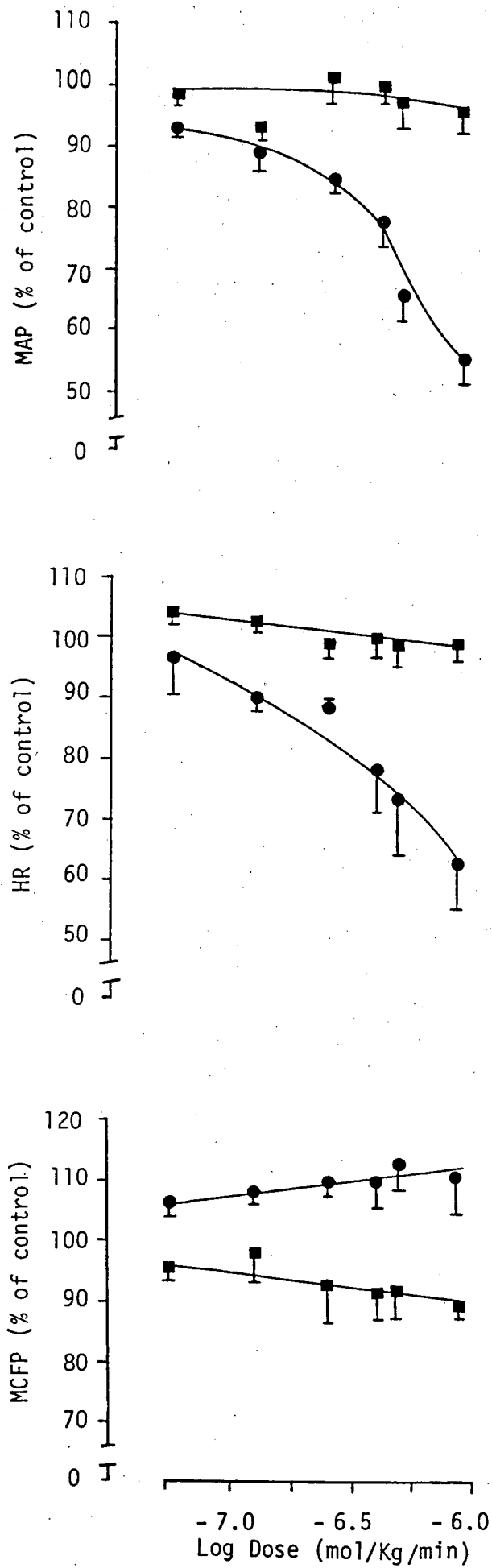


FIG. 7. Dose-response curves for the effect of verapamil (●) and saline (■) on mean arterial pressure (MAP), heart Rate (HR) and mean circulatory filling pressure (MCFP) represented as % of control values. Each point represents the mean \pm SEM; $n = 6$. Data is plotted to match the dose of drug given with its corresponding vehicle infusion rate.

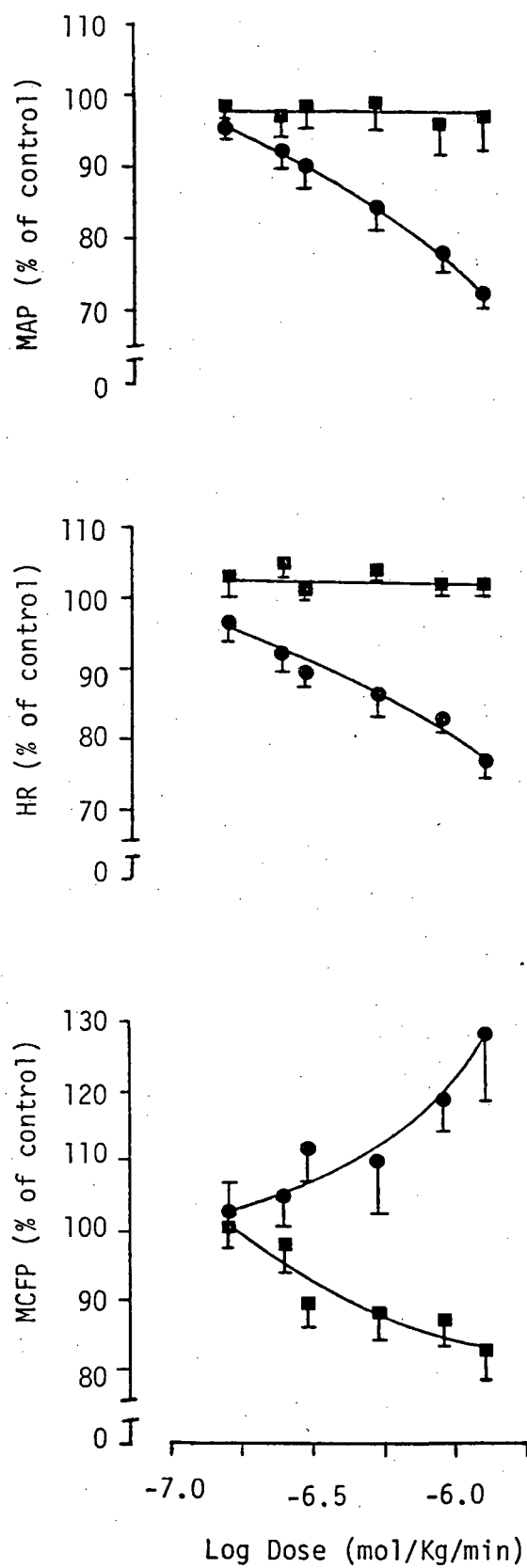


FIG. 8. Dose response curves for the effect of flunarizine (●) and tartaric acid (■) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) represented as % of control values. Each point represents the mean \pm SEM; $n = 6$. Data is plotted to match the dose of drug given with its corresponding vehicle infusion rate.

a statistically significant manner compared with ethanol-treated rats. MCFP was significantly increased by nifedipine compared with control rats. Verapamil (Fig. 7) was found to decrease both MAP and HR in a dose dependent manner and increase MCFP compared with the vehicle control. Flunarizine significantly decreased MAP and HR and increased MCFP compared with tartaric acid-treated rats (Fig. 8). Fig. 9 illustrates the effect of time on the MAP, HR and MCFP of the conscious rat. HR remains the same for the time course of the experiment while MAP decreases slightly after 100 min. MCFP was significantly decreased after 75 min. The reduction in MCFP caused by the vehicles and in the time control as a function of time are compared in Fig. 10. In all cases, MCFP is significantly reduced after 75 min.

3.5 Role of the autonomic nervous system on MCFP during verapamil administration

MAP and MCFP were significantly decreased by hexamethonium in both the hexamethonium and hexamethonium + verapamil groups (Table 5). These decreases were maintained for the duration of the experiment. HR was not affected by hexamethonium throughout the course of the experiment. Responses to verapamil or hexamethonium are presented as percent of the post-equilibration value with hexamethonium (Fig. 11).

MAP and HR were significantly decreased by verapamil compared with hexamethonium-treated control rats. MCFP was not significantly altered by verapamil compared with the hexamethonium-treated rats but the highest dose of verapamil did significantly lower the MCFP compared with its corresponding control measurement.

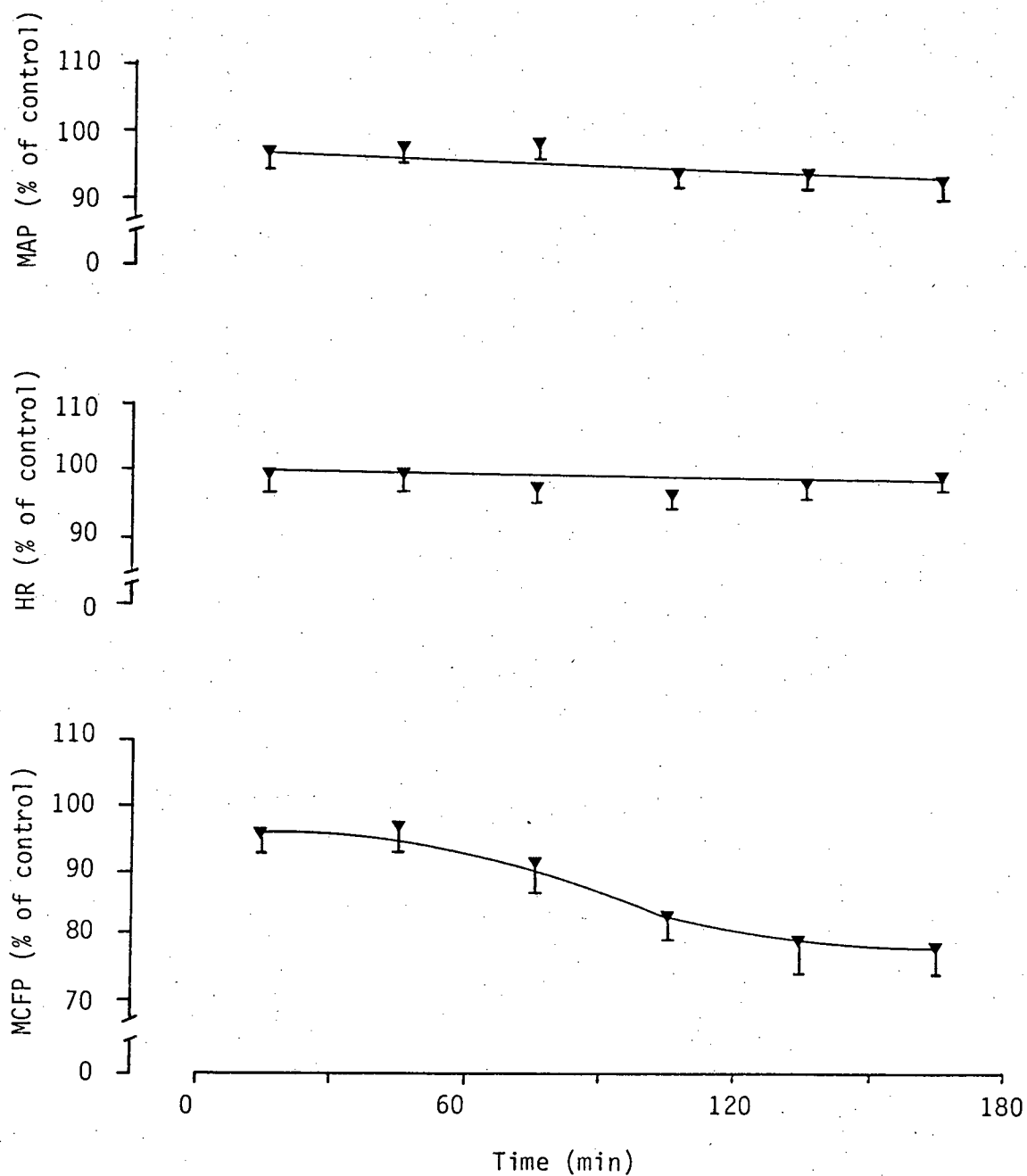


FIG. 9. The effect of time on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) represented as % of control values. Each point represents the mean \pm SEM; $n = 6$.

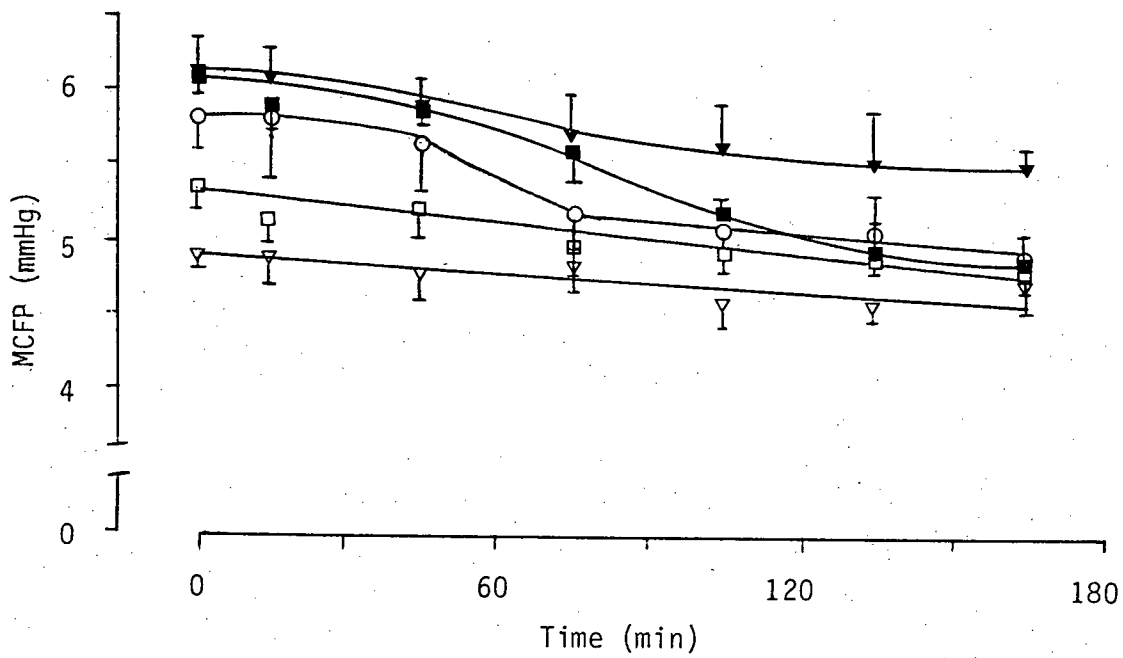


FIG. 10. The effect of ethanol (▼), saline (□), tartaric acid (○), hexamethonium (▽) and time (■) on mean circulatory filling pressure as a function of time. Each point represents the mean \pm SEM; $n = 6$.

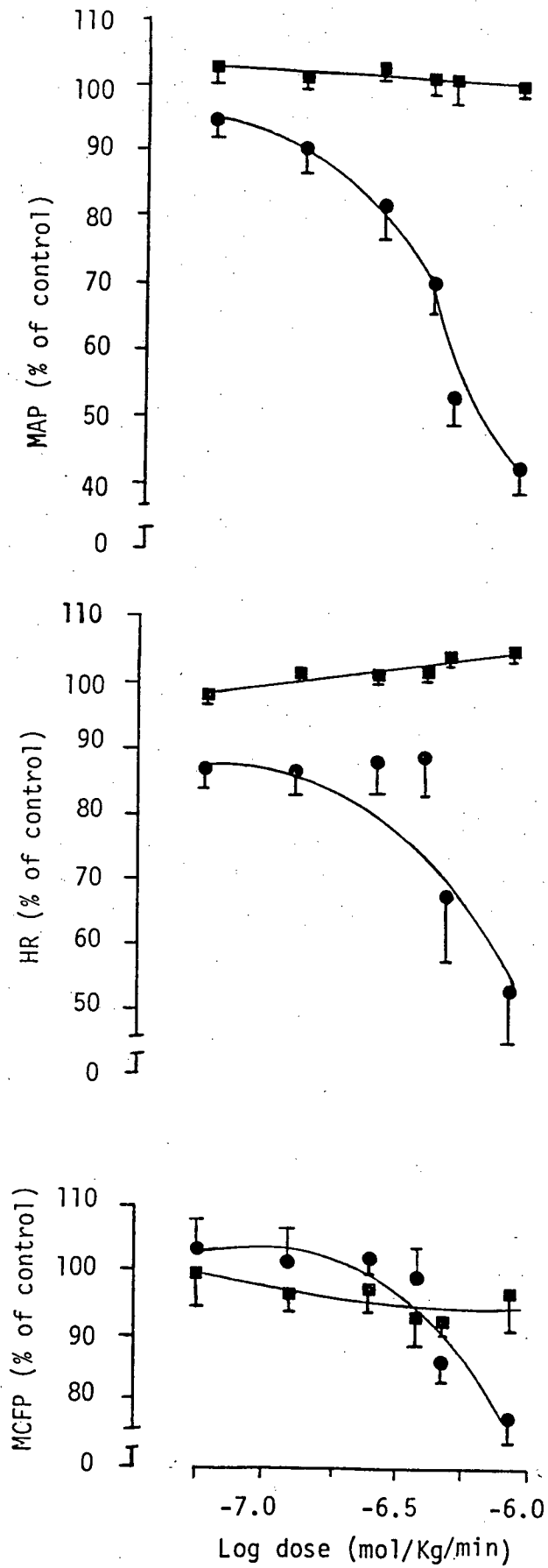


FIG. 11. Dose response curves for the effect of hexamethonium + verapamil (●) and hexamethonium (■) on the mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) represented as % of control values after equilibration with hexamethonium. Each point represents the mean \pm SEM; n = 6. Data is plotted to match the dose of drug given with its corresponding vehicle infusion rate.

4. DISCUSSION

These studies were performed to evaluate the validity of Spedding's classification of calcium antagonists with respect to their effect on the cardiovascular system of the intact rat. My findings will be discussed in relation to Spedding's classification scheme and previous studies of calcium antagonist actions.

The profile of systemic and regional hemodynamic actions were similar for the three calcium antagonists with nifedipine being the most potent, on a weight basis, and flunarizine the least. Differences in their profiles were most marked in terms of their effects on the heart. Cardiac contractility, as measured by dP/dt , was slightly but not significantly decreased by nifedipine and flunarizine and significantly decreased by verapamil. HR was reduced by verapamil and flunarizine but increased by nifedipine. Similarly, nifedipine and flunarizine had no effect on the P-R interval while verapamil lengthened the P-R interval at the high dose. Thus, at the doses given, flunarizine had more depressant effect on the SA than on the AV node, verapamil affected both while nifedipine affected neither. These effects on the heart clearly differentiate the three subclasses of calcium antagonists proposed by Spedding (1985) and also indicate that nifedipine and flunarizine are more vascular selective than verapamil.

Similar ECG results to ours were obtained in the pithed rat preparation using verapamil, diltiazem, nifedipine and cinnarizine as representative drugs from the various subclasses of calcium antagonists (Spedding 1982). Other investigators have shown that dihydropyridines increased HR and decreased LVP in the conscious rat preparation (Kanda and Flaim 1984; Drexler et al. 1985a) while diltiazem, a proposed class 2 compound, dose-dependently decreased both HR and LVP (Flaim and Zelis 1982). Flunarizine

was shown to be ineffective at inhibiting the contractile force of isolated cat papillary muscle (van Neuten et al. 1978). This is in agreement with our in vivo results which indicate that flunarazine had little effect on myocardial contractility.

In spite of a decrease in cardiac contractility, as measured by dP/dt , CO and SV were slightly increased by all agents although the increases were not statistically significant compared with vehicle-treated rats. This finding is in agreement with results using conscious rats which showed that CO and SV were not altered significantly by nisoldipine (Drexler et al. 1985a), nifedipine (Kanda and Flaim 1984) or diltiazem (Flaim and Zelis 1982). There was a tendency for CO to be increased in chloralose-anesthetized open-chest cats by PY 108-068, nicardipine, verapamil, diltiazem (Hof 1983) and nifedipine (Hof et al. 1982).

In contrast to systemic hemodynamics, the three calcium antagonists caused similar distribution of blood flow, and alterations of vascular conductances. Blood flow was increased to the lungs, heart and liver but decreased to the intestine, kidneys, skin, spleen and brain. The increase in hepatic arterial flow may be a regulatory phenomenon secondary to a decrease of portal venous flow. It has been shown that a decrease in portal venous flow leads to an increase in hepatic arterial flow such that total hepatic blood flow remains constant (Lautt 1980). Similar increases in liver blood flow have been reported in conscious rats treated with nifedipine (Kanda and Flaim 1984). Blood flow to the heart was increased, but the increase was not significantly greater than that seen in the vehicle-treated rats possibly because the large coronary vasodilator effect of the vehicle had almost maximally dilated the coronary arteries. Other investigators have shown that calcium antagonists cause an increase in coronary blood flow (Flaim and Zelis 1982; Hof et al. 1982; Hof 1983; Hof 1984; Kanda and

Flaim 1984; Drexler et al. 1985a; Hof et al. 1985).

Various calcium antagonists were found to have no effect on renal blood flow in open-chest chloralose-urethane-anesthetized cats (Hof et al. 1982; Hof 1983) and conscious rats (Flaim and Zelis 1982; Kanda and Flaim 1984; Drexler et al. 1985a). In contrast, renal blood flow was decreased by the calcium antagonist PY 108-068 in pentobarbital-anesthetized rabbits (Hof 1985), by flunarizine in pentobarbital-anesthetized rats (Gulati et al. 1983) and by nimodipine in pentobarbitone-anesthetized pigs (Duncker et al. 1986). Thus, renal blood flow was not affected by the calcium antagonists in chloralose-urethane anesthetized or conscious animals but was decreased in the present study and in other barbiturate anesthetized animals. Possibly a combination of barbiturate anesthesia and calcium antagonists hinder the ability of the kidney to autoregulate its blood flow. Renal blood flow autoregulation in anesthetized dogs has been shown to be reduced by diltiazem (Ogawa and Ono 1986a), and by verapamil (Ogawa and Ono 1986b) at a dose similar to the high dose used in this experiment. Furthermore in vitro experiments have shown that barbiturate anesthesia has depressant effects on contractile function of vascular smooth muscle, possibly due to its actions on movement or translocation of calcium (Altura and Altura 1975a,b). The reduction of blood flow to the brain upon administration of the calcium antagonists in the present experiments may also have been due to interference with autoregulation. It is well known that the renal and cerebral circulations show the most pronounced autoregulation (Shepherd and Vanhoutte 1979).

Reduction of blood flow to the skin and spleen is in agreement with previous findings using the conscious rat preparation (Kanda and Flaim 1984; Drexler et al. 1985). At the high doses of the calcium antagonists blood flow to the gastrointestinal organs was decreased probably due to

reflex vasoconstriction. This was not seen in previous experiments using the conscious rat preparation (Kanda and Flaim 1984) though the doses of nifedipine used did not lower the MAP to as great an extent as the high dose used in the present experiment.

The distribution of blood flow caused by the high dose of verapamil, nifedipine and flunarizine was similar to that caused by nimodipine in inactin-anesthetized rats (McCann et al. 1986). Verapamil and flunarizine, on the other hand, had little effect on blood flow distribution in inactin-anesthetized rats. However, nimodipine lowered MAP far more than flunarizine or verapamil. Obviously, calcium antagonist induced changes in blood flow distribution are dependent on the degree of hypotension caused by the drug.

Conductance more accurately reflects the active changes in vascular tone. When our blood flow results were corrected for changes in MAP, it was found that conductance in lungs, liver, heart and skeletal muscle were all increased by the three drugs, although the changes were only significant for the lungs and liver. Though conductances to the skin, intestine, spleen and kidneys were not significantly changed by the calcium antagonists, all showed a tendency to decrease in a dose-dependent manner possibly as a result of the release of vasoconstrictor agents following a decrease of MAP. Decreases in blood pressure have been shown to cause reflex activation of vasopressor systems including the sympathetic nervous, renin-angiotensin (Keeton and Campbell 1981) and vasopressin (Share 1976) systems. Verapamil infusion in conscious sheep (Mzail and Noble 1986) and nifedipine infusion into the renal artery of anaesthetized dogs (Imagawa et al. 1986) has been shown to increase plasma renin activity. In anesthetized rats, endogenously released vasopressin had a prominent vasoconstrictor influence in skin and stomach while angiotensin II caused the greatest vasoconstrictor influence

in skin and kidneys (Pang 1983b). The sympathetic nervous system, on the other hand, had the greatest vasoconstrictor influence in lungs and skeletal muscle (Tabrizchi and Pang 1987). Thus blood flow distribution following administration of calcium antagonists would appear to be a consequence of directly-induced vasodilatation and reflex vasoconstriction, mediated by various vasoconstrictor agents released as a result of the reduction in MAP. Variation in the dose of drug, species of animal used, the presence of an anesthetic agent and the condition of the animal may all affect the vascular response to a calcium antagonist.

Calcium antagonist administration increased the CO of pentobarbital-anesthetized rats (Fig. 1), anesthetized cats (Hof et al. 1982), anesthetized rabbits (Hof 1985), anesthetized dogs (Gross et al. 1979) and conscious rats (Flaim and Zelis 1982). CO of patients with hypertension (Elliott 1987), angina pectoris (Soward et al. 1986) and congestive heart failure (Matsumoto et al. 1980) was also increased by calcium antagonists. It is believed that the increased CO is due to the calcium antagonist induced decrease in afterload of the heart. However, verapamil decreases the contractility of the heart as well as the peripheral resistance yet CO is still increased. Possibly a reflex increase in venous return contributes to the calcium antagonist induced increase in CO. Experiments were thus designed to evaluate calcium antagonist actions on the venous side of the circulation in conscious rats.

The concept of venous return being an important determinant in the maintenance of cardiac output was first postulated by Starling (1897). Of primary importance in the control of venous return is the mean circulatory filling pressure (MCFP), an equilibrium pressure that would occur throughout the circulation if the circulation is arrested and all the pressures in the circulatory system are rapidly made to equilibrate (Guyton 1955). Theoreti-

cally MCFP has been shown to be a measure of the ratio of blood volume to the overall compliance of the circulatory system (Grodins 1959). Since venous compliance is many times greater than arterial compliance (Guyton 1973; Samar and Coleman 1978; Yamamoto et al. 1980), MCFP is inversely related to venous compliance. Thus, if the volume of the system remains constant, MCFP reflects predominantly venous tone. It has been shown experimentally that MCFP is the driving force for returning blood to the heart and, the difference between MCFP and right atrial pressure is proportional to cardiac output (Guyton 1955).

The conscious rat preparation of Yamamoto (1980) was used to determine MCFP. This preparation has the advantage of concurrently investigating the effect of drugs on MAP and total body venous tone in the absence of anaesthesia and surgery. Pentobarbital-anaesthesia has been shown to lower MCFP in dogs (Hirakawa 1975). The calcium antagonists tested all increased MCFP when compared with their respective vehicle controls. By the interpolation of the dose-response curves for the various drugs, it was found that MCFP was increased to 128%, 110% and 107 % of pretreatment values by doses of flunarizine, verapamil and nifedipine, respectively, which caused a 27% decrease in control MAP. Since calcium antagonists generally relax vascular smooth muscle, the increase in MCFP induced by these drugs was likely a result of reflex activation of the sympathetic nervous system. Nifedipine was shown to cause an increase in CO in anesthetized cats accompanied by hepatic venoconstriction and a decrease in hepatic blood volume due to reflex mechanisms (Seaman and Greenway 1983; Segstro et al. 1986). In contrast to our results, a single dose of verapamil, nifedipine and nicardipine had little effect on the MCFP of anesthetized open-chest dogs whereas diltiazem was found to decrease MCFP (Ito 1984). However, the experimental conditions in the study by Ito and Hirakawa were clearly very

different from ours.

Edema is one of the frequent side effects following the administration of calcium antagonists to patients (Elliott 1987). Increased fluid transudation and edema formation suggest that calcium antagonists decrease the ratio of pre to post-capillary resistance. This is consistent with our results of decreased arteriolar tone but increased venous tone following the administration of calcium antagonists.

In this study, HR was found to be decreased by flunarizine and verapamil but increased by nifedipine. Our results on HR with representative drugs from various subclasses of calcium antagonists are consistent with results in pithed rats (Spedding 1982) and pentobarbital-anesthetized rats (Fig. 1). Verapamil was found to dose-dependently decrease HR and induce partial AV block at high doses as shown by the sudden decreases in HR (Fig. 2). Flunarizine appeared to have a major effect on the SA node as HR was reduced dose-dependently without signs of AV-block. Nifedipine, on the other hand, caused a dose-dependent increase in HR which had usually abated by the time MCFP measurements were made 15 min. after commencing the infusion of this drug. Nifedipine was found to cause a reflex increase in heart rate in anesthetized dogs (Gross et al. 1979) and rats (Nordlander 1985) which subsequently abated after administration for more than 20 min.

The effect of time on MCFP was determined as all vehicles caused a significant reduction in MCFP after 75 min. Fig. 9 shows MAP and HR do not change over the time course of the experiment but MCFP is significantly reduced after 75 min. Thus, reduction of MCFP by all vehicles would appear to be due to a time effect. Reduction of MCFP was not seen in previous studies with respect to time (Yamamoto et al. 1980) or infusion of saline (Tabrizchi and Pang 1986) over a time period of 100 min though it was

reduced with a saline infusion after 120 min. (Tabrizchi and Pang 1986). Possibly, because MCFP was measured much more frequently in these experiments, a higher level of sympathetic nerve activity resulted and maintained the MCFP at a higher level. This explanation is supported by the observation that MCFP was lowered by time and vehicles, except ethanol, to the same level as the hexamethonium control (post-equilibration, Fig. 10). This level of MCFP was maintained in hexamethonium control rats for the duration of the experiment (3 hr). The ethanol control caused a reduction in MAP, possibly resulting in the reflex increase in venous tone which maintained MCFP at a higher level than the other vehicles.

Further experiments were conducted to investigate whether the increase in MCFP induced by verapamil was due to reflex activation of the autonomic nervous system. The administration of hexamethonium was found to decrease MAP by 10% and MCFP by 15%, but had no effect on HR. Following ganglionic blockade, the highest two doses of verapamil caused a greater decrease in MAP compared to rats not subjected to treatment with hexamethonium. HR was decreased by the lowest dose of verapamil by 10% and maintained at this level until a dose of 4.7×10^{-7} mol/kg/min when HR was decreased rapidly, probably as a result of depressed AV conduction. MCFP was significantly decreased at the highest dose of verapamil in the presence of hexamethonium as opposed to the increase seen when no ganglion blocker was present. Our results indicate that reflex activation of the autonomic nervous system did play a role in maintenance of MCFP in conscious animals following administration of the calcium antagonist verapamil.

4.1 Summary

We have found that the calcium antagonists verapamil, nifedipine and flunarizine do exhibit differential effects on chronotropy, inotropy and dromotropy of the pentobarbital-anesthetized rat. Verapamil reduced the

contractility and HR while lengthening the PR-interval. Nifedipine increased HR, had no effect on PR-interval and caused a decrease in contractility only at high doses. Flunarizine decreased HR with no effect on the other two parameters. Thus, Spedding's classification of calcium antagonists appears to be valid with respect to their actions on the heart. However, their effect on arterial smooth muscle are similar. Blood flow to the heart, lungs and liver increased while flow to the GI organs, kidneys, skin, spleen and brain decreased. Heart, lung, liver and skeletal muscle conductances increased while tissue conductance in the kidneys, spleen, skin and GI organs decreased upon administration of the calcium antagonists. This demonstrated 1) that the calcium antagonists are not general arteriolar dilators and 2) doses of the calcium antagonists which lower MAP to the same extent similarly affect the distribution of blood flow.

Results from conscious rat experiments confirmed that verapamil, nifedipine and flunarizine produced different effects on heart rate. All three drugs increased the total body venous tone of the conscious rat, as measured by MCFP, indicating that the increase of CO seen upon administration of a calcium antagonist may be due to an increase in venous tone of the animal with subsequent increase in venous return. Administration of hexamethonium to block the autonomic nervous system indicated that the increase in venous tone elicited by verapamil was due to reflex activation of the autonomic nervous system.

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