THE ROLE OF TYPE-2 SEROTONIN RECEPTORS IN MORPHINE-PRODUCED
ANALGESIA

By

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ABSTRACT

It is generally accepted that the neurotransmitter, serotonin mediates morphine-produced analgesia, however, it is not clear whether this mediation occurs at brain or spinal cord serotonin receptors. An issue that has not often been considered is the differential role that serotonin receptor types may play in morphine-produced analgesia. Paul and Phillips (1986) observed that pirenperone, a serotonin antagonist with a preferential affinity for the S2 receptor, attenuates morphine-produced analgesia. This result is particularly interesting because there are reportedly no S2 receptors in the spinal cord. The purposes of this dissertation were: to confirm the finding of Paul and Phillips, to localize the S2 receptors that mediate the anti-analgesic effect of pirenperone, and to test the hypothesis that pirenperone may exert its anti-analgesic effect through alpha-adrenergic receptors.

In each of five experiments, tail-flick latencies (the time that it takes for each rat to withdraw its tail from a 52 C water bath) were measured 0, 30, 60, 90, and 120 min after drug injection. In Experiment 1, the analgesic effect of 10 mg/kg of morphine sulphate was challenged with 0.08, 0.16, and 0.24 mg/kg of pirenperone. Each dose of pirenperone attenuated morphine-produced analgesia. Moreover, each dose of pirenperone produced hyperalgesia in rats receiving no morphine. In Experiment 2, morphine-produced analgesia was challenged with 1, 3, and 10 mg/kg of ketanserin HCl. Only the very high 10 mg/kg dose of

ketanserin significantly attenuated morphine-produced analgesia.
Because ketanserin is pharmacologically similar to pirenperone
but does not readily enter the central nervous system, this
result indicates that central S2 receptors mediate the anti-
analgesic effect of pirenperone and ketanserin. A third
experiment demonstrated that 10 mg/kg of ketanserin did not block
the analgesia produced by ketamine. Ketamine is thought to
produce analgesia by a different mechanism than morphine. Thus,
the attenuation of analgesia by S2 receptor blockers is not a
general phenomenon, and it may be specific to morphine-produced
analgesia and other analgesics that act on this system.
Experiment 4 was designed to assess whether it is S2 receptors in
the brain or in the spinal cord that mediate the anti-analgesic
effect of S2 receptor blockade. The analgesic effect of morphine
on tail-flick latencies was challenged with pirenperone in rats
with spinal cords transected at the lower thoracic level and in
sham-surgery comparison rats. Pirenperone attenuated morphine-
produced analgesia in the sham-surgery group but not in the rats
with transected spinal cords. These results indicate that brain
S2 receptors mediate the attenuation of morphine-produced
analgesia by pirenperone. In the fifth and final experiment,
morphine-produced analgesia was challenged with 10 mg/kg of
LY53857. LY53857 is an S2 antagonist which unlike pirenperone
and ketanserin has no action at alpha-adrenergic receptors. Like
pirenperone and ketanserin, LY53857 attenuated morphine-produced
analgesia. This result supports the view that S2 receptors

mediate the anti-analgesic effects of pirenperone and ketanserin.

Together, the results of these five experiments indicate that S2 receptors in the brain are important for opioid=mediated analgesia. This conclusion challenges the widely held view that only spinal cord serotonin receptors mediate morphine-produced analgesia.

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I. INTRODUCTION

One of the most important discoveries of modern neuroscience is that there are neural circuits whose primary function is to suppress the effects of normally painful stimuli. These "analgesia circuits" have been shown to be activated by opiates such as morphine, but the fact that several serotonin antagonists attenuate the analgesic effects of opiates suggests that serotonin is also involved. Further support for this notion comes from the recent finding that serotonin agonists can by themselves produce analgesia similar to that produced by morphine.

Recently, it has been shown that there are two types of serotonin receptors. The distinction between these two types of serotonin receptors is made on the basis of their relative affinity for serotonin and the serotonin antagonist, spiperone. Some serotonin receptors, called S1 receptors, have a high affinity for serotonin and a low affinity for spiperone; whereas, other serotonin receptors, now called S2 receptors, have a much lower affinity for serotonin, and a much higher affinity for spiperone than do S1 receptors.

This dissertation focuses on the contribution of S2 receptors to morphine-produced analgesia. The general introduction of this topic is divided into five major sections: The first describes several methods of measuring nociception in laboratory animals; the second comprises a description of the neural circuits that are presumed to mediate morphine analgesia;

the third focuses on the evidence that serotonin is involved in these circuits; the fourth reviews the evidence that there are two different classes of serotonin receptors; the fifth describes the study (Paul & Phillips, 1986) from which this series of experiments evolved; and the sixth, and final, section of the Introduction is a statement of the general rationale.

1. Methods of Measuring Pain Perception in Laboratory Animals

For ethical and practical reasons, the neural substrates of pain perception are most commonly investigated in laboratory animals. The methods used to measure pain perception vary greatly in terms of both the type of noxious stimulation employed and where on the body it is applied. The most widely used noxious stimuli are heat applied to the tail or paws; electric shock applied to the tails, feet, or skin; and noxious chemicals applied subcutaneously. Because laboratory rats have been the most common subjects of pain research and because they were the subjects in each of the experiments of this thesis, the most common methods of measuring pain perception in rats are briefly described in this section.

A. The Tail-Flick Test. The most common behavioral test of nociception used in animal research, and the test to be used in the experiments of this thesis, is the tail-flick test of D'Amour and Smith (1941; see also Janssen, Niemegeers, & Dory, 1963). In this test, a source of heat, usually a focused light beam or hot

water, is applied to the tail of each rat, and the time that it takes each rat to withdraw its tail (i.e. the tail-flick latency) is measured. The relative potency of analgesic drugs on this measure correlates well with their relative analgesic potency in humans (D'Amour & Smith, 1941; Smith, D'Amour, & D'Amour, 1943). Following transection of the spinal cord, the tail-flick response remains intact (Irwin et al., 1951), indicating that it is a spinally-organized reflex. Because the tail can be withdrawn after a preset time limit, there is usually not tissue damage and repeated testing does not significantly affect tail-flick latencies (Fennessy & Lee, 1975).

B. The Hot-Plate Test. A second common test of nociception is the hot-plate test (Woolfe & MacDonald, 1944), in which the subject is placed on a heated surface, and the time that it takes for the animal to raise and lick the bottom of a hindpaw is measured. Repeated testing with the hot-plate test is frequently confounded by the subjects learning to jump as soon as they are placed on the surface, and by damage to the paws (Fennessy & Lee, 1975).

C. The Flinch-Jump Test. In the flinch-jump test of nociception (Evans, 1961), an animal is placed on a grid through which discrete shocks of various intensities are administered. First, the minimal level of current intensity necessary to elicit a flinch is determined, followed by determination of the minimal level of current necessary to elicit a jump. These flinch and jump thresholds are usually determined using repeated stepwise

increases, and then decreases, in shock intensity. The entire

procedure typically requires about 90 min to complete. Thus,

this method is impractical for use in time-course studies or with

short-acting analgesics. The flinch and jump responses are

assumed to reflect spinally- and cortically-organized responses,

respectively. However, there has been no systematic test of

these assumptions.

D. <u>Formalin Writhing Test.</u> The injection of formalin into

a rat paw produces a series of characteristic responses that are

typically elicited by painful stimuli. The time that a rat

engages in each of these behaviors can be recorded and used as a

measure of the severity of the pain. Melzack and his colleagues

(e.g. Dennis & Melzack, 1979) have argued that this test is a

model of chronic pain, as opposed to the sharp, phasic pain

involved in other animal tests. Opiate drugs induce analgesia at

a much lower dose in the formalin test than in the tail-flick or

hot-plate tests (Abbott, Melzack & Leber, 1982). However,

formalin does produce tissue damage at the injection site, and

repeated testing is thus impractical.

E. <u>Noxious Stimuli Used in Electrophysiological Recording.</u>

It is often useful to record the responses of single neurons to

noxious stimuli. Various stimuli that are presumed to be painful

are used to identify neurons that mediate pain perception. These

stimuli include a pinch of the skin, an electric shock, a weak

acid applied to the skin, or a focused light beam applied to the

skin. Neurons that respond to these stimuli are considered to be

"nociceptive" neurons.

The various procedures for measuring pain in laboratory animals are differentially sensitive to experimental manipulations. Of particular relevance to the present experiments is the finding of Dennis and Melzack (1979, 1980) that the effects of serotonergic drugs on morphine-produced analgesia are dependent upon the measure of analgesia. Thus, the adoption of the tail-flick test as the basic procedure in each of the present experiments requires justification. The following are the reasons why it rather than the numerous available alternatives, was employed.

Several considerations led to the selection of the tail-flick test as the most appropriate model of nociception. First, the tail-flick test has been the most commonly used method of measuring pain in laboratory animals. This was an important consideration because a primary motivation behind this series of experiments was to determine whether differential involvement of S1 and S2 receptors in morphine-produced analgesia could account for many of the inconsistent results reported using this procedure. A second reason for the selection of the tail-flick test was that the designs of the experiments incorporated repeated testing. Consequently, tests that are greatly influenced by repeated testing were unsuitable. Rats subjected to repeated hot-plate testing learn to avoid the noxious heat stimulus by jumping as soon as they are placed in the apparatus, and formalin injection for the writhing test causes severe tissue

damage, making these two common tests impractical. In contrast, there is no systematic change in tail-flick latency with repeated daily testing, or even with repeated testing within a session (D'Amour & Smith, 1941).

All of the methods of measuring pain perception in animals described in this introduction are predictive models of analgesia. That is, the effectiveness of various drugs in altering the measure is correlated with the drugs' potencies in relieving human pain. But pain is not a unitary phenomenon. In humans, the subjective feeling of pain seems to vary with different noxious stimuli. In the animal literature, types of pain have been distinguished along a temporal dimension (e.g. Dennis & Melzack, 1979). Different types of pain may be mediated by different neural substrates (Abbott, Grimes, & Melzack, 1984; Coderre, Abbott, & Melzack; Dennis & Melzack, 1979). When we employ the tail-flick test, what type of pain are we measuring? Which neural substrates are we activating? How does this model relate to types of human pain? Because our knowledge of the neural substrate of pain and analgesia is incomplete, it is not possible to tell whether these models are subserved by neural systems that are analagous to systems that mediate human pain and analgesia. The value of the tail-flick test, or any test of analgesia, as a model of pain perception may be verified only when the circuitry involved is more completely understood.

2. Mechanisms of Morphine Analgesia

The observation that spinal transection only partially

blocks morphine-produced analgesia (Irwin et al., 1951; Takagi,

Matsumura, Yanai, & Ogiu, 1955) has led many to postulate that

morphine produces analgesia through two different systems. One

system mediating morphine-produced analgesia is assumed to

originate in the brain and to inhibit the incoming pain messages

via connections between the brain and the spinal cord segment at

which the messages enter the central nervous system. A second

morphine-analgesia system is assumed to exist entirely within the

spinal cord. According to this theory, spinal transection only

partially blocks morphine-produced analgesia because it

disconnects the first mechanism, but not the second. The

following two sections summarize the evidence for the existence

of these putative analgesia mechanisms.

A. Brainstem-to-spinal cord analgesia system. An important

step in determining where morphine acts to produce analgesia was

the isolation and localization of the nervous system's opioid

receptors. Early in vitro binding studies performed on brain

tissue homogenates found marked differences in the number of

opioid binding sites in various regions of rat and monkey nervous

systems (Kuhar, Pert, & Snyder, 1973; Hillar, Pearson, & Simon,

1973; Pert & Snyder, 1973). Some general areas, such as the

medulla and spinal cord were found to have few binding sites,

whereas high concentrations of binding sites were found in

various limbic structures and in the striatum. The main

shortcoming of these in vitro binding studies was that because they were based on the analysis of homogenized gross structures dissected from the brain, their powers of spatial resolution were low. These studies thus, provided only a very general indication of the location of opioid receptors in the brain.

The technique of autoradiography (Atweh & Kuhar, 1977a,b,c; Pert, Kuhar, & Snyder, 1975; 1976), proved to be a much more effective method of determining the distribution of opioid binding sites in the central nervous system. Rat brain and spinal cord slices were incubated in solutions of radioactively labelled drugs that bind to opioid receptors. Subsequent autoradiographs revealed high levels of radioactivity in the periaqueductal gray (PAG), interpeduncular nucleus, inferior colliculus, median raphe, amygdala, nucleus accumbens, area postrema, several diencephalic nuclei, and the dorsal horn of the spinal gray matter (Goodman, Snyder, Kuhar, & Young, 1980; see Atweh & Kuhar, 1983 for a review). These then are the likely sites of opioid receptors in the central nervous system.

Next, to establish unequivocally that these binding sites identified by autoradiography reflect the presence of opioid receptors, several investigators showed that functional changes are associated with the binding of the drug to the site. Generally speaking, injections of opiate drugs directly into these receptor-rich areas have been found to produce subsets of the effects of peripheral opiate injections. For example, morphine injected directly into the striatum was found to produce

the motor rigidity characteristic of peripheral morphine administration; whereas, injection of morphine into the area postrema was found to produce nausea and vomiting (Atweh & Kuhar, 1983).

Of direct relevance to this dissertation are those studies of opioid receptor function that focused on analgesia. Small amounts of morphine or endogenous opioids injected directly into various regions of the PAG of rats, cats, and monkeys have been shown to produce strong analgesic effects (Foster, Jenden, & Lomax, 1967; Malick & Goldstein, 1977; Pert & Yaksh, 1975; Tsuo & Jang, 1964). The fact that the analgesia produced by opiate injections into the PAG was reversed by the opiate receptor blocker, naloxone provided further evidence that the analgesia was mediated by the action of the opiates on opioid receptors. Moreover, naloxone injected into the PAG was found to reverse the analgesic effect of morphine when it was injected systemically (Yeung & Rudy, 1980a). More recently, it has been shown that analgesia can also be produced by injecting morphine into either the nucleus raphe magnus, the nucleus reticularis paragiganto-cellularis, or the nucleus reticularis paragigantocellularis lateralis of the rostral ventral medulla (Akaike, Shibata, Satoh, & Takagi, 1978; Azami, Llewelyn, & Roberts, 1982; Dickenson, Oliveras, & Besson, 1979). It thus appears that the PAG is not the only brainstem nucleus with morphine receptors involved in the mediation of analgesia (Atweh & Kuhar, 1983; Basbaum & Fields, 1984); however, it is considered to be the primary one.

It has been hypothesized (Abols & Basbaum, 1981; Behbehani &
Pomeroy, 1978; Behbehani, Pomeroy & Mack, 1981; Gallager & Pert,
1978; Mantyh, 1983) that the contribution of the PAG to morphine-
produced analgesia is mediated via connections between the PAG
and the nucleus raphe magnus, commonly referred to as the NRM.
Support for this theory comes from several lines of evidence:
Proudfit and Anderson (1975) found that lesions of the NRM
attenuate analgesia produced by injection of morphine into the
PAG. Pomeroy and Behbehani (1979) found that electrical
stimulation of the PAG produced excitation of neurons in the NRM.
Behbehani and Pomeroy (1978) found that injections of morphine
into the PAG alter the firing rate of NRM neurons. Finally,
Beitz (1982a,b) showed that some of the neurons that project from
the PAG to the NRM contain either serotonin or neurotensin. On
the basis of this combined evidence, Basbaum and Fields (1978,
1984) suggested that the analgesic effect of morphine is mediated
by its binding to opioid receptors in the PAG and the subsequent
activation of the NRM via serotonergic and/or neurotensinergic
neurons.

Studies demonstrating that the analgesia produced by
injections of morphine into the PAG can be substantially
attenuated by transection of the spinal cord or lesions of the
dorsolateral funiculus, a descending tract of the spinal cord, is
strong evidence that a major component of morphine analgesia is
mediated by brain-to-spinal cord connections (Jurna & Grossman,
1976; Kitahata, Yosaka, Taub, Bonikos, & Hoffert, 1974; LeBars,

Menetrey, Conseiller, & Besson, 1975; Murphin, Bennett, & Mayer,
1976). Also consistent with this view is the observation that
the NRM, as well as two other nuclei of the rat rostral ventral
medulla, the nucleus reticularis paragigantocellularis and the
nucleus reticularis paragigantocellularis lateralis, that receive
input from the PAG (Beitz, 1982a,b; Mantyh, 1983) all project to
the spinal cord via the dorsolateral funiculus in several
different species (Basbaum & Fields, 1979; Leichnetz, Watkins,
Griffin, Martin, & Mayer, 1978; Martin, Jordan, & Willis, 1978).
Evidence for a second, more direct, brain-to-spinal cord
analgesia pathway is provided by a recent demonstration that
there are also extensive projections from the PAG directly to the
spinal cord of rats, cats, and monkeys (Mantyh & Peschanski,
1982).

Axons descending from cell bodies in the PAG and from those
in the NRM and the other rostral ventral medulla nuclei terminate
in the marginal layer and substantia gelatinosa of the dorsal
horn of the spinal cord (Basbaum, Clanton, & Fields, 1978; Mantyh
& Peschanski, 1982). These two areas of the dorsal horn are
where the axons of primary nociceptive neurons enter the central
nervous system and synapse on ascending spinal neurons (see
Willis, 1985 for a review). The rapid-conducting Ao nociceptive
primaries, which tend to carry information about mechanical pain,
project to the marginal layer of the dorsal horn; whereas, the
slow-conducting C-polymodal fibers synapse in the substantia
gelatinosa of the dorsal horn (e.g. Beal & Bicknell, 1981;

Rethelyi & Capowski, 1977). This convergence of the descending

axons of the analgesia circuits with the incoming nociceptive

fibers in the marginal layer and substantia gelatinosa of the

dorsal horn implies that it is at these sites that the analgesia

circuits exert their inhibitory effects (Basbaum & Fields, 1984;

Fields & Basbaum, 1978; Melzack, 1973; Melzack & Wall, 1965).

Supporting this view is the observation that activation of the

NRM or other rostral ventral medulla nuclei by intracerebral

injection of morphine or by electrical stimulation inhibits the

responses of dorsal horn neurons that have been identified to

transmit nociceptive information from the spinal cord to the

brain (Liebeskind, Guilbaud, Besson, & Oliveras, 1973; Willis,

Haber, & Martin, 1977), but has no effect on the firing rates of

neurons that are only responsive to non-noxious tactile stimuli

(see Besson & Le Bars, 1978 for a review).

 B. <u>Spinal Mechanisms of Opiate Analgesia</u>. As previously

mentioned, transection of the spinal cord significantly

attenuates, but does not eliminate morphine analgesia in those

areas of the body that are served by spinal cord segments that

are below the transection (Irwin et al., 1951; Takagi et al.,

1955). This observation has led to the conclusion (e.g. Soja &

Sinclair, 1983) that opiates may also produce analgesia by acting

directly on the opioid receptors of the dorsal horn. This theory

is supported by the fact that intrathecal administration of

morphine, that is, injection of morphine into the spinal

subarachnoid space, also has strong analgesic effects (Yaksh,

1981; Yaksh & Reddy, 1981; Yaksh & Rudy, 1977). Opioid receptors have been identified on the presynaptic terminals of primary afferent axons entering the dorsal horn (Atweh & Kuhar, 1977a; Fields, Emson, Leigh, Gilbert, & Iversen, 1980; Hiller, Simon, Crain, & Peterson, 1978; LaMotte, Pert, & Snyder, 1976). Moreover, iontophoretic administration of morphine onto these presynaptic opioid receptors has been shown to produce changes in excitability of both Ao and C primary afferents (Belcher & Ryall, 1978; Calvillo, Henry, & Neuman, 1974; Dostrovshy & Pomeranz, 1976; Duggan, Hall, & Hedley, 1977, Duggan, Johnson, & Morton, 1981; Zieglgansberger & Bayerl, 1976). Hence, there is support for a presynaptic inhibitory role for opioids in the spinal cord.

An alternative to this "presynaptic" interpretation is that direct postsynaptic inhibition of nociceptive transmission by morphine can produce analgesia by blocking the transmission of nociceptive input to the spinothalamic tract (Basbaum & Fields, 1984). Consistent with this view is the demonstration of Ruda (1980) that neurons that contain endogenous opioid pentapeptides contact spinothalamic tract neurons. Accordingly, morphine may act within the dorsal horn of the spinal cord in one of two ways, or perhaps in both, by presynaptic inhibition of the nociceptive primaries or by direct postsynaptic inhibition of ascending sensory neurons.

3. <u>The Involvement of Serotonin in Morphine Analgesia</u>

Over the past 20 years there has been growing evidence that the neurotransmitter serotonin plays an important role in the mediation of morphine analgesia (see Messing & Lytle, 1977 for a review). The first two parts of this section will review the effects on morphine-produced analgesia of serotonin antagonists and agonists, respectively. The third part reviews data relevant to the question of whether the critical serotonin receptors are located in the spinal cord or in the brain.

A. <u>Antagonism of Serotonergic Activity and Morphine Analgesia</u>. The idea that serotonin is a critical neurotransmitter in the mediation of morphine-produced analgesia is primarily based on evidence that morphine-produced analgesia is attenuated by manipulations that decrease the action of serotonin. Tennen (1968) was the first to propose a role for serotonin in morphine-produced analgesia. He based his hypothesis on his findings that blockade of serotonin biosynthesis with para-chlorophenylalanine attenuated the analgesic effect of morphine and that the blockade was reversed by restoration of serotonin levels with injections of 5-hydroxytryptophan, a serotonin precursor. Although these findings have been frequently replicated (Berge, Hole, & Ogren, 1983; Fennessy & Lee, 1970; Gorlitz & Frey, 1972; Reigle & Barker, 1983; Tilson & Rech, 1974; Tulunay, Yano, & Takemori, 1976; Vogt, 1974), some investigators have not observed the usual inhibitory effect of para-chlorophenylalanine on morphine

analgesia (Buxbaum, Yarborough, & Carter, 1973; Fennessy & Lee, 1980; Harvey, Schlosberg, & Yunger, 1974; Sugrue, 1979). This inconsistency has received considerable formal discussion (Berge et al., 1983; Fennesy & Lee, 1970; Tilson & Rech, 1974), but it has yet to be convincingly resolved.

The selective destruction of serotonin-containing neurons has also been found to attenuate the analgesic effect of morphine. For example, the amphetamine derivatives para-chloroamphetamine and fenfluramine have both been shown to produce a severe and permanent reduction in brain serotonin by destroying serotonergic neurons. Rats exposed to either of these drugs for a sufficiently long time to destroy serotonergic neurons no longer exhibit morphine-produced analgesia (Berge et al., 1983; Duncan & Spencer, 1973; Sugrue, 1979; Takemori, Tulunay, & Yano, 1975; Tulunay et al., 1976) Another selective serotonergic neurotoxin, 5,6-dihydroxytryptamine has also been shown to attenuate morphine-produced analgesia (Vogt, 1974). Because 5,6-dihydroxytryptamine causes a long-lasting loss of serotonin from the spinal cord but has only a temporary effect on brain serotonin (Baumgarten, Evetts, Holman, Iversen, Vogt, & Wilson, 1972), this finding suggests that serotonergic neurons in the spinal cord mediate morphine-produced analgesia. Sugrue (1979) reported that the similar neurotoxin 5,7-dihydroxytryptamine produced a 60% reduction in brain serotonin without significantly disrupting morphine-produced analgesia.

The support for the hypothesis that serotonin mediates

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morphine-produced analgesia that has come from the use of serotonin receptor blockers has been inconsistent. Cyproheptidene, a serotonin antagonist, which is also a potent blocker of histamine receptors (Stone, Wenger, Ludden, Stavorski, & Ross, 1961), has been shown to block the analgesia produced by peripheral administration of morphine in mice (Gorlitz & Frey, 1972); whereas, the more selective serotonin receptor blockers methysergide, mianserin and metergoline have not (Berge, Fasmer, & Kjell, 1983; Fennessy & Lee, 1970). However, these selective serotonin receptor blockers have been found to attenuate the analgesia produced by injections of morphine into the PAG of the rat (Yaksh, DuChateau, & Rudy, 1976), or NRM (Dickenson et al., 1972).

In summary, procedures that deplete serotonin stores, destroy serotonin neurons, or block serotonin receptors have been found to attenuate morphine-produced analgesia. There have, however, been notable exceptions to this general rule.

B. <u>Serotonin Agonists and Analgesia</u>. The observation that antagonism of serotonergic activity tends to reduce the analgesic effect of morphine has led many to assess the analgesic effects of serotonin agonists. For example, quipazine and 5-methoxy-N, N-diamethyltryptamine, which act as serotonin agonists by stimulating central nervous system serotonin receptors, were found to have analgesic properties in rats (Berge, Hole, & Dahle, 1980; Samanin, Bernasconi, & Quattrone, 1976). Quipazine-produced analgesia is reversed by metergoline, a serotonin

receptor blocker (Samanin et al., 1976). However, the analgesic effect of 5-methoxy-N,N-diamethyltryptamine is reversed by noradrenalin depletion, but not by serotonin depletion, suggesting that this drug produces analgesia via alpha-adrenergic receptors (Archer, Minor, & Post, 1985). Another serotonin agonist, fluoxetine, which facilitates transmission at serotonergic synapses by inhibiting uptake of serotonin from the synapse, also has been shown to produce analgesia by itself (Messing, Fisher, Phebus, & Lytle, 1976; Messing, Phebus, Fisher, & Lytle, 1975) and to facilitate the analgesia produced by morphine (Larson & Takemori, 1977).

The demonstration that serotonin agonists produce analgesia is not by itself strong evidence for the serotonergic mediation of morphine-produced analgesia. Serotonin could be involved in an independent, non-opioid analgesia system without being active in the circuitry that is activated by morphine. Stronger evidence for the involvement of serotonin in morphine-produced analgesia is provided by the finding that the serotonin precursor 5-hydroxytryptophan potentiates morphine-produced analgesia without having any analgesic action of its own (Gardiner & Eberhart, 1970; Takagi, Takashima, & Kimura, 1964; Tulunay et al., 1976).

C. Anatomical Localization of Serotonin Receptors that are Thought to Mediate Morphine-Produced Analgesia. Because the NRM is part of the neural circuit mediating the analgesic effect of morphine and is also the source of serotonergic neurons that

project to the spinal cord (Basbaum et al., 1976; Bowker, Westlund, & Coulter, 1981; Dahlstrom & Fuxe, 1965), Fields & Basbaum (1978) proposed that serotonin's role in morphine-produced analgesia is mediated by the serotonin receptors on the dorsal horn neurons that receive input from axons descending from the NRM. Others have claimed (Berge et al., 1983; Genovese, Zonta, & Mantegazza, 1973; Roberts, 1984) that it is serotonergic synapses in the brain that are important for morphine-produced analgesia. Two main strategies have been used to localize the serotonin receptors mediating the analgesic effects of morphine: (1) the comparison of intrathecal and intracerebroventricular injections of serotonin agonists and antagonists, and (2) the selective depletion of spinal cord or brainstem serotonin.

Intrathecal injections of neither 5,6-dihydroxytryptamine or 5,7-dihydroxytryptamine, which have been found to selectively destroy spinal cord serotonin-containing neurons without affecting forebrain neurons, have been shown to block the analgesic effect of morphine (Deakin & Dostrovsky, 1978; Kuraishi, Harada, Aratani, Satoh, & Takagi, 1983). In contrast, injections of 5,7-dihydroxytryptamine into the dorsal raphe nuclei of rats apparently has no effect on analgesia (Deakin & Dostrovsky, 1978). Taken together, these results are commonly used as evidence for the role of spinal cord serotonin in the mediation of morphine-produced analgesia. However, Romandi, Esposito, and Samanin (1985) report that 5,7-dihydroxytryptamine injections into neither the ventromedial tegmentum, which deplete

forebrain serotonin, nor the ventral raphe, which deplete spinal serotonin, affect morphine-produced analgesia. Moreover, some investigators have failed to confirm that lesions of spinal cord serotonergic neurons by intrathecal injection of 5,6-dihydroxytryptamine result in a blockade of morphine-produced analgesia (Kuraishi et al., 1983; Proudfit & Yaksh, 1980), and injections of 5,6-dihydroxytryptamine into the cerebral ventricles also have been shown to block morphine-produced analgesia (Genovese et al., 1973).

Berge et al. (1983) have proposed that many conflicting results obtained with various methods of serotonin depletion may be accounted for by considering that para-chlorophenylalanine treatment produces a depletion of both ascending and descending serotonin pathways, whereas para-chloroamphetamine treatment preferentially destroys cerebral serotonin nerve terminals (Kohler, Ross, Srebro, & Ogren, 1978; Ogren et al., 1981), leaving spinal nerve terminals intact. They reported that pretreatment with either para-chlorophenylalanine or para-chloroamphetamine blocks morphine-produced analgesia, which would implicate forebrain serotonin. Ogren et al. (1981) also reported that the serotonin uptake inhibitor, zimeledine injected before para-chloroamphetamine preferentially protects against the neurotoxic effects in forebrain terminals, but has only a weak prophylactic effect on brainstem terminals. Thus, treatment with zimelidine and para-chloroamphetamine produces a rat with a selective brainstem serotonin lesion. Berge et al. (1983)

reported that such lesions also block morphine-produced analgesia, and they thus concluded that brainstem serotonergic synapses contribute to morphine-produced analgesia.

Llewelyn, Azami, and Roberts (1983) provided further evidence that brainstem serotonergic receptors contribute to nociception and analgesia by demonstrating that serotonin injected into the NRM produces analgesia. Furthermore, the serotonin uptake blocker zimelidine or the serotonin releasing agent, fenfluramine, both produce potent analgesia when injected into the NRM (Llewelyn, Azami, & Roberts, 1984).

Yaksh (1979) and Yaksh et al. (1976) found that intrathecal administration of the serotonin antagonists, cinanserin or mianserin block the analgesia produced by injections of morphine into the PAG (Yaksh, 1979; Yaksh et al., 1976). However, Proudfit and Hammond (1981) found that intrathecal injections of methysergide, a serotonin antagonist, did not significantly attenuate analgesia produced by subcutaneous injections of morphine. This latter result suggests that spinal serotonin receptors do not play an major role in analgesia produced by peripherally injected morphine, but leaves open the possibility of a role for supraspinal serotonin receptors. Considered together, the Yaksh et al. and the Proudfit and Hammond findings emphasize the danger in the common practice of generalizing from data collected following central injections of morphine to the analgesia produced by peripheral morphine injections.

Intrathecal administration of serotonin or the serotonin

agonists, quipazine and MK 212 produce strong analgesia that is reversed by serotonin antagonists (Wang, 1977; Yaksh & Wilson, 1979), but this does not necessarily implicate serotonin receptors in morphine-produced analgesia. These intrathecal injections might have activated an independent analgesia system unrelated to morphine.

Peripheral injections of serotonin antagonists have been shown to increase the responsiveness of dorsal horn neurons to noxious stimulation (Rivot, Calvino, & Besson, 1987). These results implicate a tonically active (i.e., normally firing at a high rate) serotonergic system that inhibits nociceptive afferent neurons in rats. Similar injections have been shown to block the inhibition of nociceptive neurons by electrical stimulation of the PAG or NRM (Yezierski, Wilcox & Willis, 1982; Carstens, Fraunhoffer, & Zimmerman, 1981).

In summary, the evidence from the study of serotonin antagonists and agonists supports the notion that serotonergic neurons are involved in some forms of analgesia. Although the evidence is far from unequivocal, there is support from studies involving selective lesions and local injections that both spinal and cerebral serotonergic mechanisms are involved. Although serotonin is widely believed to be involved in the analgesic effects of systemically administered morphine--see the influential recent texts of Kandel and Schwartz (1981) and Carlson (1981)--there is in fact little direct evidence for this view.

4. Serotonin Receptor Types

The recent discovery that there are at least two types of
serotonin receptors, S1 and S2 (Peroutka, Lebovitz, & Snyder,
1981; Peroutka & Snyder, 1979) raised the question of which
receptor type mediates morphine analgesia. The following three
parts of this section: A) summarize the evidence for the
distinction between S1 and S2 receptors, B) review the current
knowledge of the localization of each of the receptor types
within the central nervous system, and C) discuss the functions
that are thought to be mediated by each of the types.

A. The S1-S2 Distinction. Radioactively labelled serotonin
has been shown to bind to homogenized membranes from the central
nervous system. Because this bound radioactive serotonin is
displaced by unlabelled serotonin or by drugs that are thought to
bind to serotonin receptors, but is not easily displaced by other
transmitters, it has been proposed that these binding sites
represent receptors that are specific to serotonin. The finding
that radioactively-labeled spiperone also binds to these same
sites was an important step toward the discovery of S1 and S2
serotonin receptor types. Peroutka and Snyder (1979) observed
that after washing spiperone-labeled membranes with a
concentration of serotonin that completely eliminated labelled
serotonin binding, approximately half of the labelled spiperone
remained. A low concentration of spiperone easily displaced this

remaining labelled spiperone, but it required a 100-fold increase

over the original serotonin concentration in the wash to

accomplish the same task. The opposite was true of labelled

serotonin that was incubated with spiperone. Thus, to put it

concisely, Peroutka and Snyder found that serotonin binds with

high affinity to some serotonin receptors, which they named S1

receptors; whereas spiperone binds with high affinity to others,

which they referred to as S2 receptors.

Further evidence for the idea that there are two distinct

serotonin receptor types comes from the observation that some

drugs that are active at serotonin receptors easily displace

labelled serotonin, but not labelled spiperone; whereas other

drugs easily displace labelled spiperone but not labelled

serotonin (Peroutka & Snyder, 1979). For example, the

concentration of the serotonin agonist, 5-methoxytryptamine that

is required to displace spiperone is 250 times greater than that

required to displace serotonin. On the other hand, cinanserin is

100 times more effective in displacing spiperone than serotonin.

Because both serotonin and spiperone bind to the same sites, but

are differentially displaced from subgroups of the sites,

Peroutka and Snyder (1979) concluded that there must be at least

two distinct populations of serotonin receptors.

B. Distribution of Serotonin Binding Sites. Early

information from studies comparing membrane binding in

homogenized tissue indicated that S1 and S2 binding sites are

differentially distributed within various central nervous system

areas. The hippocampus, striatum, raphe nuclei and spinal gray matter showed dense S1 binding (Blackshear, Steranka, & Sanders-Bush, 1981; Peroutka & Snyder, 1981), and S2 binding was found to be particularly dense in frontal and occipital cortex, accumbens, striatum, and olfactory tubercle, with no detectable binding in the hypothalamus or spinal gray matter (Blackshear et al., 1981; Leysen, Niemegeers, Van Nueten, & Laduron, 1982; Monroe & Smith, 1983; Peroutka & Snyder, 1981).

Autoradiography of sections of rat brain tissue incubated in radioactively-labelled serotonin (Biegon, Rainbow, & McEwan, 1982; Young & Kuhar, 1980) has shown that serotonin binding sites are most concentrated in the hippocampus, septum, medial and dorsal raphe, and interpeduncular nucleus. There is also dense binding in the frontal cortex, some amygdaloid and hypothalamic nuclei, dorsal tegmentum, caudate, olfactory tubercle, median central gray, and in the dorsal horn of the spinal cord. But, because radiolabeled serotonin binds to all serotonin receptors, these studies did not distinguish between serotonin receptor types.

Refined autoradiographic techniques that used drugs that are selective for serotonin receptor types allowed the visualization of S1 and S2 binding (Desamukh, Yamamura, Woods, & Nelson, 1983; Laduron, Janssen, & Leysen, 1982; Marcinkiewicz, Verge, Gozlan, Pichat, & Hamon, 1984; Pazos, Cortes, & Palacios, 1985; Pazos & Palacios, 1985; Slater & Patel, 1983). Results from these studies have generally confirmed and extended results obtained

with the less precise membrane binding techniques. For example,
the binding sites in the median raphe have been shown to be
primarily S1 sites, and the sites in the dorsal raphe are
primarily S2 sites. The S1 binding seen in the spinal gray
matter is almost entirely restricted to the dorsal horn, with the
binding sites most concentrated in the substantia gelatinosa.
Thus, binding to both S1 and S2 receptors has been localized to
areas thought to be associated with analgesia.

    C. Behavioral Correlates of S1 and S2 Binding Sites.
Although the mediation of many behavioral effects of serotonin
receptor stimulation have been attributed to either S1 or S2
receptors, there are few relevant data. Administration of
serotonin or serotonin agonists produces a variety of behavioral
effects, which include head-twitching, forepaw treading, tremor,
hindlimb abduction, and Straub tail (Jacobs, 1976). This entire
syndrome is blocked by metergoline and methysergide, two
serotonin antagonists with partial action at both S1 and S2
receptors (Colpaert & Janssen, 1983; Ortmann, Bischoff, Radeke,
Buech, & Delini-Stula, 1982). In contrast, the selective S2
antagonists ketanserin, pirenperone, and pipamperone blocked the
head twitches (Colpaert & Janssen, 1983; Green, 1984; Lucki,
Nobler, & Fraser, 1984; Ortmann et al., 1982) but had no effect
on the other behavioral symptoms (Green, 1984; Lucki et al.,
1984).

    Mendelson and Gorzalka (1985a,b; 1986b) found that blockade
of S2 receptors with selective antagonists inhibits the sexual

behavior of female rats; whereas, quipazine, an agonist with
relatively high affinity for S2 receptors, was found to
facilitate it. In contrast, S1 agonists have been shown to
inhibit female sexual behavior (Mendelson & Gorzalka, 1986a).
Moreover, Mendelson & Gorzalka (1986a) found that S1 receptors
may serve an opposite function in the sexual behavior of male
rats than in female rats; the S1 agonist 8-hydroxy-
2,dipropylaminotetralin inhibited the sexual behavior of female
rats, but facilitated that of males. On the basis of their
research, Mendelson and Gorzalka have speculated that serotonin
receptor types appear to play antagonistic roles in females, but
work in concert in males.

5. Paul and Phillips (1986)

Little attention has been given to the relative roles of S1
and S2 receptors in morphine-produced analgesia. Because it has
been hypothesized that spinal serotonin receptors mediate
morphine-produced analgesia (Basbaum & Fields, 1984; Fields &
Basbaum, 1978; Messing & Lytle, 1977; Samanin et al., 1978) and
because spinal cord serotonin receptors are almost exclusively S1
receptors (Monroe & Smith, 1983; Pazos et al., 1985), many have
assumed that spinal S1 receptors mediate morphine-produced
analgesia (e.g. Pazos et al., 1985). However, the data of
Zemlan, Kow, and Pfaff (1983) suggest that stimulation of spinal
S1 receptors produces hyperalgesia. They found that systemic
administration of the serotonin agonists quipazine and 5-methoxy-

N,N-diamethyltryptamine to rats with transected spinal cords facilitated responding to noxious stimuli. Furthermore, cinanserin, a serotonin antagonist with a preferential affinity for S2 receptors, has been shown to block analgesia produced by injections of morphine into the PAG (Yaksh, 1979; Yaksh et al., 1976). However, recent data indicate that cinanserin may have agonistic properties at S1 receptors as well as its S2 receptor blocking effect (Janssen, 1983). Because virtually no S2 receptors have been found in the spinal cord, the blockade seen by Yaksh et al. may have been produced by stimulation of S1 receptors within a system that facilitates nociceptive transmission, and would thus support the hypothesis of Zemlan et al. that S1 receptors in the spinal dorsal horn facilitate nociceptive transmission. Thus, although a direct implication of the widely accepted PAG-NRM-spinal cord model is that serotonin directly or indirectly inhibits ascending nociceptive information, there may be two spinal serotonergic systems: the first inhibiting ascending nociceptive fibers, and the second facilitating local spinal withdrawal reflexes.

I have recently published (Paul & Phillips, 1986) a first attempt to assess the effect of S2 receptor blockade on the analgesia produced by systemic injection of morphine. Rats were injected with 0.04, 0.08, or 0.16 mg/kg (SC) of the selective S2 receptor blocker, pirenperone, followed 60 min later by 10 mg/kg (IP) of morphine sulfate. Each rat was tested for analgesia 15 min after the morphine administration by measuring the amount of

time that it took to remove its tail from a hot water bath, that is by measuring its tail-flick latency. As expected, morphine by itself substantially increased tail-flick latencies, thus indicating strong morphine-produced analgesia. When morphine administration was preceded by injection of 0.16 mg/kg of pirenperone, but not 0.04 or 0.08 mg/kg, there was a significant attenuation of morphine-produced analgesia. These results suggest that S2 receptors mediate morphine-produced analgesia.

6. General Rationale and Purposes

Clearly, our theories of how the neurotransmitter serotonin is involved in the mediation of pain and morphine-produced analgesia are far from unequivocal. The large number of contradictory findings suggest that some crucial variable has not been considered. The recent findings that S1 receptors may serve to enhance nociception (Zemlan et al., 1983), whereas S2 receptors may mediate inhibition (Paul & Phillips, 1986), led me to conclude that the study of nociception and analgesia may also benefit from a closer examination of the differential roles of S1 and S2 receptors. This approach has recently proven successful in resolving some of the inconsistencies in the study of serotonin's role in sexual behavior (Mendelson & Gorzalka, 1985a,b; 1986a,b).

The experiments in the present thesis had two general purposes. The first was to more closely determine where in the nervous system the selective S2 receptor blocker pirenperone acts

to block morphine-produced analgesia. The second general purpose
of this series of experiments was to test the alternative
hypothesis that the anti-analgesic effect of pirenperone and
other S2 antagonists is due to the reported action of these drugs
at alpha-adrenergic receptors (Janssen, 1983).

II. GENERAL METHOD

This section describes the methods common to all five experiments of this thesis. Any specific modifications or additions to this general methodology are described in the method section of each experiment.

1. Subjects

Serving as subjects in each experiment were 300-to-450g male rats housed individually with free access to Purina lab chow and water.

2. Apparatus

All tail-flick tests were conducted in a 6.5 x 6.5 x 20 cm chamber. Each rat's tail was drawn through a 2 cm wide opening at the rear of the chamber, and approximately 5 cm of the tail was submerged in a 52 C water bath. The time that it took each rat to remove its tail from the bath, that is the tail-flick latency, was recorded electronically. On the few trials that a subject did not respond within 10 sec, its tail was removed from the bath by the experimenter to prevent tissue damage, and a tail-flick latency of 10 sec was assigned. All testing occurred in the colony room during the last 5 hr of the light phase of the 12/12 hr light/dark cycle.

3. Procedure

Baseline tail-flick latencies were recorded on 5 or 6 consecutive days. Each daily test session consisted of five tail-flick tests administered at 30-min intervals. Each subject spent the 30-min intertest intervals in its home cage. The first

of the four drug-test sessions occurred on the day after the last
baseline session, and the remaining three occurred at 4-day
intervals thereafter. Baseline sessions were conducted on each
of the 3 days between consecutive drug-test days. The drug-test
sessions were identical to the baseline sessions except that
immediately after the first tail-flick test on each drug-test
day, each rat was injected with either the appropriate dose of a
serotonin antagonist or its vehicle, followed either by an
analgesic or its saline vehicle. Thus, there were four basic
conditions in each study: vehicle-vehicle, analgesic-vehicle,
vehicle-antagonist, and analgesic-antagonist. In each study,
each subject was tested under all four of these treatment
combinations in a counterbalanced sequence. Table 1 lists each
drug used in these experiments with its source, vehicle,
concentration, injection volume, dose, and route of
administration.

4. Statistical Analysis

For each dose of the antagonist an ANOVA was used to assess
the significance of the within-group differences in tail-flick
latencies for the four post-injection intervals. In all cases,
the main effects for test interval and treatment, and the
interval x treatment interaction were significant at the .05
alpha level. Newman-Keul's post-hoc comparisons were then used
to assess the significance of treatment differences and
differences at specific test intervals. The alpha level was .05
for all post-hoc comparisons. A 2 x 2 ANOVA was also used to

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Table 1. The vehicle, concentration, injection volume, dose, route of administration, and source of each drug used in this series of experiments. The pH was adjusted to between 6 and 7 with NaOH when necessary.

DRUG	VEHICLE	CONCENTRATION (mg/ml)	VOLUME (ml/kg)	DOSE (mg/kg)	ROUTE	SOURCE
ANALGESICS						
Morphine Sulphate	Saline	10	1	10	IP	BDH
Ketamine HCl	Saline	50	2	100	IP	Parke-Davis
S2 ANTAGONISTS						
Pirenperone	Citrate	0.4	0.2	0.08	SC	Janssen
		0.8	0.2	0.16		
		1.2	0.2	0.24		
Ketanserin HCl	Citrate	5	0.2	1	SC	Janssen
		15	0.2	3		
		50	0.2	10		
LY53857	Saline	10	1	10	IP	Lilly

assess the possibility that a shift in baseline caused by the antagonist may account for any observed attenuation of analgesia. This analysis was used on data at the peak of the analgesic (60 min for morphine and 30 min for ketamine).

III. <u>EXPERIMENT 1</u>:   EFFECT OF PIRENPERONE ON MORPHINE ANALGESIA

The first experiment was designed to confirm the preliminary
finding (Paul & Phillips, 1986) that the selective S2 antagonist,
pirenperone (Colpaert & Janssen, 1982; Colpaert et al., 1983;
Leysen et al., 1981) blocks morphine-produced analgesia.
Confirmation of this finding was a particularly important step in
this thesis because it was inconsistent with current theories of
morphine-produced analgesia and because each of the experiments
of the thesis was based on it.

Another reason for "replicating" the Paul and Phillips
(1986) study was that it was not conducted according to the
procedures adopted for this series of experiments. Briefly, in
the present experiments, pirenperone and morphine were injected
concurrently, and the rats were tested repeatedly throughout the
2 hr session; whereas, in the Paul and Phillips experiment,
pirenperone was injected 60 min before morphine, and the rats
were tested only once, 15 min after the morphine injection.

Method

Following 5 days of baseline testing the 39 rats serving as
subjects were randomly divided into three groups. The analgesic
effect of 10 mg/kg of morphine or saline injected
intraperitoneally was challenged with 0.08 (n=12), 0.16 (n=14),
or 0.24 mg/kg (n=13) of pirenperone or its citrate vehicle
administered subcutaneously.

<u>Results</u>

Figure 1 illustrates the results of Experiment 1. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as indicated by substantially increased tail-flick latencies at the 30-, 60-, and 90-min test intervals in the morphine-vehicle condition compared to the vehicle-vehicle condition at all three doses of pirenperone. The major finding of this study was that when 0.08, 0.16 or 0.24 mg/kg of pirenperone was injected with the morphine, the morphine-produced analgesia was attenuated. A second important finding was that each of the three doses of pirenperone produced hyperalgesia when administered by themselves; that is, the tail-flick latencies in the vehicle-pirenperone condition were significantly shorter than those in the vehicle-vehicle control condition.

For all three groups, the overall ANOVAs revealed significant main effects for treatment (0.08 mg/kg group, $F(3,33)=15.1$, $p<.0001$; 0.16 mg/kg group, $F(3,39)=17.0$, $p<0.0001$; 0.24 mg/kg group, $F(3,36)=31.7$ $p<.0001$). The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle treatment and the morphine-vehicle condition for each of the three groups (all three Newman-Keul's $p<.05$). In each group, morphine produced a significant increase in mean tail-flick latency at the 30-, 60-, and 90-min test intervals (all nine Newman-Keul's $p<.05$).

The tail-flick latencies of rats at the 30-, 60-, and 90-min test intervals were significantly shorter when they were injected

Figure 1. The analgesic effect of 10 mg/kg of morphine sulphate challenged with three doses of pirenperone. Mean tail-flick latencies were assessed at 0, 30, 60, 90, and 120 min after the injections in each of the four treatments. The three graphs illustrate the effects of 0.08, 0.16, and 0.24 mg/kg doses of pirenperone (n=12, 14, and 13, respectively). The analgesic effect of morphine is illustrated by the difference between the VEH-VEH and MOR-VEH conditions. The effect of pirenperone by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-PIR conditions. The attenuation of morphine-produced analgesia by pirenperone is illustrated by the difference between the MOR-VEH and MOR-PIR conditions.
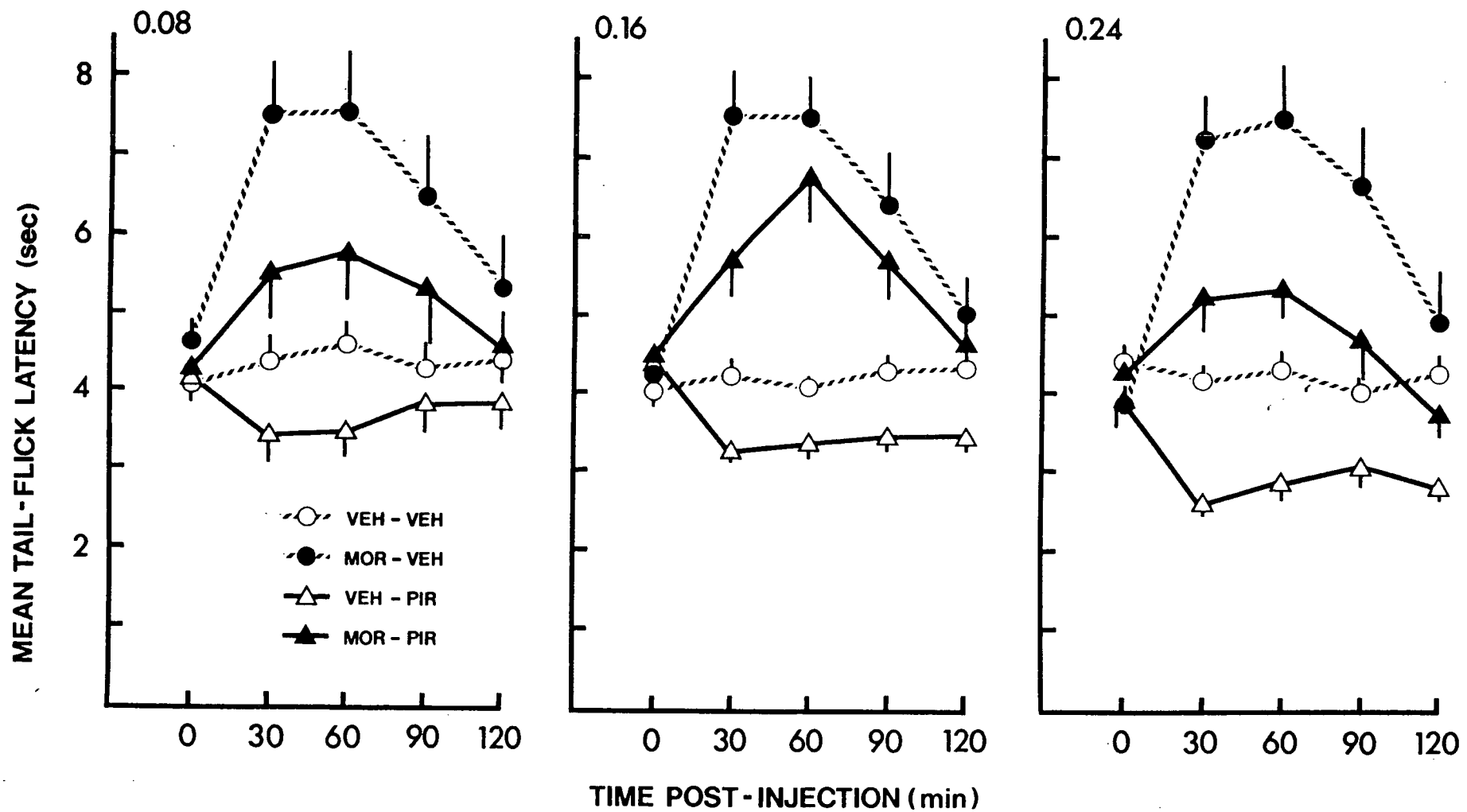
with morphine and any of the three doses of pirenperone than whenthey were treated with morphine by itself (all Newman-Keul's p<.05). —

Although the mean tail-flick latencies of rats treated with each of the three doses of pirenperone by itself were shorter than when they received the vehicle-vehicle injections at every interval (excluding the 0-min interval), this effect reached statistical significance (Newman-Keul's p<.05) only at the 30- and 60-min test intervals after the 0.08 mg/kg dose of pirenperone and at all four intervals after the 0.24 mg/kg dose.

The 2 X 2 ANOVAs revealed that the shifts in baseline following the vehicle-pirenperone injections may account for the observed attenuation of morphine-produced analgesia. Although the main effect for pirenperone was significant at the 0.08 and 0.24 mg/kg doses (0.08 mg/kg group, $F(1,11)=17.21$, $p<.005$; 0.16 mg/kg group, $F(1,13)=3.85$, $p>.05$; 0.26 mg/kg group, $F(1,12)=27.99$, $p<.001$), the morphine x pirenperone interactions were not ($F(1,11)=0.56$; $F(1,13)=0$; $F(1,12)=0.71$, respectively; all three $p>.05$).

Discussion

The results of Experiment 1 confirm the finding of Paul and Phillips (1986) that the selective S2 receptor blocker, pirenperone attenuates the analgesic effect of morphine. Moreover, pirenperone, when administered by itself, produced hyperalgesia. Because pirenperone has a preferential affinity for S2 receptors, this pattern of results provides further

evidence that S2 receptors play a significant role in the perception of pain and in the analgesic effect of morphine.

Considering the observation that S2 binding sites have not been demonstrated in the spinal cord (Monroe & Smith, 1983; Pazos et al., 1985), these results are surprising. Serotonin is generally thought to exert control over nociception and analgesia through spinal cord receptors (e.g. Carlson, 1981; Kandel & Schwartz, 1981). Accordingly, the fact that a selective S2 blocker such as pirenperone can attenuate morphine-produced analgesia suggests that serotonin may be producing this effect through blockade of S2 receptors in the brain or peripheral nervous system. It is, however, possible that the reduction in baseline tail-flick latencies following vehicle-pirenperone injections may account for the observed anti-analgesic effect of pirenperone.

IV. EXPERIMENT 2: EFFECT OF KETANSERIN ON MORPHINE ANALGESIA

Experiment 2 was designed to assess the effect of the selective S2 antagonist ketanserin on morphine-produced analgesia. Pirenperone is a derivative of ketanserin and these two drugs have comparable affinities for the same receptors. Although ketanserin and pirenperone have affinities for the same receptors (Table 2), ketanserin does not easily enter the central nervous system (Laduron et al., 1982). Because it is only at very high doses that ketanserin affects receptors in the central nervous system, any effects of low doses can be reasonably attributed to its action at peripheral receptors.

The differential abilities of pirenperone and ketanserin to enter the central nervous system provided a convenient way to test whether morphine-produced analgesia is mediated by S2 receptors in the central or peripheral nervous system. Blockade of morphine-produced analgesia by a low doses of ketanserin would implicate peripheral S2 receptors, whereas, blockade restricted to high doses would support a role for central receptors.

Method

Following 6 days of baseline testing, the 32 rats serving as subjects were randomly divided into three groups. The analgesic effect of 10 mg/kg of morphine or saline injected intraperitoneally was challenged with 1 (n=10), 3 (n=11), or 10 mg/kg (n=11) of ketanserin HCl or its citrate vehicle administered subcutaneously.

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Table 2. The inhibition constants of ketanserin and pirenperone
for S1, S2, histamine type-1 (H1), alpha, dopamine (DA), and
muscarinic acetylcholine (Ach-m) receptors as determined by in
vitro receptor binding techniques (adapted from Janssen, 1983).
Values are expressed in nanomoles.

| Drug	S2	S1	H	alpha	DA	Ach-m
Ketanserin	2.1	–	10	10	220	–
Pirenperone	2.0	–	14	6.8	16	–

<u>Results</u>

Figure 2 illustrates the results of Experiment 2. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as seen by substantially increased tail-flick latencies at the 30-, 60-, and 90-min test intervals in the morphine-vehicle condition compared to the vehicle-vehicle condition at all three doses of ketanserin. The major finding of this study was that 10 mg/kg of ketanserin, but not 1 or 3 mg/kg, attenuated morphine-produced analgesia.

For all three groups, the overall ANOVAs revealed significant main effects for treatment (1 mg/kg group, $F(3,27)=10.6$, $p<.0001$; 3 mg/kg group, $F(3,30)=17.5$, $p<.0001$; 10 mg/kg group, $F(3,30)=19.3$, $p<.0001$). The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle condition and the morphine-vehicle treatment for all three groups (all three Newman-Keul's $p<.05$). In the 3 and 10 mg/kg groups, morphine produced a significant increase in mean tail-flick latency at the 30-, 60-, and 90-min test intervals; and in the 1 mg/kg group, it produced significant increases at the 30- and 60-min intervals (all eight Newman-Keul's $p<.05$).

The tail-flick latencies of rats at the 30-, 60-, and 90-min test intervals were significantly shorter when they were injected with morphine and 10 mg/kg of ketanserin than when they were treated with morphine by itself (all three Newman-Keul's $p<.05$). In contrast, 1 or 3 mg/kg of ketanserin did not significantly

Figure 2. The analgesic effect of 10 mg/kg of morphine sulphate challenged with three doses of ketanserin HCl. Mean tail-flick latencies were assessed at 0, 30, 60, 90, and 120 min after injections of rats in the four treatment conditions. The three graphs illustrate the 1, 3, and 10 mg/kg doses of ketanserin (n=10, 11, and 11, respectively). The analgesic effect of morphine is illustrated by the difference between the VEH-VEH and MOR-VEH conditions. The effect of ketanserin by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-KET conditions. The attenuation of morphine-produced analgesia by ketanserin is illustrated by the difference between the MOR-VEH and MOR-KET conditions.

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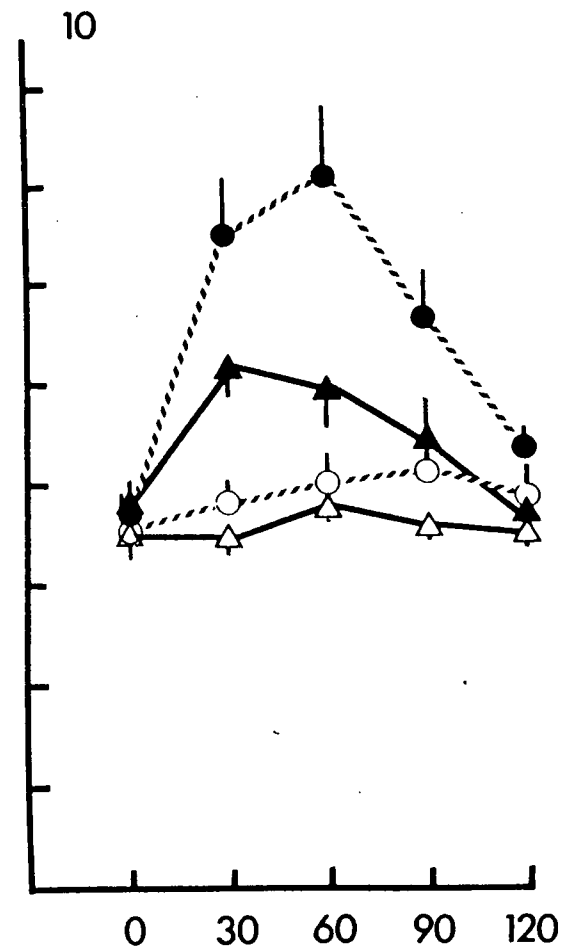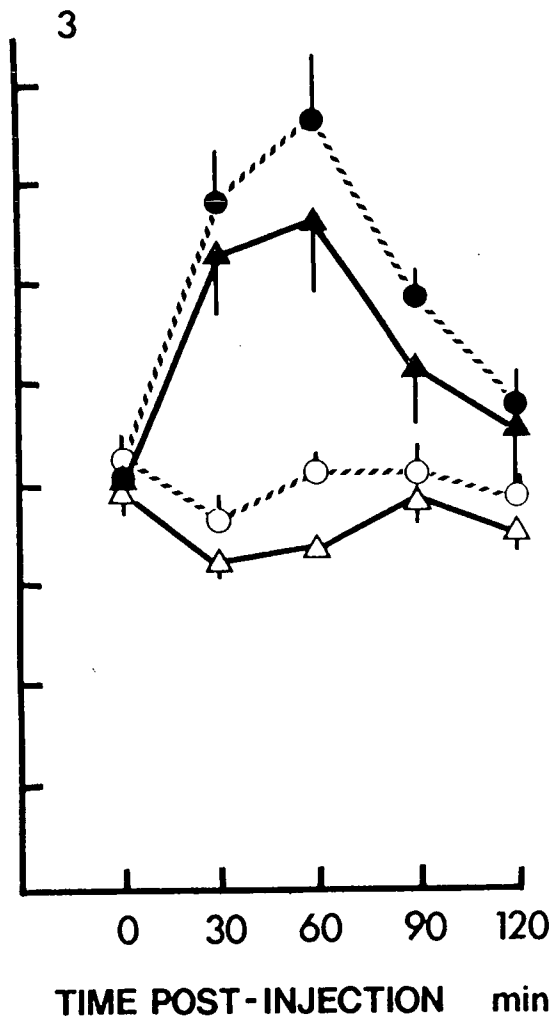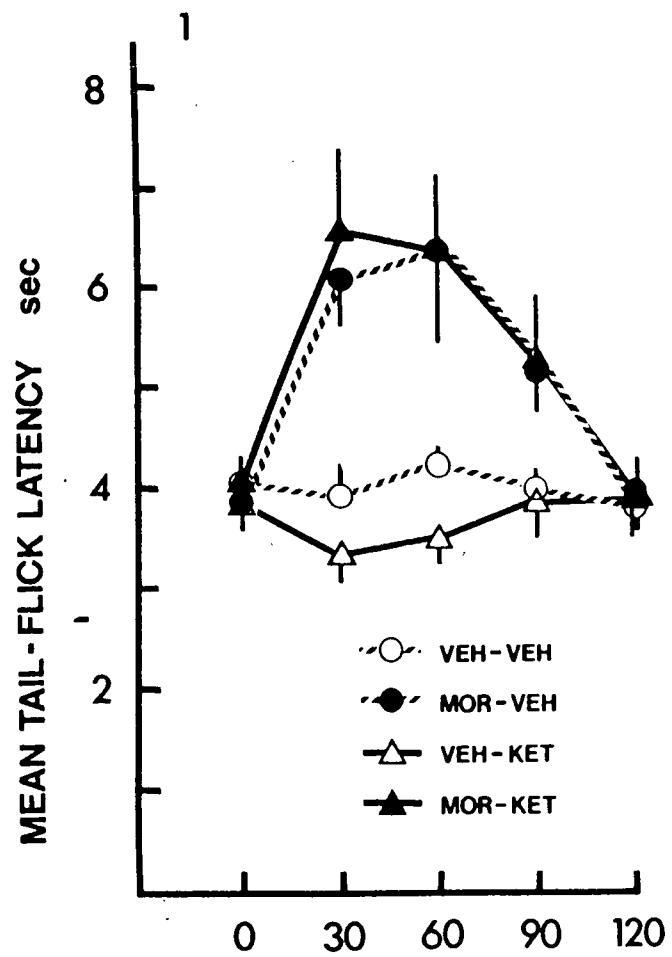
reduce the tail-flick latencies of morphine-treated subjects at any of the test intervals.

Although the mean tail-flick latencies of rats treated with each of the three doses of ketanserin and the vehicle were consistently shorter than when they received the vehicle-vehicle injections, this effect never reached statistical significance.

The 2 X 2 ANOVAs revealed that the shifts in baseline following the vehicle-ketanserin injections do not account for the observed attenuation of morphine-produced analgesia. The main effect for ketanserin was significant at the highest dose (1 mg/kg group, $F(1,9)=0.57$, $p>.05$; 3 mg/kg group, $F(1,10)=1.86$, $p>.05$; 10 mg/kg group, $F(1,10)=24.05$, $p<.001$), as was the morphine x pirenperone interaction ($F(1,9)=1.11$, $p>.05$; $F(1,10)=0.05$, $p>.05$; $F(1,10)=5.02$, $p<.05$, respectively).

Discussion

The fact that ketanserin significantly attenuated morphine-produced analgesia at the high 10 mg/kg dose provides further evidence that S2 receptors are important for the mediation of morphine-produced analgesia. In Experiment 1, pirenperone, a drug that is pharmacologically similar to ketanserin (see Table 2), blocked morphine-produced analgesia at a dose much lower than the doses of ketanserin that failed to block it in this experiment. Because pirenperone easily enters the central nervous system, whereas ketanserin does not, this pattern of results provides evidence that the S2 receptors that are important for morphine-produced analgesia are in the central

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nervous system.

In Experiment 1, pirenperone by itself caused a decrease in baseline tail-flick latencies. This result raised the ⁻ possibility that the observed anti-analgesic effect of the S2 antagonist is nothing more than a reflection of the baseline shift. The results of Experiment 2, however, challenge this hypothesis. Because 10 mg/kg of ketanserin significantly attenuated morphine-produced analgesia but produced only a slight, nonsignificant decrease in baseline tail-flick latency, it seems likely that the attenuation of morphine-produced analgesia and the hyperalgesia produced by these S2 antagonists are dissociable.

V. EXPERIMENT 3:   EFFECT OF KETANSERIN ON KETAMINE ANALGESIA

Experiment 3 was designed to assess the effect of S2 receptor blockade on analgesia that is not mediated by a descending inhibitory system activated by supraspinal opioid receptors. The analgesic, ketamine appears to exert its analgesic effect by acting directly on spinal receptors (Okuda, 1986; Smith, Perrotti, Mansell, & Monroe, 1985). Three findings suggest that it does not produce its analgesic effect by activating a descending inhibitory pathway. First, although transection of the spinal cord attenuates morphine-produced analgesia (Irwin et al., 1951; Takagi et al., 1955), spinal cord transection produces a 9-fold increase in the potency of ketamine as an analgesic (Pekoe & Smith, 1982). Second, spinal cord transection enhances the inhibitory effect of ketamine on dorsal horn nociceptive neurons (Okuda, 1986). And third, naloxone injected into the PAG attenuates morphine-produced analgesia but not ketamine-produced analgesia (Smith et al., 1985).

Method

The 15 rats serving as subjects received 5 days of baseline testing. Then the analgesic effect of 100 mg/kg ketamine hydrochloride was challenged with 10 mg/kg ketanserin, the dose of ketanserin that was found in Experiment 2 to block morphine-produced analgesia.

Results

Figure 3 illustrates the results of Experiment 3. Injection of 100 mg/kg of ketamine produced a potent analgesia at the 30- and 60-min test intervals. The major finding of this experiment was that a dose of ketanserin that attenuated morphine-produced analgesia in Experiment 2, did not significantly attenuate ketamine-produced analgesia.

The overall ANOVA revealed a significant main effect of treatment ($F(3,45)=13.8$, $p<.0001$). The analgesic effect of ketamine was confirmed by the significance of the overall difference between the vehicle-vehicle and the ketamine-vehicle conditions (Newman-Keul's $p<.05$). Ketamine produced a significant increase in tail-flick latency at the 30- and 60-min test intervals (both Newman-Keul's $p<.05$).

The lack of significant effect of ketanserin on ketamine-produced analgesia was indicated by the lack of significant differences between the mean tail-flick latencies of the rats in the ketamine-ketanserin condition and the ketamine-vehicle condition (all four Newman-Keul's $p>.05$).

As in Experiment 2 the mean tail-flick latencies of rats in the vehicle-ketanserin condition were all shorter than those in the vehicle-vehicle condition although none of these differences was statistically significant (all five Newman-Keul's $p>.05$).

Figure 3. The analgesic effect of 100 mg/kg of ketamine HCl

challenged by 10 mg/kg of ketanserin HCl. Mean tail-flick

latencies (N=15) were assessed 0, 30, 60, 90, and 120 min after

injections in the four treatment conditions. The analgesic

effect of ketamine is illustrated by the difference between the

VEH-VEH and KTA-VEH conditions. The effect of ketanserin by

itself on mean tail-flick latency is illustrated by the

difference between the VEH-VEH and VEH-KET conditions. The

attenuation of ketamine-produced analgesia by ketanserin is

illustrated by the difference between the KTA-VEH and KTA-KET

conditions.
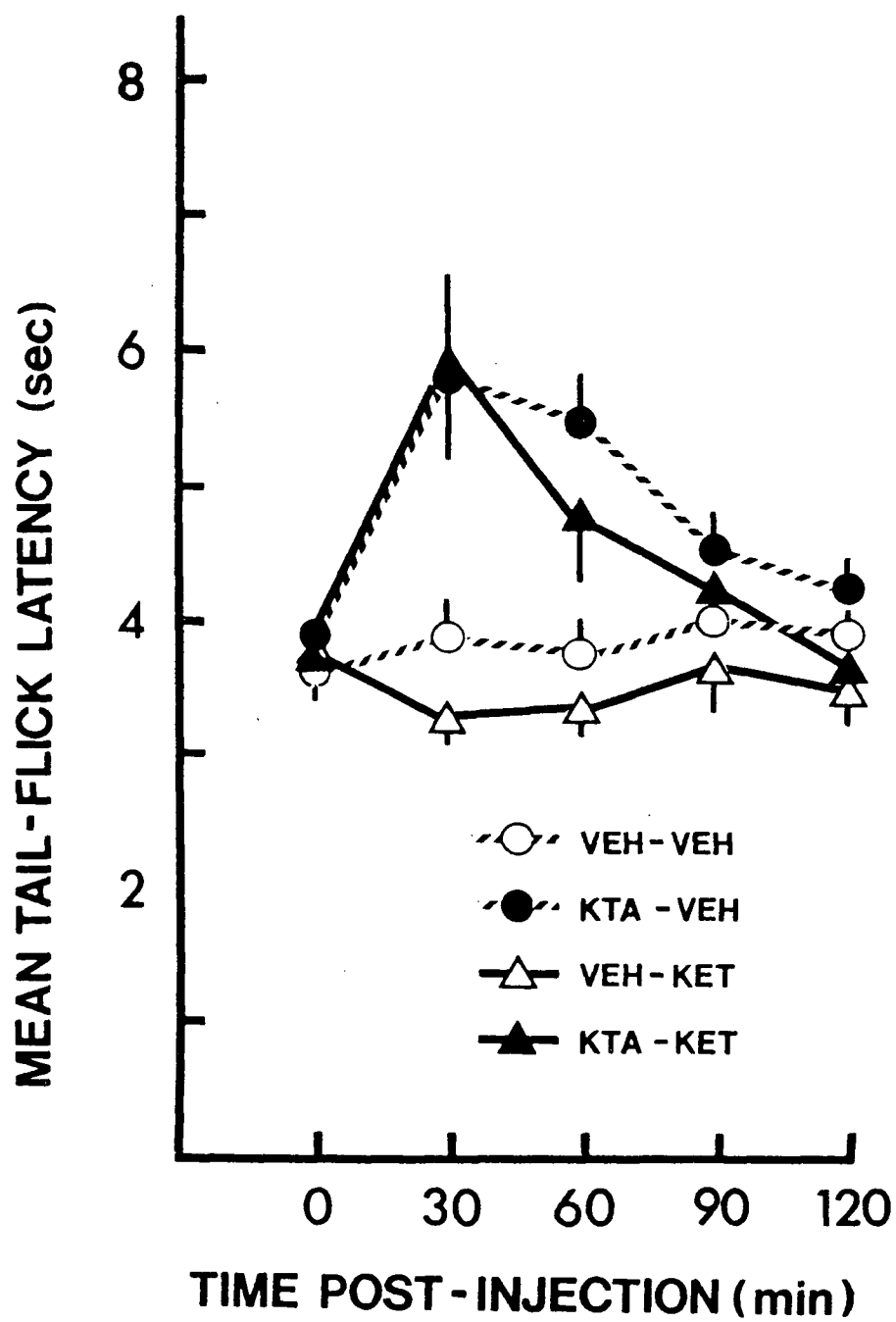
The 2 X 2 ANOVA revealed that the shift in baseline following the vehicle-ketanserin injections does not account for the observed attenuation of ketamine-produced analgesia. The main effect for ketanserin was not significant $(F(1,15)=0.56, p>.05;)$. Likewise, the ketamine x pirenperone interaction was not significant $(F(1,15)=1.65, p>.05)$.

Discussion

The results of Experiment 3 show that blockade of S2 receptors with ketanserin does not attenuate ketamine-produced analgesia. This finding established that the anti-analgesic effect of ketanserin is not completely general and suggests that it might be specific to morphine-produced analgesia.

VI. EXPERIMENT 4: EFFECT OF PIRENPERONE ON MORPHINE ANALGESIA IN SPINAL RATS

Because S2 receptors have not been found in the spinal cord, the finding of Experiment 1 that pirenperone blocks morphine-produced analgesia suggests that S2 receptors that mediate the effect are in the brain or the peripheral nervous system, or both. In Experiment 2, ketanserin, a pirenperone-like drug that does not readily enter the central nervous system, attenuated morphine-produced analgesia only at high doses, thus suggesting that the S2 receptors that mediate morphine-produced analgesia are in the central nervous system. Together, the results of Experiments 1 and 2 provide evidence that S2 receptors that mediate morphine-produced analgesia are in the brain. The finding of Experiment 3 that even a high dose of ketanserin did not attenuate the analgesic effect of ketamine, a drug that produces its analgesic effect by acting directly on spinal cord receptors, provided further support for a this hypothesis.

Experiment 4 was designed to test the cerebral S2 hypothesis of morphine-produced analgesia by assessing the effect of S2 receptor blockade with pirenperone on morphine-produced analgesia in rats with transected spinal cords. Transection of the spinal cord isolates the opioid analgesia circuitry of the spinal cord from the influence of descending inhibition or excitation originating in the brain. If the hypothesis that pirenperone produces its anti-analgesic effect via supraspinal receptors is correct, then pirenperone should have no effect on morphine-

produced analgesia in rats with spinal cord transections. On the other hand, if pirenperone is found to block morphine-produced analgesia, then a role for spinal receptors would be implicated.

Method

The analgesic effect of 10 mg/kg of morphine or saline injected intraperitoneally was assessed in rats with or without spinal cord transections that were injected subcutaneously with 0.24 mg/kg of pirenperone or its citrate vehicle.

Following 6 days of baseline testing, the 141 rats serving as subjects were assigned to either the spinal cord transection condition or the sham-surgery condition as needed. Surgery was carried out under pentobarbitol anesthesia. Spinal cords were transected at the T12-L1 level by exposing the vertebrae, removing the spinous process, and transecting the cord with a spatula. Complete transection was confirmed visually before suturing the muscle and closing the incision with wound clips. The vertebrae of the sham-surgery controls were exposed, but the spinous process was left intact. All testing was conducted not less than 6 hr but not more than 12 hr after surgery.

Eleven rats died from the anesthetic and forty-four rats died or did not respond to the hot water after spinal cord transection. The eighty-six rats surviving the operation were assigned to one of four treatment conditions in each of the two surgical conditions. There were 10 rats with transections in the vehicle-vehicle and the vehicle-pirenperone groups and 11 rats in the morphine-vehicle and the morphine-pirenperone groups. There

were 11 sham-operated rats in each of the four treatment groups.

Results

The results of Experiment 4 are illustrated in Figure 4. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as indicated by the substantially greater tail-flick latencies of the rats in the morphine-vehicle group relative to rats in the vehicle-vehicle group at the 30-, 60-, and 90-min test intervals. Pirenperone had two major effects. First, it significantly attenuated the morphine-produced analgesia in the sham-surgery animals but not in the rats with spinal cord transections. Second, pirenperone by itself produced a significant hyperalgesia in the sham-surgery animals, but again it did not do so in the subjects with spinal cord transections.

For both surgery conditions, the overall ANOVAs revealed significant main effects for treatment (sham-surgery condition, $F(3,40)=14.0$, $p<.0001$; transection condition, $F(3,38)=5.8$, $p<0.01$). The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle group and the morphine-vehicle group for each of the two conditions (both Newman-Keul's $p<.05$). In both conditions, morphine produced a significant increase in mean tail-flick latency at the 30-, 60-, and 90-min test intervals (all six Newman-Keul's $p<.05$).

For rats in the sham-surgery condition, the tail-flick latencies at the 30-, 60-, and 90-min test intervals were

Figure 4. The analgesic effect of 10 mg/kg of morphine sulphate
challenged with 0.24 mg/kg of pirenperone in rats with transected
spinal cords and in sham-surgery controls. Mean tail-flick
latencies were assessed at 0, 30, 60, 90, and 120 min after
injections in each of the four treatment conditions. The graph
labeled SHAM represents the rats in the sham-surgery group and
the graph labeled SPINAL represents the rats with transected
spinal cords (n=10 for the VEH-VEH and VEH-PIR treatments in the
SPINAL condition, n=11 for all other treatments). The analgesic
effect of morphine is illustrated by the difference between the
VEH-VEH and MOR-VEH groups. The effect of pirenperone by itself
on mean tail-flick latency is illustrated by the difference
between the VEH-VEH and VEH-PIR groups. The attenuation of
morphine-produced analgesia by pirenperone is illustrated by the
difference between the MOR-VEH and MOR-PIR groups.

SHAM   SPINAL

MEAN TAIL-FLICK LATENCY (sec)

TIME POST-INJECTION (min)

VEH – VEH
MOR – VEH
VEH – PIR
MOR – PIR

58
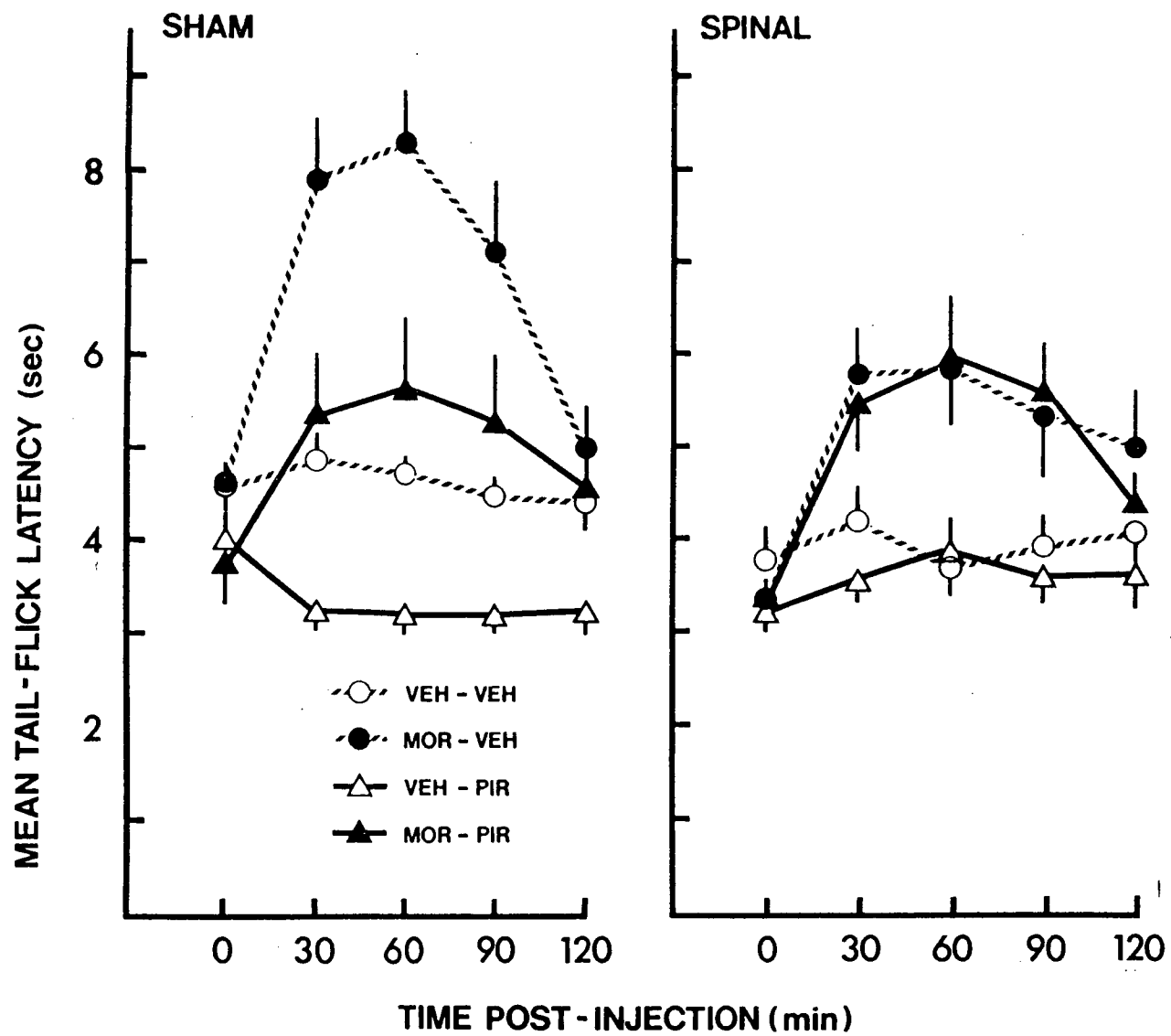
significantly shorter in animals injected with morphine and pirenperone than in animals treated with morphine by itself (all three Newman-Keul's p<.05). In contrast, at no test interval were the tail-flick latencies of rats in the transection condition that were injected with morphine and pirenperone significantly different than the latencies of those injected with morphine alone (all four Newman-Keul's p>.05).

Although in the sham-surgery condition, the mean tail-flick latencies of rats that were treated with pirenperone alone were significantly shorter than those of rats receiving the vehicle-vehicle combination of injections (all four Newman-Keul's p<.05), rats in the spinal-cord-transection condition injected with this dose of pirenperone did not differ significantly from those in the vehicle-vehicle treatment group at any of the post-injection intervals (all four Newman-Keul's p>.05).

The 2 X 2 ANOVA revealed that the shift in baseline following the vehicle-pirenperone injections in the sham-surgery group may account for the observed attenuation of morphine-produced analgesia. Although the main effect for pirenperone was significant (F(1,40)=17.76, p<.005), the morphine x pirenperone interactions was not (F(1,40)=1.49 p>.05). Neither the main effect for pirenperone nor the morphine x pirenperone interaction was significant in the spinal transection condition (both p>.05).

Discussion

In Experiment 4, the selective S2 receptor blocker, pirenperone attenuated the analgesic effect of morphine in

control rats but not in those with transected spinal cords. Moreover, in contrast to the hyperalgesic effect of pirenperone seen in rats in the sham-surgery condition, injection of pirenperone and the vehicle did not affect nociception in the transection condition. The findings in the sham-surgery condition confirm those of Experiment 1.

Because transection of the spinal cord isolates the opioid analgesia circuitry of the spinal cord from the influence of descending inhibition or excitation originating in the brain, these results provide further evidence that S2 receptor blockade does not produce its anti-analgesic and hyperalgesic effects via spinal cord receptors. Considering the lack of S2 receptors in the spinal cord (Monroe & Smith, 1982; Pazos et al., 1985) these results are not surprising. They do, however, run counter to the prevailing theory that serotonin mediates morphine-produced analgesia and modulates nociception via spinal cord serotonergic receptors.

There is, however, another possible interpretation for this finding. The rats with transected spinal cords had substantially shorter tail-flick latencies, and these short baselines may have been less sensitive to anti-analgesic effects. This possibility could be assessed by decreasing the temperature of the water bath to establish higher baselines.

VII. EXPERIMENT 5: EFFECT OF LY53857 ON MORPHINE ANALGESIA

The serotonin antagonists, pirenperone and ketanserin are potent blockers of S2 receptor sites but have virtually no activity at S1 sites. However, these two serotonin antagonists have been recently found to be moderately potent blockers of alpha-adrenergic receptors (Janssen, 1983). Approximately 10% of the binding of pirenperone and ketanserin is at alpha-receptors. Moreover, some of the effects of ketanserin have been attributed to this drug's activity as a blocker of alpha-receptors, rather than to its activity at S2 receptors (Fozard, 1982; Vanhoutte et al., 1986).

In Experiments 1, 2, and 4 (see also Paul & Phillips, 1986), pirenperone and ketanserin blocked morphine-produced analgesia. However, it is not clear whether these effects were the result of the effects of these drugs on S2 receptors or the result of their recently discovered effects on alpha-adrenergic receptors (Neal & Sparber, 1986; Paul & Phillips, 1986). Accordingly, Experiment 5 assessed the effect on morphine-produced analgesia of LY53857, a selective S2 antagonist with a 300,000-fold greater affinity for S2 receptors than for alpha-adrenergic receptors (Cohen, Fuller, & Kurtz, 1983).

Method

The 10 rats serving as subjects received 6 days of baseline testing. Then they were injected with 10 mg/kg (IP) of morphine sulphate or its saline vehicle in combination with 10 mg/kg (IP) of LY53857 or its saline vehicle. Because of the low solubility

of LY53857, this drug was dissolved to a concentration of 10 mg/ml in saline, and injected intraperitoneally in a volume of 1 ml/kg, rather than the 0.2 mg/ml SC injections used throughout these experiments.

Results

Figure 5 illustrates the results of Experiment 5. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as indicated by the substantially increased mean tail-flick latencies at the 30-, 60-, and 90-min test intervals in the morphine-vehicle condition in comparison to those in the vehicle-vehicle condition. The major finding of this study was that when LY53857 was injected with the morphine, the morphine-produced analgesia was attenuated. A second important finding was that LY53857 injected by itself produced hyperalgesia; that is, the tail-flick latencies in the vehicle-LY53857 condition are significantly shorter than those in the vehicle-vehicle control condition.

The overall ANOVA revealed a significant main effect for treatment ($F(3,27)=14.7$, $p<.0001$). The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle treatment and the morphine-vehicle condition (Newman-Keul's $p<.05$). Morphine produced a significant increase in mean tail-flick latency at the 30-, 60-, and 90-min test intervals (all three Newman-Keul's $p<.05$).

Although the mean tail-flick latencies of rats injected with

Figure 5. The analgesic effect of 10 mg/kg of morphine sulphate challenged with 10 mg/kg of LY53857. Mean tail-flick latencies (n=10) were assessed 0, 30, 60, 90, and 120 min after injections in the four treatment conditions. The analgesic effect of morphine is illustrated by the difference between the VEH-VEH and MOR-VEH conditions. The effect of LY53857 by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-LY53857 conditions. The attenuation of morphine-produced analgesia by LY53857 is illustrated by the difference between the MOR-VEH and MOR-LY53857 conditions.
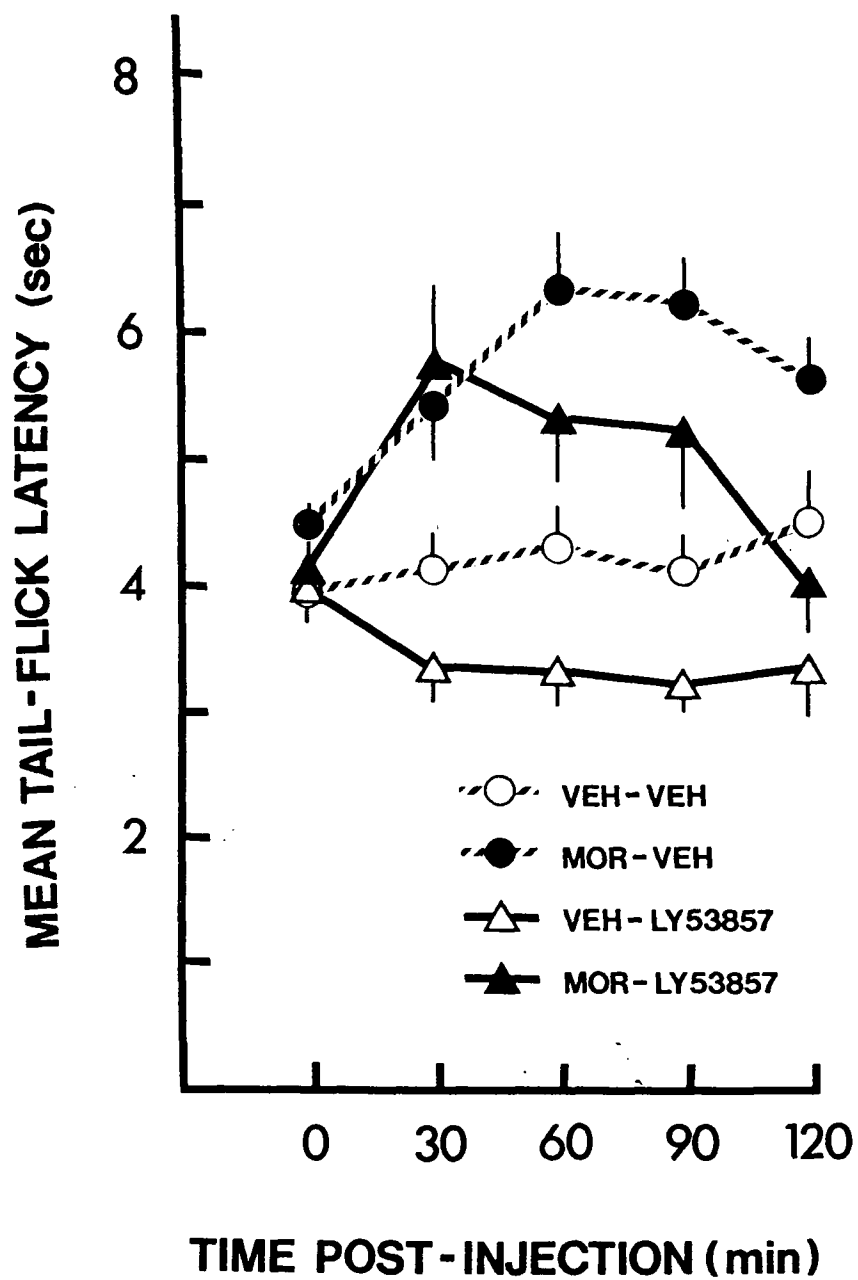
morphine and LY53857 were shorter at the 60-, 90-, and 120-min intervals than when they were treated with morphine alone, this difference reached significance only at the 120-min interval (Newman-Keul's p<.01).

The hyperalgesic effect of LY53857 was confirmed by the significance of the overall difference between the vehicle-vehicle condition and the vehicle-LY53857 condition (Newman-Keul's p<.05). However, only the comparison at the 90-min test interval was significant (Newman-Keul's p<.05).

Discussion

In Experiment 5, LY53857 produced a significant overall attenuation of morphine-produced analgesia, and it produced a significant overall hyperalgesia. Because LY53857 is an S2 receptor blocker with no activity at alpha-adrenergic receptors, this pattern of results provides evidence that the attenuation of morphine-produced analgesia by pirenperone, ketanserin, and LY53857 is not due to the effect of these drugs on alpha-adrenergic receptors. Likewise, because LY53857 by itself produced hyperalgesia, this study provides evidence that the hyperalgesia produced by S2 receptor blockers is not mediated by alpha-receptors. It seems that the hyperalgesic and anti-analgesic effects of S2 receptor blockers are indeed mediated by S2 receptors.

LY53857 produced a significant overall attenuation of morphine-produced analgesia and a significant overall hyperalgesia, that was significant only at the 120-min interval.

It appears, then, that this drug may have a different time course than pirenperone and ketanserin. But it is also possible that the anti-analgesic effect of LY53857 was not observed until the later test intervals because this drug was injected intraperitoneally, rather than subcutaneously.

VIII. <u>DISCUSSION</u>

The general goal of this dissertation was to clarify the role of S2 receptors in the production of morphine analgesia. Five experiments were conducted to achieve this goal. The first four experiments were designed to localize the S2 receptors that are involved in the attenuation of morphine-produced analgesia; whereas, the fifth was designed to test the possibility that the attenuation of morphine-produced analgesia by S2 receptor blockers is due to their action at alpha-adrenergic receptors. The general discussion is divided into four sections. In the first section, the results are summarized and integrated, and it is argued that together they provide strong evidence for the major conclusion of the dissertation: that S2 receptors in the brain are involved in morphine-produced analgesia. The second section describes the results of a recently completed pilot study that supports this view. The third considers the implications of this conclusion for current theories of nociception and analgesia. And the fourth considers its general implications for future directions of psychopharmacological research.

1. <u>Summary and General Discussion of the Experiments</u>

The experiments described in this dissertation had two general purposes. The purpose of the first four experiments was to determine the location of the S2 receptors involved in the attenuation of morphine-produced analgesia. The purpose of Experiment 5 was to test the hypothesis that the anti-analgesic effect of pirenperone and other S2 antagonists is due to their

putative action at alpha-adrenergic receptors (Janssen, 1983).
Accordingly, the results of the first four experiments and the
fifth experiment are separately summarized in the following two
subsections.

A. General Discussion of Experiments 1, 2, 3, and 4.
Experiment 1 assessed the effect of the S2-selective receptor
blocker, pirenperone on morphine-produced analgesia. The results
of this experiment confirmed and extended the finding of Paul and
Phillips (1986) that pirenperone attenuates the analgesic effect
of morphine. However, in contrast to the findings of Paul and
Phillips, pirenperone by itself was found to produce significant
hyperalgesia. The reason for this inconsistency is not clear,
but it could be attributable to methodological differences. Paul
and Phillips assessed the effect of pirenperone on tail-flick
latencies in a single test, 75 min after injection, whereas in
Experiment 1 of the present thesis this effect was assessed four
times at 30 min intervals. Because pirenperone-produced
hyperalgesia was also observed in Experiment 4, there is little
doubt that this is a bona fide effect.

Because S2 receptors have been shown to exist in the
periphery (Van Nueten et al., 1981) as well as in the brain, it
was not clear whether the anti-analgesic effect of pirenperone
was mediated by central or peripheral receptors. In Experiment
2, the analgesic effect of morphine was challenged with the S2
receptor blocker, ketanserin. This drug is pharmacologically
similar to pirenperone, however, it does not readily enter the

central nervous system (Laduron et al., 1982). Because ketanserin attenuated morphine-produced analgesia only at a very high dose (10 mg/kg), Experiment 2 provided evidence that the anti-analgesic effect of S2 receptor blockade is mediated by receptors within the central nervous system. Although ketanserin administered by itself slightly reduced mean tail-flick latencies at all test intervals after drug injection, this hyperalgesic effect did not reach statistical significance.

It was not clear from the first two experiments whether the anti-analgesic effect of S2 receptor blockers is specific to morphine-produced analgesia. Can S2 receptor blockers affect analgesia produced by agents thought to act through different circuits? Morphine is thought to produce at least part of its analgesic effect through a PAG-to-NRM-to-spinal cord descending inhibitory system (Fields & Basbaum, 1978; Basbaum & Fields, 1978, 1984; Mayer & Price, 1976). In contrast, ketamine is thought to produce its analgesic effect through a direct action on spinal cord receptors (Okuda, 1986; Smith, Perrotti, Mansell, & Monroe, 1985). Accordingly, In Experiment 3, ketamine-produced analgesia was challenged with a dose of ketanserin that had attenuated morphine-produced analgesia in Experiment 2 (10 mg/kg). The finding that ketanserin did not attenuate ketamine-produced analgesia established that the anti-analgesic effect S2 receptor blockade is not general and that it may be specific to analgesia produced by morphine or other drugs that act on the same system. As in Experiment 2, ketanserin, injected alone,

produced a reduction in mean tail-flick latencies at all post-injection test intervals, although again this effect was not statistically significant. Ketanserin has produced a nonsignificant reduction in tail-flick latency on the eight occasions that I have assessed its effects (the 3 doses of ketanserin in Experiment 2, once in Experiment 3, and in 4 experiments in Paul, Symons, and Pinel, unpublished observations). This suggests that ketanserin has a slight hyperalgesic effect that may be dissociable from its inhibitory effect on morphine-produced analgesia.

The finding of Experiment 2 that the anti-analgesic effect of S2 receptor blockers is mediated by their effect in the central nervous system, in combination with the apparent lack of S2 receptors in the spinal cord implicates brain S2 receptors in the mediation of the anti-analgesic effects of pirenperone and ketanserin. Experiment 4 addressed this issue directly. In order to confirm that the blockade of morphine-produced analgesia by S2 recptor blockers is mediated by S2 receptors in the brain, Experiment 4 assessed the effect of pirenperone on morphine-produced analgesia in rats with transected spinal cords. Transection of the spinal cord at the lower thoracic level isolates the spinal circuitry that mediates the tail-flick reflex from the influence of descending inhibition or excitation originating in the brain. In Experiment 4, pirenperone attenuated morphine-produced analgesia in sham-surgery control rats but not in rats with transected spinal cords. This confirms

that it is S2 receptors in the brain that mediate the attenuation of morphine-produced analgesia by pirenperone. Although pirenperone produced hyperalgesia in both Experiments 1 and 4 in intact rats, it did not produce hyperalgesia in the rats with transected spinal cords in Experiment 4. This result suggests that S2 receptors in the brain also mediate pirenperone-produced hyperalgesia. It is possible, however, that the reduced baseline tail-flick latencies observed in rats with transected spinal cords may have disguised a hyperalgesic effect of pirenperone.

In summary, when considered together, the results of Experiments 1, 2, 3, and 4 provide evidence that the anti-analgesic effects of the S2 receptor blockers, pirenperone and ketanserin are mediated by S2 receptors in the brain. It is not clear, however, if the mild hyperalgesia produced by these S2 antagonists is mediated by these same supraspinal S2 receptors.

B. General Discussion of Experiment 5. The serotonin antagonists, pirenperone and ketanserin are selective in the sense that they are potent blockers of S2 receptor sites but have virtually no activity at S1 sites (Colpaert & Janssen, 1982; Colpaert et al., 1983; Leysen et al., 1982). However, recently both pirenperone and ketanserin have been found to also be moderately potent blockers of alpha-adrenergic receptors (Janssen, 1983). Thus, it is not clear whether the attenuation of morphine-produced analgesia by pirenperone and ketanserin observed in Experiments 1, 2, and 4 was mediated by their blockade of S2 receptors or by their action at alpha-adrenergic

receptors. The recently synthesized serotonin receptor blocker, LY53857 binds with high affinity to S2 receptors but has virtually no action at either S1 receptors or at alpha-adrenergic receptors (Cohen et al., 1983). Accordingly, the purpose of Experiment 5 was to test the "alpha-adrenergic" alternative hypothesis by assessing the effect of LY53857 on nociception and morphine-produced analgesia. Like pirenperone and ketanserin, LY53857 attenuated morphine-produced analgesia, and it produced significant hyperalgesia. These results indicate that both the anti-analgesic and hyperalgesic effects of S2 receptor blockers are mediated by S2 receptors rather than alpha-adrenergic receptors.

2. Results of a Pilot Experiment to Test Directly Whether Central or Spinal S2 Receptors Mediate the Blockade of Morphine-Produced Analgesia by S2 Receptor Blockers

Experiment 4 provided evidence that spinal receptors do not mediate the attenuation of morphine-produced analgesia by pirenperone, thus implicating by elimination supraspinal S2 receptors in this effect. The relative contribution of brain and spinal cord receptors to the hyperalgesic and anti-analgesic effects of S2 receptor blockade was assessed in a recent pilot study (Paul, Pfaus, & Pinel, 1987). In this study, the analgesic effect of morphine was challenged with ketanserin injected into the cerebral ventricles or into the spinal subarachnoid space. Intraventricular injection of 5 ug of ketanserin attenuated

analgesic effect of 10 mg/kg of morphine sulphate (IP) at the 30 min test interval. In contrast, 5 ug of ketanserin injected intrathecally enhanced morphine-produced analgesia. The finding that intraventricular injection of ketanserin attenuates morphine-produced analgesia provides evidence that S2 receptors in the brain mediate the anti-analgesic effect of ketanserin and pirenperone. Because there is no precedent for the observed enhancement of morphine-produced analgesia by intrathecal injection of ketanserin this effect requires replication. Neither intraventricular nor intrathecal injection of ketanserin by itself had an effect on tail-flick latencies. If it proves to be replicable, this finding would implicate peripheral receptors in the mediation of the hyperalgesia produced by S2 receptor blockers.

3. Implications of the Present Findings for Theories of Pain Perception and Analgesia

It is widely believed that morphine produces most of its analgesic effect by binding to opioid receptors in the PAG, which in turn activate an inhibitory system that descends to the spinal cord via synapses in the NRM (cf. Soja & Sinclair, 1983). This system is presumed to inhibit incoming pain signals carried by primary somatosensory neurons in the dorsal horn of the spinal cord. It is these dorsal horn inhibitory synapses that are assumed to be serotonergic (Mayer & Price, 1976; Fields & Basbaum, 1978; Basbaum & Fields, 1984; Messing & Lytle, 1977).

The view that spinal cord serotonergic synapses mediate morphine-
produced analgesia is supported by the fact that the NRM has been
implicated in the analgesic effect of morphine and the axons of
serotonergic NRM cell bodies have been shown to project into the
dorsal horn (Bowker, Westlund, & Coulter, 1981; Dahlstrom & Fuxe,
1965). Moreover, intrathecal injections of 5,6-
dihydroxytryptamine or 5,7-dihydroxytryptamine, which have been
found to selectively destroy spinal cord serotonin-containing
neurons without affecting forebrain neurons, have been shown to
block the analgesic effect of morphine (Deakin & Dostrovsky,
1978; Kuraishi et al., 1983). However, despite the wide
acceptance of this view (see Carlsson, 1981; Kandel & Schwartz,
1981), the evidence that morphine-produced analgesia is mediated
by serotonergic synapses in the dorsal horn remains equivocal
(Berge et al., 1983; Roberts, 1984; Zemlan, 1983). Some
investigators have failed to confirm that lesions of spinal cord
serotonergic neurons by intrathecal injection of 5,6-
dihydroxytryptamine result in a blockade of morphine-produced
analgesia (Kuraishi et al., 1983; Proudfit & Yaksh, 1980).

Although it has not received wide recognition, several
authors (Berge et al., 1983; for a review see Roberts, 1984) have
provided evidence that the well documented interaction of
morphine-produced analgesia and serotonin occurs via receptors in
the brain. For example, Berge et al. (1983) found that in rats
pretreated with the serotonin uptake blocker zimelidine, para-
chloroamphetamine produced selective lesions of serotonin-

containing terminals in the brainstem. These selective lesions attenuated the analgesic effect of morphine, thus implicating brainstem serotonin receptors in the mediation of morphine-produced analgesia.

The results of the present experiments constitute what is arguably the strongest evidence against the current view that the serotonergic involvement in morphine-produced analgesia is primarily spinal. Because there are no S2 receptors in the spinal cord (Monroe & Smith, 1983; Pazos et al., 1985), the evidence provided here that the serotonergic mediation of morphine-produced analgesia involves S2 receptors rules out the possibility that only spinal serotonin receptors mediate morphine-produced analgesia. The hypothesis of Berge et al. (1983) that brainstem serotonin synapses are crucial for the mediation of morphine-produced analgesia is consistent with the dense S2 binding that has been found in the dorsal raphe nuclei (Desamukh et al., 1983; Laduron et al., 1982; Marcinkiewicz et al., 1984). This area has been implicated in the mediation of morphine-produced analgesia. The fact that many of the neurons that project from the PAG to raphe nuclei contain serotonin (Beitz, 1982b), provides further support for the hypothesis that morphine-produced analgesia is partially mediated by a PAG-to-raphe serotonergic system that synapses on S2 receptors in the raphe nuclei.

Because there is strong evidence that serotonergic synapses in the spinal cord mediate both inhibitory and excitatory

modulation of nociception (Jordan, Kenshalo, Martin, Haber, & Willis, 1979; Yaksh et al., 1976; Yaksh & Wilson, 1979; Zemlan et al., 1983), I am not proposing that the serotonergic involvement in morphine-produced analgesia is entirely cerebral; my point is that there is a major cerebral component. In fact, existing evidence suggests that there are three different systems that use serotonin to modulate pain perception. One of these systems is a spinal system that facilitates nociceptive reflexes. The existence of this system is supported by the findings of Zemlan et al. (1983) that systemic injections of serotonin agonists in rats with transected spinal cords reduces tail-flick latencies. A second serotonergic system is thought to inhibit ascending nociceptive neurons. Evidence for this system comes from the findings that intrathecal injection of serotonin or serotonin agonists suppresses withdrawal reflexes to painful stimuli (Wang, 1977; Yaksh & Wilson, 1979). Both of these systems are assumed to involve S1 receptors because there are no S2 receptors in the spinal cord. The results of the present experiments suggest that there is a third, cerebral serotonergic system mediating the analgesic effect of morphine, which involves S2 receptors. It is also possible that supraspinal S1 receptors are involved. Indirect evidence suggests that this system involves serotonergic neurons projecting from the PAG to the raphe. Clearly, an important direction for future research is to more rigidly define the roles of spinal and supraspinal S1 receptors in nociception and morphine-produced analgesia.

4. General Implications and Future Directions

With the discovery that there are several types of receptors for some neurotransmitters there has been a concerted effort to determine the functions of the different receptor types. The synthesis of drugs with a preferential affinity for specific receptor types has greatly facilitated this line of research (Carlsson, 1983). Investigation of the functional role of receptor types has been particularly intense in the case of opioid receptors. Four types of opioid receptors have been identified: mu, delta, kappa, and sigma. Many investigators have attempted to attribute subsets of the multiple effects of opiate drugs to each of these receptor types by administering peptides that are selective for a particular type. For example, mu receptors are thought to mediate the cataleptic effect of opiates, whereas, delta receptors are thought to mediate the respiratory depressant effect (Pasternak et al., 1983).

Recently, similar functional investigations have been carried out for the serotonin receptor types. The development of S1 receptor agonists and S2 receptor antagonists has greatly facilitated this line of research. Mendelson and Gorzalka's (1985a,b; 1986a,b) analysis of the differential role of serotonin receptor types in male and female sexual behavior has accounted for many of the contradictory data in this area of research. Similarly, the line of research on specific serotonin receptor types and morphine-produced analgesia initiated here may eventually resolve some of the controversies in this field.

The use of receptor-selective compounds in behavioral research is not, however, without its problems. The main problem is that there is always some question about the selectivity of any compound. It seems that the selectivity of many drugs has proven to be inversely proportional to the elapsed time since its original synthesis. This is a somewhat flippant way of pointing out that the more a drug is studied, the greater the number of effects it is found to have. However, drugs do not have to be totally selective to be of value in psychopharmacological research. Pirenperone and ketanserin are a case in point. They were originally reported to be highly selective antagonists of S2 receptors, with no activity at S1 receptors (Colpaert & Janssen, 1982; Colpaert et al., 1983; Leysen et al.,1981). This selectivity for the S2 receptor type relative to the S1 type has held, but recently these drugs have been reported to also be moderately potent blockers of alpha-adrenergic receptors (Janssen, 1983). Fortunately, the more selective S2 receptor blocker, LY53857 was synthesized and it has turned out to have no activity at alpha-adrenergic receptors (Cohen, Fuller, & Kurtz, 1983). Thus, by comparing the effects on morphine-produced analgesia of pirenperone, ketanserin, and LY53857, the hypothesis that an action at alpha-adrenergic receptors was responsible for the anti-analgesic effect of pirenperone and ketanserin could be discounted.

Another problem encountered when using receptor-selective drugs is the continual discovery of additional receptor types.

For example, the existence of a third serotonin receptor type
(Fozard, 1984; cf. Feuerstein & Hertting, 1986), known as the M
receptor or S3 receptor, raises the possibility that the
attenuation of morphine-produced analgesia by pirenperone,
ketanserin, and LY53857 may be mediated by an action at this
third serotonin receptor site. However, this hypothesis is
unlikely for two reasons. First, there is no firm evidence that
S3 receptors exist in the central nervous system. Because the
present experiments implicate central receptors in the anti-
analgesic effect of S2 receptor blockers, this would argue
against a role for S3 receptors. However, firm conclusions must
await autoradiographic localization of S3 receptors. Second,
Feuerstein and Hertting (1986) recently found that the selective
S3 receptor blockers MDL 72222 and ICS 205-930 attenuated
serotonin-induced transmitter release, whereas ketanserin did
not. This suggests that ketanserin does not act at S3 receptors.

The present studies also took advantage of differences in
the pharmacological properties of two similar drugs to roughly
localize S2 receptors that mediate the anti-analgesic effect of
S2 receptor blockade. Pirenperone and ketanserin are
pharmacologically similar, except that pirenperone readily enters
the central nervous system, whereas ketanserin does not (Laduron
et al., 1982). By exploiting this difference, it was possible to
show that the S2 receptors responsible for the attenuation of
morphine-produced analgesia are in the central nervous system.
This strategy is not widely used because it is rare for two drugs

of highly similar structure to differ only in their disposition. The greater the differences in the two drugs, the greater the number of possible interpretations.

A recent trend in psychopharmacology has been to combine the use of receptor-selective drugs with neurosurgical techniques to precisely localize receptor populations that subserve a particular drug effect. For example, Zemlan et al. (1983) systemically administered the selective S1 agonist, 5-methoxy-N,N-dimethyltryptamine to rats with transected spinal cords and observed a facilitation of nociception. Experiment 4 of the present thesis, used the technique of spinal cord transection to assess the effect of S2 receptor blockade on the isolated spinal cord opiate analgesia system and found that pirenperone injected systemically had no effect on spinally-mediated morphine-produced analgesia thus implicating supraspinal S2 receptors in the anti-analgesic effect of pirenperone.

Cerebral S2 receptors have been shown to be particularly abundant in the accumbens, striatum, olfactory tubercle, dorsal raphe, and in the frontal and occipital cortex (Desamukh et al., 1983; Laduron et al., 1982; Marcinkiewicz et al., 1984; Pazos et al., 1985; Pazos & Palacios, 1985; Slater & Patel, 1983). A traditional physiological method that could be used to more precisely localize the receptors that mediate the anti-analgesic effect of S2 receptor blockers is the "cerveau isole" preparation, in which transection of the brain between the superior and inferior colliculi isolates the brainstem from the

midbrain and forebrain. If midcollicular transections eliminated
the attenuation of morphine-produced analgesia by S2 receptor
blockers, midbrain or forebrain S2 receptors would be implicated.
Conversely, if midcollicular transection failed to eliminate the
attenuation of morphine-produced analgesia by S2 receptor
blockers then brainstem S2 receptors would be implicated. Once
the general areas mediating the anti-analgesic effect of S2
receptor blockers have been identified, direct injection of S2
receptor blockers into brain regions that are rich in S2
receptors may precisely localize the receptors that mediate the
attenuation of morphine-produced analgesia by pirenperone,
ketanserin, and LY53857.

In summary, in this series of experiments current
psychopharmacological research strategies were adapted for the
purpose of characterizing the nature and location of the
serotonin receptors involved in the mediation of morphine-
produced analgesia. By challenging morphine-produced analgesia
with selective S2 receptor blockers, it was firmly established
that S2 receptors that mediate the analgesic effect of morphine.
By exploiting the differences in the three antagonists used in
these experiments, the attenuation of morphine-produced analgesia
was localized to the central nervous system, and the alternative
hypothesis that this effect was due to alpha-adrenergic receptor
blockade was eliminated. By using the physiological technique of
spinal cord transection, the mediation of the anti-analgesic
effect of S2 receptor blockers was localized to receptors in the

brain. These conclusions stand in marked contrast to current

views of the involvement of serotonin in morphine-produced

analgesia.   —

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