AN ABSTRACT OF THE THESIS OF

Christopher J. Hopkins for the degree of Master of Science in Pharmacy presented on August 8, 1986. Title: Pharmacological Characterization Of Endogenous Spinal Adenosine Involvement In Antinociception.

Abstract approved: Redacted for Privacy

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Endogenous spinal adenosine is implicated in the indirect action of morphine intracerebroventricularly (i.c.v.)-induced antinociception. The present investigations were designed to pharmacologically characterize spinal adenosine modulation of pain pathways and adenosine interactions with specific descending antinociceptive pathways stimulated by morphine.

Mice were injected intrathecally (i.t.) with various doses of adenosine agonists, 5'-N-ethylcarboxamidoadenosine (NECA), 2-chloroadenosine (CADO), R- and S-phenylisopropyladenosine (R-PIA and S-PIA), 10 or 15 minutes before testing for antinociception on tail flick and hot plate assays. Each adenosine agonist (i.t.) dose-dependently increased tail flick and hot plate latencies. Motor impairment induced by adenosine agonists (i.t.) as measured by the rotorod assay, was clearly differentiated from antinociception as measured by tail flick assay, since both potency and time of peak

of peak action for PIA isomers varied between assays. Antinociception and motor impairment induced by adenosine agonists (i.t.) were antagonized dose-dependently by theophylline, an adenosine receptor antagonist, in each assay. pA2 values for theophylline/adenosine agonist interactions, indicated interactions at a common receptor type. Furthermore, rank order potencies for adenosine agonists (i.t.), support involvement of A2 adenosine receptors. Differences in time courses for antinociception and motor impairment induced by adenosine agonists (i.t.), however, suggest that spinal sites mediating adenosine induced antinociception are more superficial than sites of action for motor effects.

Accumulation of data supports spinal adenosine involvement in morphine (i.c.v.)-induced antinociception. Therefore, experiments were designed to characterize adenosine involvement with specific descending neuronal pathways.

A subantinociceptive dose of NECA (0.030 nmol) (i.t.) potentiated morphine (i.c.v.) dose-response curve 3.9 fold to the left and a similar potentiation occurred with 20% antinociceptive doses of each adenosine agonist. The rank order potency for adenosine agonist (i.t.)-potentiation of the morphine (i.c.v.) dose-response curve again implicates interactions with A2 adenosine receptors. These results suggest that A2 adenosine receptors which mediate

antinociception induced by adenosine agonists (i.t.) are the same A2 adenosine receptors responsible for adenosine agonist (i.t.)-potentiation of morphine (i.c.v.)-induced antinociception.

A separate set of experiments were performed with monoaminergic and purinergic receptor antagonists administered i.t. to further characterize the descending pathways stimulated by morphine.

Mice were injected i.c.v. with various doses of morphine. Five minutes before testing for antinociception in tail flick and hot plate assays, the same mice were administered methysergide , phentolamine or theophylline i.t. in varying doses and combinations. Morphine (i.c.v.)-induced antinociception was antagonized by each antagonist in the tail flick assay. Moreover, the combination of 1 ug phentolamine and 111 nmol theophylline produced significantly greater antagonism than the combination 1 ug methysergide and 111 nmol theophylline. All antagonists (i.t.) were significantly less effective in antagonizing morphine (i.c.v.)-induced antinociception as measured by the hot plate assay. These results suggest that spinal norepinephrine and adenosine may act in a similar manner. In order to differentiate the involvement between the postsynaptic action of descending antinociceptive pathways and spinal adenosine, mice were injected i.t. with various doses of NECA, norepinephrine, serotonin, methysergide,

phentolamine or theophylline. Serotonin (i.t.) dose-response curve was antagonized by 111 nmol theophylline, however, a subantinociceptive dose of NECA had no significant effect, suggesting that serotonin induces the release of adenosine. A subantinociceptive dose of norepinephrine (1.22 nmol) (i.t.) potentiated the NECA (i.t.) or serotonin (i.t.) dose-response curves, but no antagonism of either dose-response curves was observed by phentolamine (i.t.) as measured by the tail flick assay. These results suggest that the mechanism of action for NECA (i.t.)- or serotonin (i.t.)-induced antinociception is different than the mechanism for norepinephrine (i.t.)-induced antinociception. This hypothesis is supported by experiments performed with norepinephrine. Norepinephrine (i.t.) dose-response curve was potentiated by a subantinociceptive dose of NECA (i.t.) or serotonin (58.8 nmol) (i.t.), however, no antagonism was observed with either theophylline (i.t.) or methysergide (i.t.).

These studies demonstrated antinociception and motor impairment induced by adenosine agonists administered i.t. was mediated by A2 adenosine receptors and that separate populations of A2 adenosine receptors is the most probable explanation for these different effects. In addition, spinal A2 adenosine receptors responsible for mediating the antinociceptive effects of adenosine agonists (i.t.), are probably the same

receptors responsible for mediating antagonism of morphine (i.c.v.)-induced antinociception by theophylline. Morphine (i.c.v.)-induced antinociception was potentiated by i.t. administration of adenosine agonists. This multiplicative interaction between supraspinal and spinal sites involves adenosine interactions with the descending antinociceptive noradrenergic pathway and not the serotonergic pathway. Serotonin may, however, be involved in the activation of adenosine release, suggesting that endogenous spinal serotonin and adenosine function within the same pathway which differs from the pathway and mechanism of action that norepinephrine utilizes to induce antinociception.

Pharmacological Characterization Of Spinal Adenosine Involvement In Antinociception

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed August 8, 1986

Commencement June 1987

APPROVED:

Redacted for Privacy

Professor of Pharmacology in charge of major

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Date thesis is presented August 8, 1986

Typed by Christopher Hopkins for Christopher Hopkins

Acknowledgements

The undertaking and completion of this project would not have been possible without the love and support from my family. Each one of you has given me something which I carry with me each day, thank you all very much. I thank Dr. Gary DeLander for his guidance, instruction and friendship. In two and a half short years he taught me the meaning of science; observation, theory and experimentation (note that the sequence is important), not too mention how to line up a putt. I also thank Deb for making my graphs and for providing long distance encouragement, and Glen and Cathy for the use of their home, computer and word processing expertise. I would like to thank my fellow graduate students for their advice and friendship which will always be remembered and the pharmacy office and stockroom staff for their help and cooperation. Finally to Lori, my nurse, my raquetball partner, my best friend, my wife, thank you for your encouragement. I find endless love in your heart and joy in your eyes, I dedicate this thesis to you.

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PHARMACOLOGICAL CHARACTERIZATION OF SPINAL ADENOSINE INVOLVEMENT IN ANTINOCICEPTION

INTRODUCTION

Endogenous pain pathways are considered to be among the most complex neuronal circuits found in the human body, consisting of components from both central and peripheral nervous systems. Elaborate peripheral receptors and neuronal circuits transmit painful stimuli along primary afferents into the dorsal horn of the spinal cord. Neurotransmitters, such as substance P (Nicoll et al., 1980), are released from primary afferents to activate secondary neurons which travel up the spinal cord to higher brain centers where pain perception occurs.

Several sites within the brainstem are thought to contain cell bodies of neurons that descend into the spinal cord and can be activated to endogenously inhibit pain transmission. Endogenous pain modulatory centers include the periaqueductal gray (PAG), nucleus raphe magnus (NRM), nucleus reticularis paragigantocellularis (NRPG) and locus ceruleus (LC) (Basbaum and Fields, 1978). Many drugs, including opioids, appear to interact with these centers to produce analgesia.

The use of opioids to relieve pain dates back many centuries. The mechanism of action for opioid-induced analgesia is not wholly understood, but involves both

direct interaction of opioids with specific receptors in the central nervous system (CNS) and activation of descending spinal pathways. Adenosine, a putative neurotransmitter, has been implicated in opioid-induced analgesia (Ho et al.,. 1972). It is hypothesized that morphine causes the release of adenosine to induced analgesia (Sawynok and Jhamandas, 1976). The goal of this thesis was to pharmacologically characterize antinociception induced by adenosine in the spinal cord and the possible involvement of adenosine in antinociception induced by morphine.

A. Mechanisms Of Opioid Induced Antinociception

Opiate alkaloids, such as morphine, interact with sterospecific and saturable receptors in the CNS, which are also sites of action for a number of endogenous opioid peptides (Jaffe and Martin, 1985). Opioid receptors have been localized at a variety of sites within the central and peripheral nervous systems, including the limbic system, PAG, NRM, dorsal horn of the spinal cord and gastrointestinal tract (Jaffe and Martin, 1985). At least three different opioid receptors have been identified and are referred to as mu, kappa and delta opioid receptors (Jaffe and Martin, 1985). Several additional receptors, including sigma and epsilon receptors, have been

theorized, but await additional verification (Takemori and Portoghese, 1985). Interactions of opioids with these receptors may inhibit the transmission of nociceptive impulses postsynaptically or inhibit the release of nociceptive neurotransmitters presynaptically. Opioids also induce antinociception indirectly by activating additional antinociceptive pathways.

Indirect modulation of nociception by opioids involves supraspinal stimulation of descending antinociceptive pathways which inhibit the transmission of nociceptive stimuli at the spinal level. The PAG, LC, NRM and the NRPG have each been implicated in the regulation of nociceptive stimuli (Basbaum and Fields, 1978). Activation of PAG, NRM and NRPG by electrical stimulation, microinjections of morphine or glutamate has been observed to produce antinociception (Akaike et al., 1978; Jensen and Yaksh, 1984). Antinociception is antagonized by intrathecal administration of serotonergic or noradrenergic antagonists, suggesting the involvement of serotonergic and noradrenergic neurons projecting from the brainstem to the spinal cord (Kuraishi et al., 1979; Hammond et al., 1980; Sagen and Proundfit, 1981; Hammond and Yaksh, 1984). NRM and NRPG are thought to be sources of serotonin and norepinephrine neurons, respectively (Hammond and Yaksh, 1984). It is the cummulative direct and indirect actions of opioids which are responsible for synergism observed

when intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections of opioids are administered concurrently (Yeung and Rudy, 1980a; b; Roerig et al., 1984).

Purines have been implicated in the antinociceptive actions of morphine since Ho et al. (1972, 1973) demonstrated antagonism of morphine-induced antinociception by methylxanthines, adenosine receptor antagonists. These results have been replicated in vitro (Sawynok and Jhamandas, 1979; Jurna, 1984) and in vivo (Ahlijanian and Takemori, 1985; DeLander and Hopkins, 1986). The mechanism of action for morphine/adenosine interactions is hypothesized to involve opioid-induced release of adenosine (Fredholm and Vernet, 1978; Sawynok and Jhamandas, 1979; Stone and Perkins, 1979; Phillis et al., 1979; Wu et al., 1982b). Recent studies have suggested additional types of interactions between adenosine and morphine. The present investigations were designed to further characterize adenosine involvement in antinociception.

ADENOSINE

A. Synthesis, Release And Uptake Of Adenosine

Adenosine is chemically designated as a nucleoside, consisting of the purine base adenine and the sugar

ribose. The purine structure components come from several sources, formate, carbon dioxide, and the amino acids aspartic acid, glutamate and glycine (Stryer, 1980). A number of biosynthetic mechanisms exist for adenosine (Snyder, 1985).

The release of adenosine triphosphate (ATP), adenosine and other purines from peripheral and central nervous tissue is well established (Snyder, 1985). Pull and McIlwain (1977) find that adenosine comprises the majority of tritium labeled purines released from electrically stimulated or potasium depolarized brain slices incubated with tritium labeled adenine. Adenosine release is calcium-dependent (Phillis and Wu, 1981; Stone, 1981). To this date, no direct evidence for purine-containing neurons in the CNS or peripheral nervous system exist, however, it is known that ATP is stored and co-released from cholinergic (Fredholm and Hedqvist, 1980) and noradrenergic (Snyder, 1985) presynaptic nerve terminals.

Adenosine deaminase, an intracellular cytoplasmic enzyme, degrades adenosine to inosine (Snyder, 1985). Deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl-) adenine (EHNA) are two potent inhibitors of adenosine deaminase and are used as two pharmacological tools to increase endogenous levels of adenosine (Zetterstorm et al., 1982).

As is the case for biogenic amines and amino acid

neurotransmitters, specific high affinity, sodium dependent uptake mechanisms in nerve terminals are primarily responsible for terminating the effects of adenosine. Bender et al. (1981) differentiates adenosine uptake into discrete rapid and slow uptake systems. The slow system consists of high and low affinity components with respective Km values of 1 and 5 micromolar. Bender et al. concludes that there are two functional pools of adenosine in synaptosomes which may reflect different uses (i.e. metabolism or release). In nervous tissue, nitrobenzylthioinosine (NBI) is a potent inhibitor of adenosine reuptake sites, which are distinct from adenosine receptors mediating pharmacologic effects of adenosine (Bareris et al., 1981). NBI is the drug of choice over other adenosine reuptake inhibitors, such as dipyridamole, papaverine and 2-deoxyadenosine, due to their lack of specificity (Geiger et al., 1985).

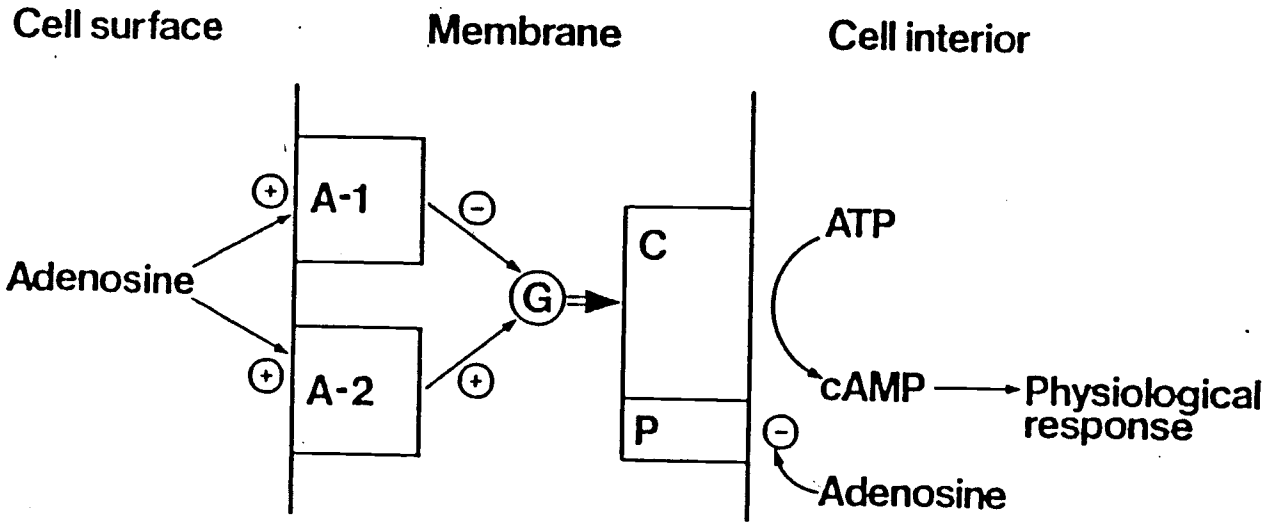
B. Adenosine Receptors

The observation that methylxanthines block effects of adenosine and not ATP allowed Burnstock (1981) to postulate that effects of adenosine and ATP are mediated by different receptors. Burnstock classified purinergic receptors into two types, P1 for adenosine and P2 for ATP.

P1 adenosine receptors have been broken down into

Figure 1. Schematic of adenosine receptor function. Abbreviations: G = G protein of adenylate cyclase; C= catalytic unit of adenylate cyclase; + = activation; - = inhibition. (Adapted from Williams, 1984.)

FIGURE 1


Cell surface
Membrane
Cell interior
A-1
A-2
Adenosine
G
C
P
ATP
cAMP
Physiological response
Adenosine

two distinct extracellular cell surface receptors. A1 receptors interact with nanomolar concentrations of adenosine, whereas A2 receptors require micromolar concentrations. Daly (1977) has characterized the P1 receptor subtypes based on their ability to influence adenylate cyclase. Stimulation of A2 adenosine receptors leads to an increase in adenylate cyclase activity and intracellular cyclic adenosine monophosphate (cAMP) levels, while A1 adenosine receptors are coupled to adenylate cyclase in an inhibitory manner (fig. 1). In addition to the differential effects on adenylate cyclase, A1 and A2 receptor sites can be differentiated by the relative potency of adenosine agonists. Rank order potency of adenosine agonists interacting at an A1 adenosine receptor is characterized by R(-) N (2-phenylisopropyl) adenosine (R-PIA) > 2-chloroadenosine (CADO) > adenosine > 5'-(N-ethyl)-carboxamido-adenosine (NECA) > D(+) N (2-phenylisopropyl) adenosine (S-PIA) (Williams,1984), while A2 adenosine receptors are recognized by the rank order potency NECA > CADO > R-PIA > S-PIA > adenosine. Methylxanthines, such as theophylline and caffeine, antagonize A1 and A2 receptors with similar potencies.

Daly et al. (1984) has proposed further division of adenosine A2 receptors into two classes, based on observations that some A2 adenosine receptors have EC50

values for adenosine in the high nanomolar range as opposed to the characteristic micromolar range. High affinity A2 receptors have thus far been localized in the striatum and nucleus accumbens whereas low affinity A2 receptors exist in almost all areas of the brain (Bruns et al., 1986).

Londos et al. (1980) demonstrated that intracellular P2 sites activated by adenosine related agents with intact purine rings, could reduce accumulation of cyclic AMP. 2',5'-dideoxyadenosine is a potent agonist at intracellular sites, while adenosine is weak and PIA is relatively inactive. Unlike A1 and A2 receptor sites, intracellular sites are uneffected by methylxanthines (Londos et al., 1980). The physiologic role of the intracellular site is unknown.

C. Biochemical, Physiological And Pharmacological Actions Of Adenosine

Biochemically adenosine plays an important role in the biosynthesis of nucleic acids (DNA and RNA), ATP and cAMP, but it also has a wide variety of effects in its native state.

Peripherally administered adenosine has significant actions on the cardiovascular system causing vasodilation, hypotension, negative chronotropic (Drury and

Szent-Gyorgyi, 1929), inotropic and dromotropic effects (Schrader et al., 1977). Adenosine induced bronchial constriction, inhibition of platelet aggregation and lipolysis (Snyder, 1985), and inhibition of acetylcholine release at the neuromuscular junction (Fredholm and Hedqvist, 1980) have also been observed.

Central effects of adenosine and adenosine analogs include sedation, hypothermia and increased convulsive thresholds (Phillis and Wu, 1981). Inhibition of presynaptic neurotransmitter release by adenosine has been well characterized in brain slices (Harms et al., 1979; Hollins and Stone, 1980; Phillis and Wu, 1981), and is antagonized by methylxanthines (Phillis and Wu, 1981). Adenosine mediated inhibition of presynaptic neurotransmitter release has been reported for acetylcholine, dopamine, norepinephrine, serotonin and gama-aminobutyric acid (Snyder, 1985).

Decreased release of excitatory neurotransmitters induced by adenosine, appears to involve the blockade of calcium ion influx into nerve terminals, since calcium uptake by synaptosomal preparations is inhibited by adenosine and restored by methylxanthines (Ribeiro et al., 1979; Wu et al., 1982a). Moreover, Dunwiddie et al. (1981) and Dunwiddie and Frehold (1984) find that calcium is involved in presynaptic inhibitory electrophysiologic effects of adenosine acting at A1 adenosine receptors.

D. Adenosine Involvement In Opioid-Induced Antinociception

As discussed previously, several investigations support the hypothesis that morphine, after interacting with its receptor, increases extracellular adenosine, which in turn contributes to the antinociceptive effects of morphine. Recent results also demonstrate that adenosine at spinal sites appears to cause antinociception and interact with opioid-stimulated descending antinociceptive pathways (DeLander and Hopkins, 1986). Morphine (i.c.v.)-induced antinociception is dose dependently antagonized by intrathecal (i.t.) administration of theophylline, an adenosine receptor antagonist (DeLander and Hopkins, 1986). The results were replicated with two additional methylxanthines, caffeine and isobutylmethylxanthine (IBMX). Antagonism of morphine (i.c.v.)-induced antinociception by methylxanthines appears to be mediated via spinal adenosine receptors and not via inhibition of phosphodiesterase enzymes. The actual mechanism involved in spinal methylxanthine-mediated antagonism of morphine (i.c.v.)-induced antinociception or spinal adenosine-mediated antinociception, however, is poorly characterized.

Administration of adenosine or adenosine analogs also induces antinociception. Vappatalo et al. (1975) was the

first to demonstrate antinociception induced by adenosine or adenosine analogs following systemic administration of R-phenylisopropyladenosine (R-PIA) in rats. Their results have since been replicated in rats (Holmgren et al., 1983) and in mice (Ahlijanian and Takemori, 1985). Yarbough and McGuffin-Clineschmidt (1981) demonstrate antinociception after i.c.v. injections of adenosine, 5'-N6-ethylcarboxamidoadenosine (NECA) or 2-chloroadenosine (CADO) to rats. Preliminary studies suggest adenosine agonists administered i.t. also induce antinociception in rats (Holmgren et al., 1984; Post, 1984) and in mice (DeLander and Hopkins, 1986). The present investigations were designed to pharmacologically characterize spinal adenosine involvement in pain pathways and adenosine interactions with specific descending antinociceptive pathways.

METHODS

Animals

Male, Swiss-Webster mice weighing 20 to 30 grams (Simonsen; Gilroy Ca.) were housed in our vivarium on a twelve hour light/dark schedule and allowed free access to food and water. Each animal was used once.

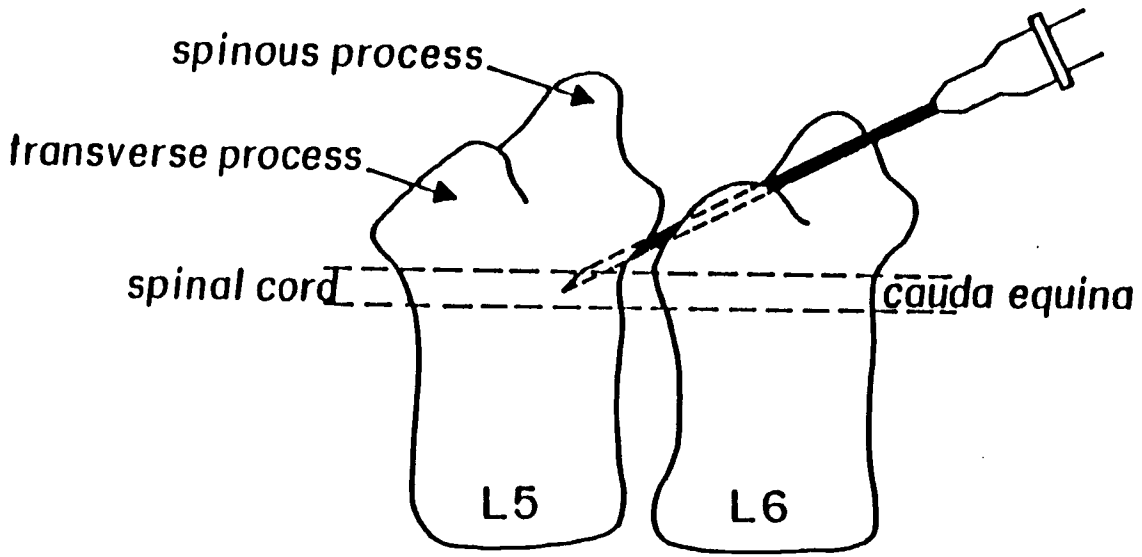
Drugs And Drug Administration

Intracerebroventricular injections (i.c.v.) were performed as described by Haley and McCormick (1957). Intrathecal injections (i.t.) were performed using a modification of the method described by Hylden and Wilcoxon (1980) (fig. 2). Drugs administered i.c.v. and i.t. were prepared such that the desired dose was in 5 microliters. Interactions of multiple drugs at spinal sites were administered such that each drug reached their peak time for drug action concurrently.

Adenosine agonists, 2-chloroadenosine (CADO), 5'-(N-ethyl)-carboxamido-adenosine (NECA), R(-)N (2-phenylisopropyl) adenosine (R-PIA) and D(+)N (2-phenylisopropyl) adenosine (S-PIA) were solubilized by adding 0.5 ml of 0.1 molar hydrochloric acid to a

Figure 2. Schematic of intrathecal injections in mice. Insertion of a 30 gauge needle, along spinious and transverse processes, into spinal canal in lumbar region of mouse spinal cord. (Illustration is reproduced with permission of Drs. Hylden and Wilcox.)

FIGURE 2


spinous process
transverse process
spinal cord
cauda equina
L5
L6

solution of the desired adenosine agonist concentration in 4.5 ml of normal saline. NECA, however, only required 0.3 ml of dilute hydrochloric acid with gentle heating. Nitrobenzylthioinosine (NBI) was prepared by dissolving 21 mg in 100% dimethylsulfoxide (DMSO) and then making 1:1 dilutions with normal saline to reach desired concentrations. All other drugs were dissolved directly into normal saline. CADO, NECA, R-PIA, S-PIA, clonidine, caffeine, methysergide, phentolamine and theophylline required gentle heating to achieve complete solubilization. Morphine sulfate, adenosine, norepinephrine, 5-hydroxytryptamine and NBI required no heating.

Adenosine hemisulfate salt, (-) arterenol (norepinephrine), caffeine, 2-chloroadenosine (CADO), clonidine hydrochloride, 5-hydroxytryptamine, S-(p-nitrobenzyl)-6-thioinosine (NBI) and theophylline were obtained from Sigma Chemical Company (St. Louis, Mo.). 5'-(N-ethyl)-carboxamido-adenosine (NECA) was obtained from Boehringer Mannheim (Mannheim, West Germany). R(-)N (2-phenylisoproyl) adenosine (R-PIA) and D(+)N (2-phenyliso-propyl) adenosine (S-PIA) were obtained from Reseach Biochemicals Inc. (Wayland, Ma.). Methysergide maleate, phentolamine and morphine sulfate were obtained from Sandoz Pharmaceuticals (Hanover, N.J.), CIBA GEIGY Corp. (Summit, N.J.) and Mallinckrodt Chemical Works (St.Louis,

Mo.), respectively.

Behavioral Assays

Assessment Of Antinociception

Two assays were employed to assess antinociception. The tail flick assay of D'Amour and Smith (1941) utilizes a high intensity light focused on the tail of a mouse. The first tail movement is accepted as the endpoint and the typical mean control latency was 2.5 seconds. The hot plate assay described by Eddy and Leimbach (1953) involves placing a mouse within a cylinder upon a copper surface heated to 55 C by a circulating water bath. Licking of the front paws twice, back paws once or jumping were used as endpoints and a mean control latency of 8.0 seconds was typical. The tail flick assay is generally thought to measure the spinal reflec arc (Hammond and Yaksh, 1984), while the hot plate assay is thought to measure pain pathways that require supraspinal processing. Mice were considered to be positive for antinociception after drug treatment if differences between test and control latencies for a mouse were greater than three standard deviations of mean of control latencies for all groups tested that day. A cut-off time of 5.0 and 30.0 seconds was used for tail flick and hot plate assays, respectively, to avoid lasting

injury to animals in the absence of a response. Ten mice were used per dose and ED50 values were generally determined using the procedure described by Litchfield and Wilcoxon (1949) using a computer (Pharm Basic. pcs; Life Sci. Assc., Bayport, NY). ED50 values and potency ratios for adenosine agonists, however, were determined using the Finney statistical methods for biological assay (1964).

Assessment Of Motor Function

Mice were confined to a 3 centimeter section of a rod rotating at 6 revolutions per minute and given three opportunities to demonstrate their ability to remain on the rod for 60 seconds. Animals were used only if they succeeded in trials. An animal was considered to have significant hindlimb paralysis if the animal was unable to remain on the rotorod for 60 seconds after drug treatment.

Experimental Protocol

Characterization Of Spinal Adenosine Involvement In Antinociception And Motor Function

Mice were injected intrathecally (i.t.) with various doses of adenosine, CADO, NECA, R-PIA or S-PIA. Ten minutes after NECA, R-PIA or S-PIA i.t. injections, animals

were tested for antinociception on the tail flick asay. NECA treated animals were also tested for antinociception on the hot plate assay 10 minutes after i.t. injections. The time of peak effect for R-PIA and S-PIA in the hot plate assay was 15 minutes after intrathecal injections. Time of peak antinociception effect for adenosine and CADO (i.t.)-treated animals occurred at five minutes for tail flick and hot plate assays.

Antagonism of adenosine agonist (i.t.)-induced antinociception was determined by comparing the results of experiments above to those obtained when theophylline, an adenosine receptor antagonist, was coadministered i.t. with each adenosine agonist.

Effects of adenosine at spinal sites was determined by i.t. coadministration of adenosine and nitrobenzylthioinosine (NBI), an adenosine reuptake inhibitor. Ten minutes after i.t. injections, mice were tested for antinociception.

Characterization of spinal adenosine involvement in motor function was performed by injecting mice i.t. with various doses of adenosine agonists or combinations of adenosine agonists and theophylline. Adenosine, CADO and NECA (i.t.) treated animals were tested for motor function in the rotorod assay five, five and ten minutes after i.t. injections, respectively. Animals treated with R-PIA or S-PIA were tested 30 minutes after i.t. injections.

Characterization Of Spinal Adenosine Interactions With Specific Descending Antinociceptive Pathways

The influence of opioid stimulated descending noradrenergic antinociceptive pathways was determined by administering phentolamine, a noradrenergic antagonist, i.t. ten minutes after morphine i.c.v. injections. Fifteen minutes after morphine injections animals were tested for antinociception. Involvement of opioid stimulated descending serotonergic pathways was assessed in a similar manner using methysergide, a serotonergic antagonist, in place of phentolamine. Interactions between spinal adenosine and each of these pathways was determined by comparing the results above to those obtained when theophylline was coadministered i.t. with either phentolamine or methysergide.

Interactions between adenosine agonists and descending antinociceptive pathways were also investigated to verify results obtained with the adenosine receptor antagonist theophylline. Morphine (i.c.v.) treated animals were administered i.t. injections of subantinociceptive doses of NECA or 20% antinociceptive doses of NECA, R-PIA or S-PIA five minutes after morphine. Twenty percent antinociceptive doses of CADO were administered i.t. ten minutes after morphine. Fifteen minutes after morphine treatment, animals were tested for antinociception. The

involvement of endogenous spinal adenosine was determined by injecting nitrobenzylthioinosine (NBI), an adenosine reuptake inhibitor, i.t. ten minutes before testing morphine (i.c.v.)-treated mice for antinociception.

A separate set of experiments were designed to determine the influence of spinal adenosine upon antinociception induced by stimulation of postsynaptic spinal noradrenergic or serotonergic receptors. Spinal noradrenergic pathways were investigated by administering norepinephrine i.t. to mice, and testing for antinociception five minutes later. Interactions between spinal noradrenergic pathways and spinal serotonergic or purinergic pathways were determined by i.t. administration of norepinephrine in combination with phentolamine, serotonin, methysergide or theophylline. Interactions between norepinephrine and NECA required that norepinephrine be administered five minutes after NECA and antinociception be determined ten minutes after NECA. Similar experiments were performed with clonidine, a relatively selective alpha 2 receptor agonist, in place of norepinephrine.Interactions between the spinal serotonergic and purinergic pathways were assessed in a similar manner.

RESULTS

Time Course Of Antinociception And Motor Impairment Induced By Intrathecal Administration Of Adenosine Agonists

Behavioral experiments were performed to determine time of peak effect and ED50 values for adenosine and four adenosine analogs. Time courses for effects induced by i.t. injections of adenosine, NECA and R-PIA are shown in figures 3, 4 and 5 respectively. Peak time for drug action of adenosine (i.t.) was five minutes for all assays and all animals returned to control latencies by 30 minutes. Peak time for drug action of NECA (i.t.) was ten minutes for tail flick and hot plate assays, but 15 minutes on the rotorod assay. NECA (i.t.) treated animals had returned to control latencies by 60 minutes. Peak time for R-PIA-induced effects on tail flick, hot plate and rotorod assays occurred at 10, 15 and 30 minutes, respectively. All R-PIA treated animals returned to control latencies on tail flick and hot plate assays by 120 minutes. It should be noted that determining behavioral endpoints on tail flick and hot plate assays over a 120 minute time course is difficult. Onset of respiratory depression and hind limb paralysis induced by adenosine agonists added to the difficulty. Hind limb paralysis-induced by R-PIA (i.t.) was very long lasting due to the high doses of R-PIA

Figure 3. Time course for behavioral responses induced by adenosine administered i.t. Adenosine was administered i.t. in doses which typically induced 50% response; 31.6 nmol for the tail flick (●) assay and 126.4 nmol for hot plate (■) and rotorod (▲) assays.

FIGURE 3

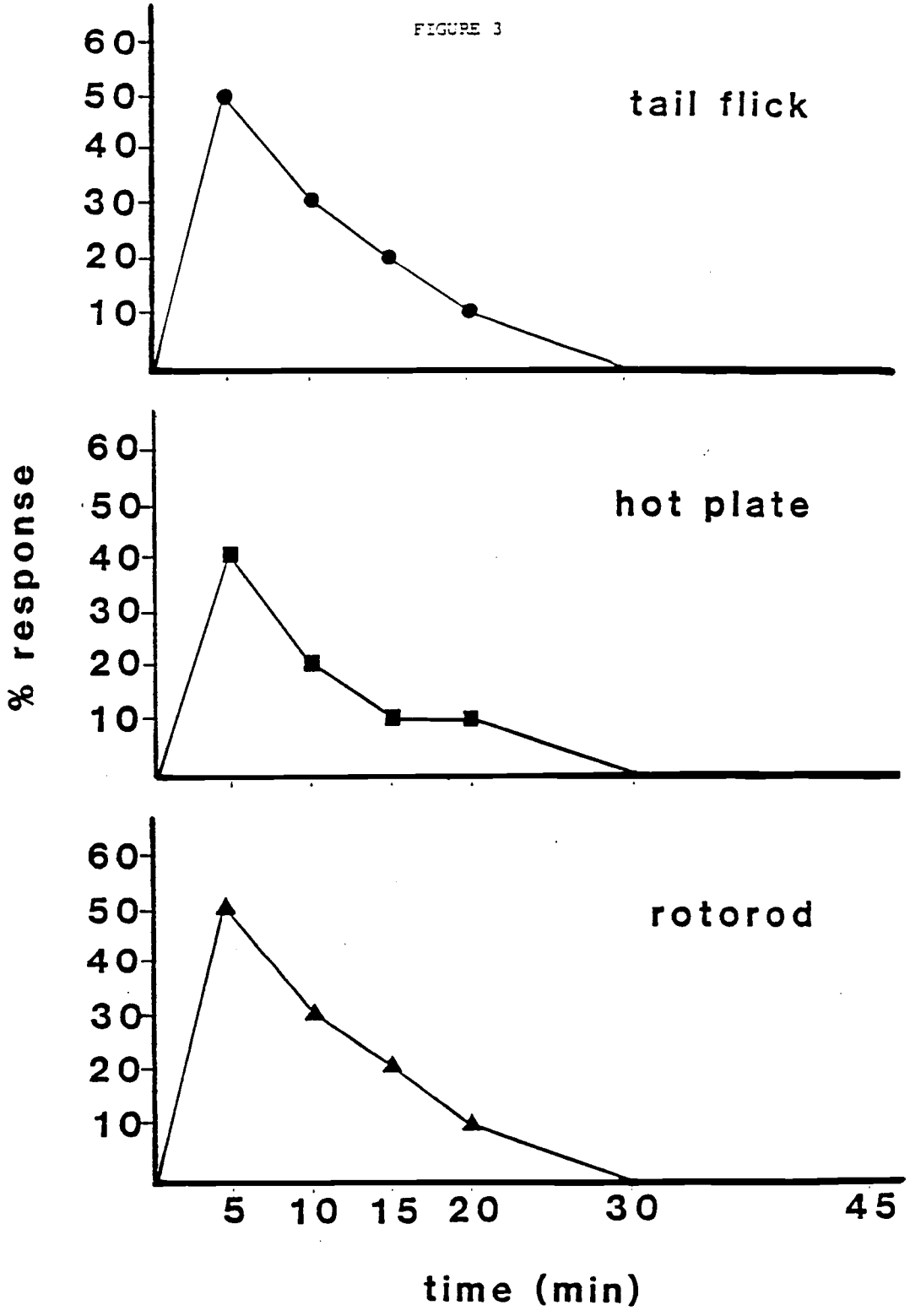

60
50
40
30
20
10
tail flick
hot plate
rotorod
% response
5
10
15
20
30
45
time (min)

Figure 4. Time course for behavioral responses induced by NECA administered i.t. NECA was administered i.t. in a dose which typically induced 50% response (0.122 nmol NECA). Responses were observed in tail flick (●), hot plate (■) and rotorod (▲) assays.

FIGURE 4

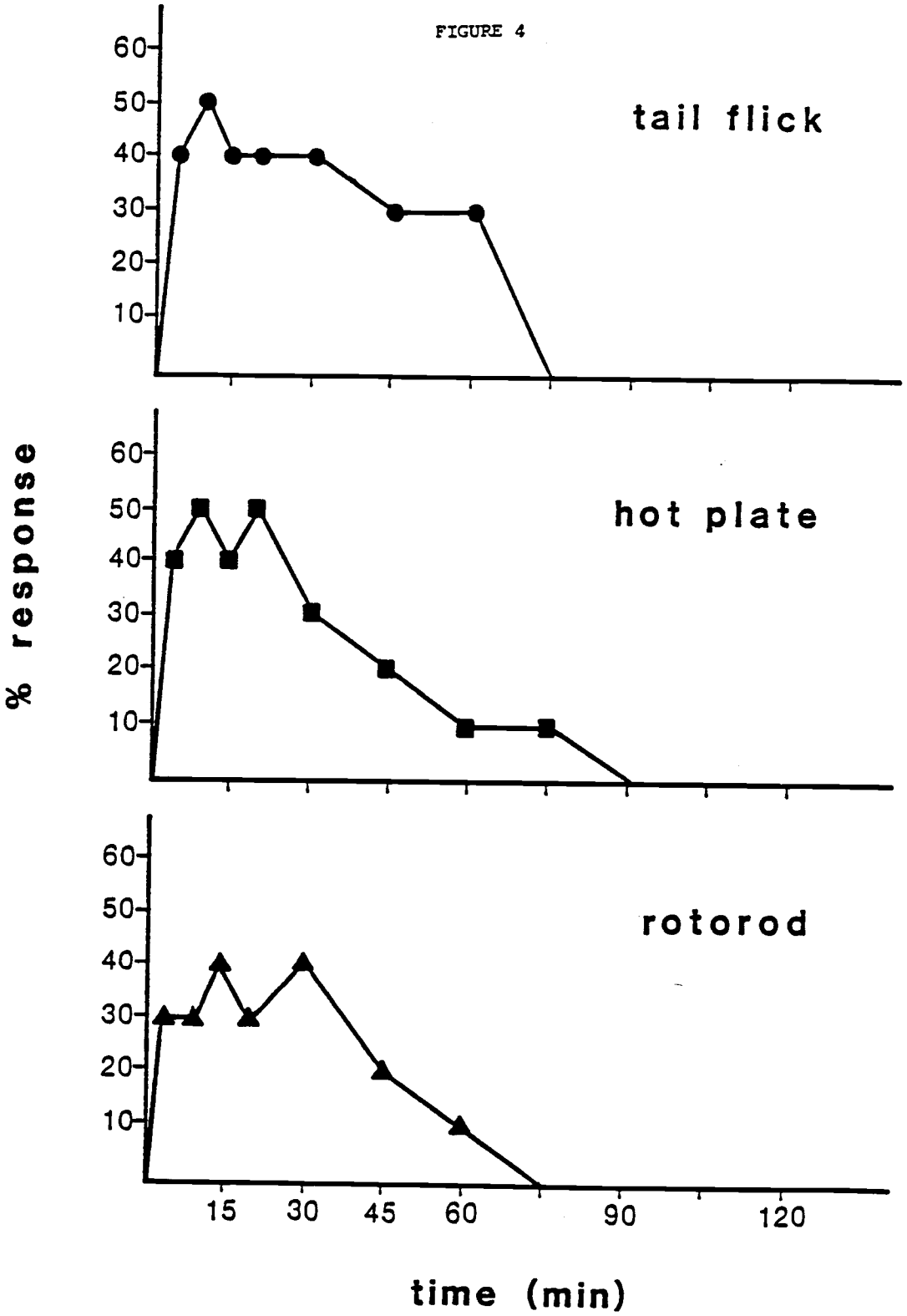
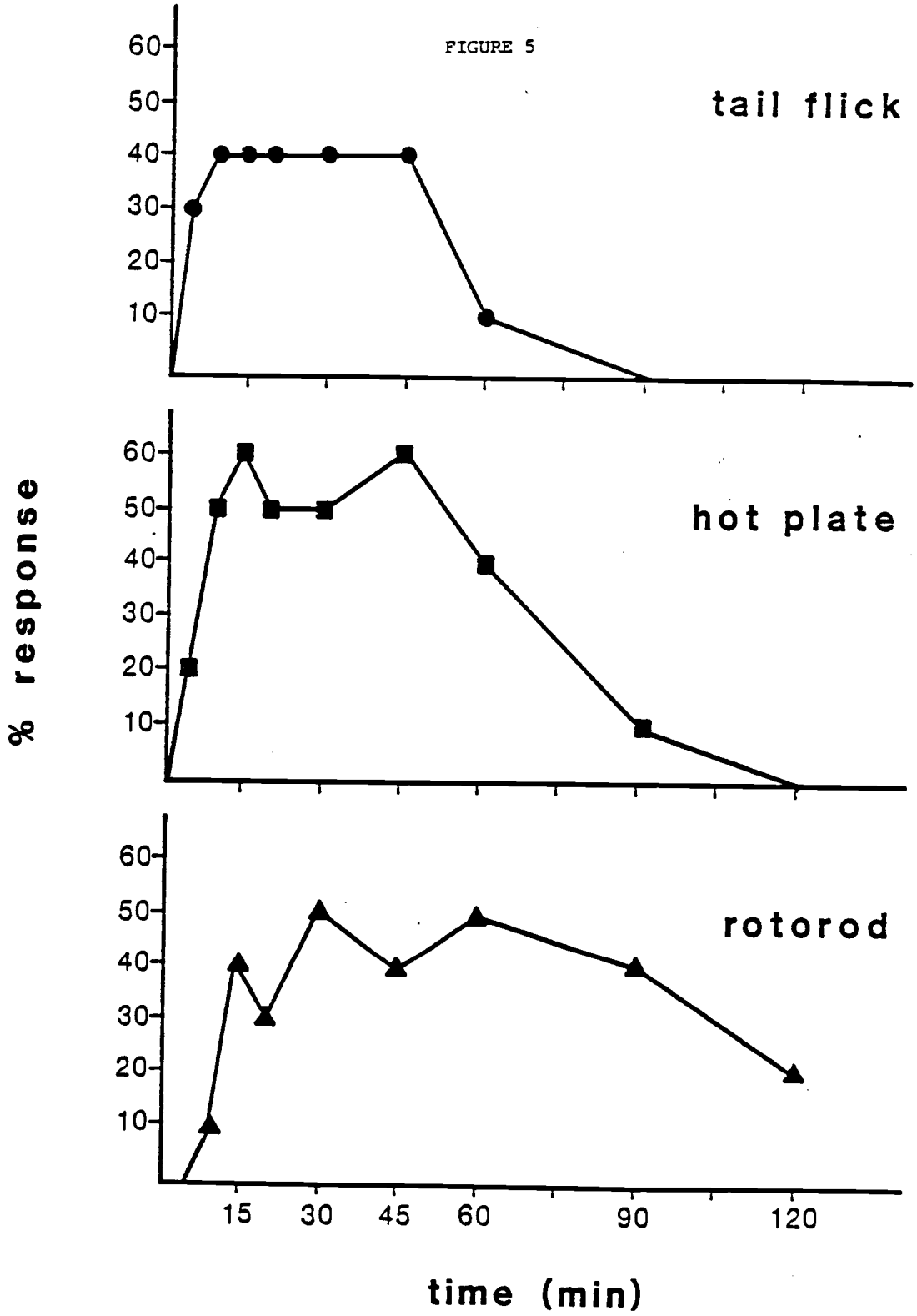

tail flick
hot plate
rotorod
% response
60
50
40
30
20
10
15
30
45
60
90
120
time (min)

Figure 5. Time course for behavioral responses induced by R-PIA administered i.t. R-PIA was administered i.t. in a dose which typically induced 50% response; 0.243 nmol for the tail flick (●) assay and 0.973 nmol for hop plate (■) and rotorod (▲) assays.

FIGURE 5


tail flick
hot plate
rotorod
% response
60
50
40
30
20
10
15
30
45
60
90
120
time (min)

used, however, all treated animals had returned to control values by 24 hours.

Incomplete time courses for CADO and S-PIA were performed to determine their peak time of drug action. CADO had a similar peak time for drug action as adenosine, five minutes for all assays, while S-PIA had similar peak times for drug action as R-PIA, 10, 15 and 30 minutes on the tail flick, hot plate and rotorod assays, respectively.

Antinociception And Motor Impairment Induced By Intrathecal Administration Of Adenosine Or Adenosine Agonists

All adenosine agonists produced dose-dependent increases in tail flick and hot plate latencies (fig. 6 and 7). Dose-dependent hind limb paralysis was also observed for each adenosine agonist (fig. 8). Rank order potencies for antinociception and motor impairment induced by adenosine agonists administered i.t. were the same for all assays, NECA > R-PIA > CADO > S-PIA > adenosine. Table 1 contains ED50 values and the corresponding 95% confidence limits for each adenosine agonist administered i.t. in each assay. Adenosine (i.t.) was the least potent compound in all assays (table 1). Co-administration of adenosine and 25 nmol NBI i.t. significantly increased the potency of adenosine (ED50 value = 17.4 nmol, with a 95% confidence interval of 11.6 to 26.0) as measured by the tail flick

Figure 6. Antinociception induced by i.t. injections of adenosine agonists as measured by the tail flick assay. Mice were injected i.t. with various doses of NECA (□), R-PIA (O) or S-PIA (+) 10 minutes before testing for antinociception in the tail flick assay. Intrathecal injections of CADO (△) were made 5 minutes before testing in the tail flick assay.

Figure 6

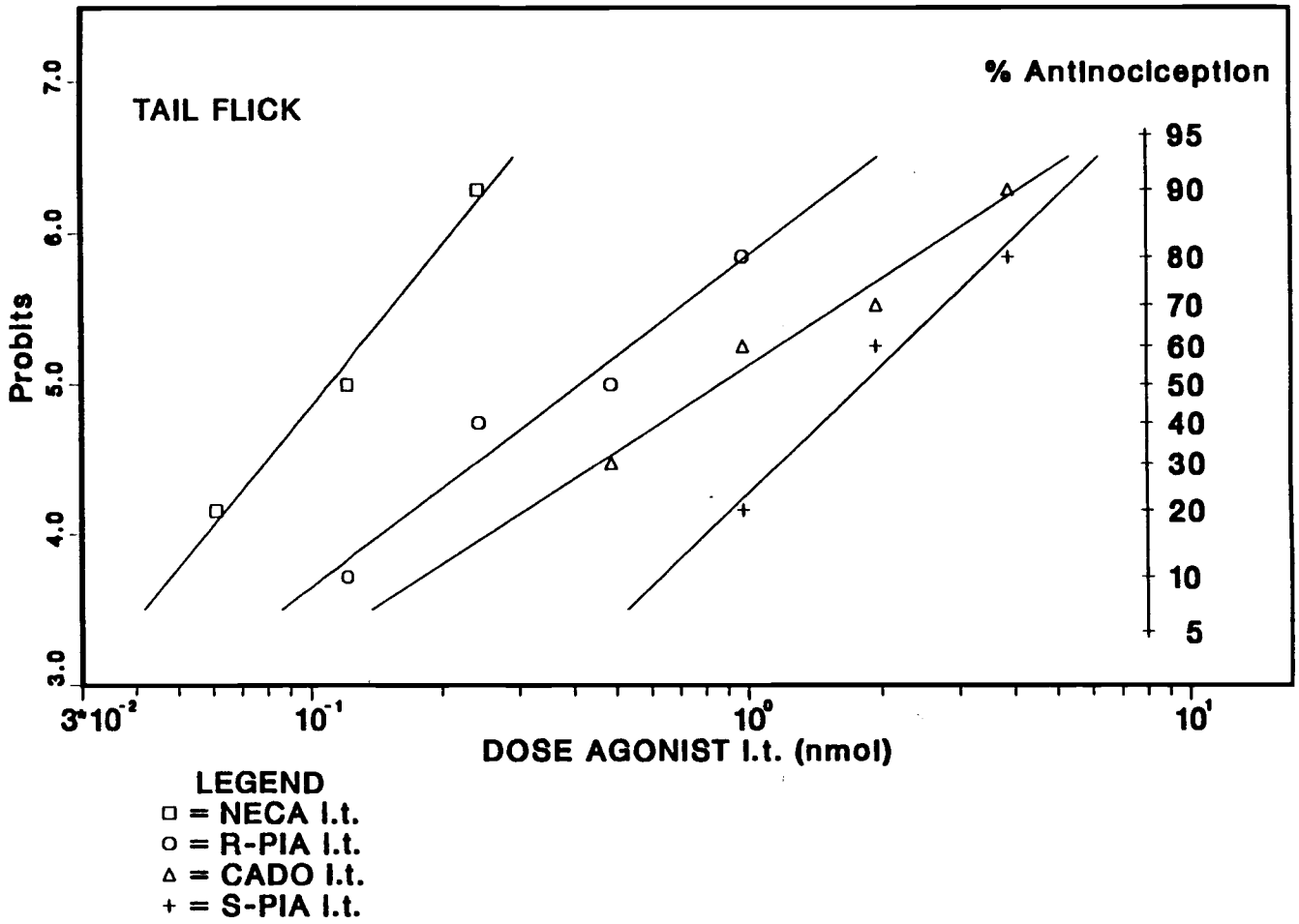
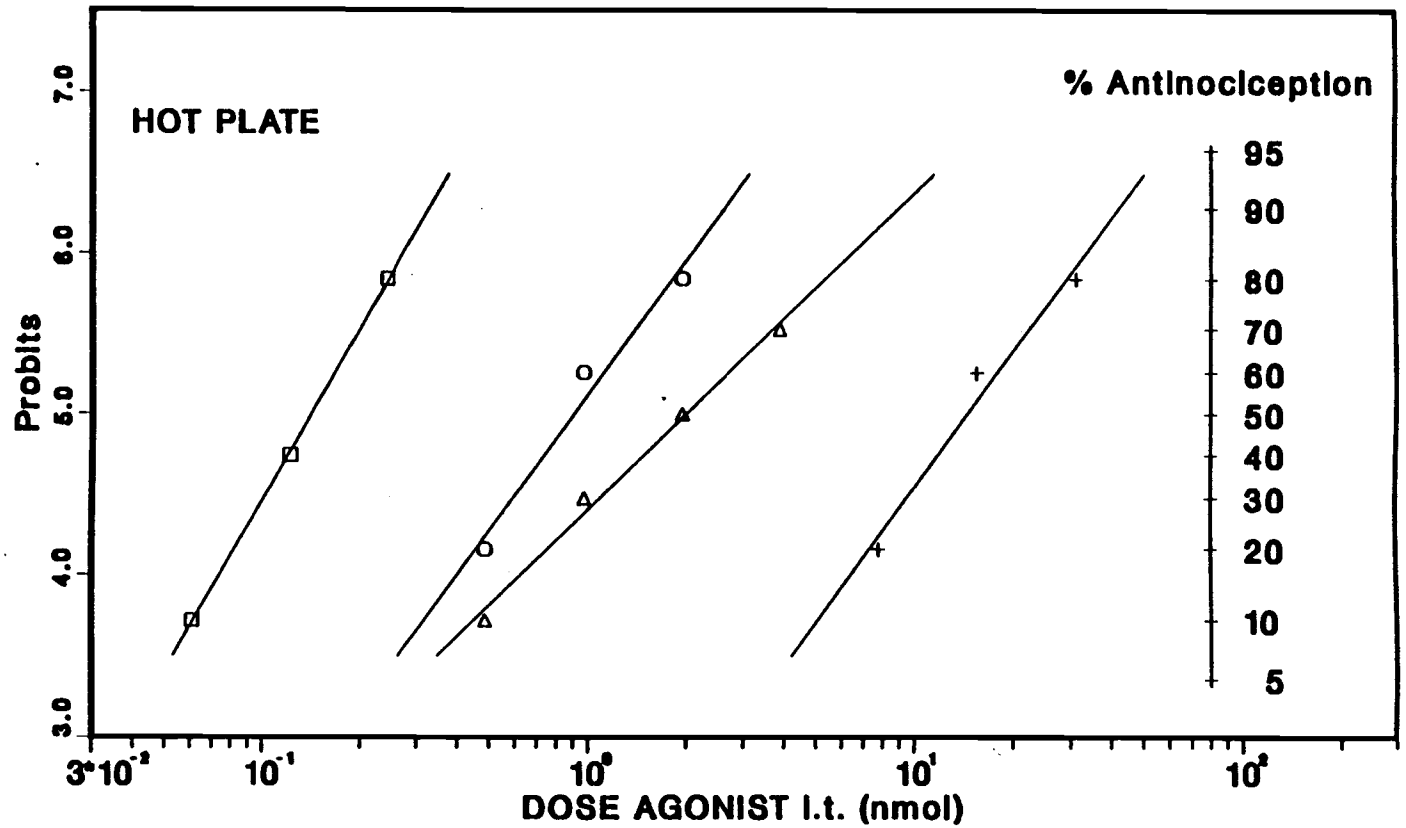

TAIL FLICK
% Antinociception
Probits
7.0
6.0
5.0
4.0
3.0
95
90
80
70
60
50
40
30
20
10
5
3*10⁻²
10⁻¹
10⁰
10¹
DOSE AGONIST I.t. (nmol)
LEGEND
□ = NECA I.t.
o = R-PIA I.t.
Δ = CADO I.t.
+ = S-PIA I.t.

Figure 7. Antinociception induced by i.t. injections of adenosine agonists as measured by the hot plate assay. Mice were injected i.t. with various doses of CADO (△) or NECA (□) 5 or 10 minutes, respectively, before testing for antinociception in the hot plate assay. Intrathecal injections of R-PIA (O) or S-PIA (+) were made 15 minutes before testing in the hot plate assay.

Figure 7



LEGEND
□ = NECA I.t.
○ = R-PIA I.t.
△ = CADO I.t.
+ = S-PIA I.t.

Figure 8. Motor impairment induced by i.t. injections of adenosine agonists. Mice were injected i.t. with various doses of NECA (□) or CADO (△) 5 minutes before testing for motor impairment on the rotorod assay. Intrathecal injections of R-PIA (O) or S-PIA (+) were made 30 minutes before testing.

Figure 8

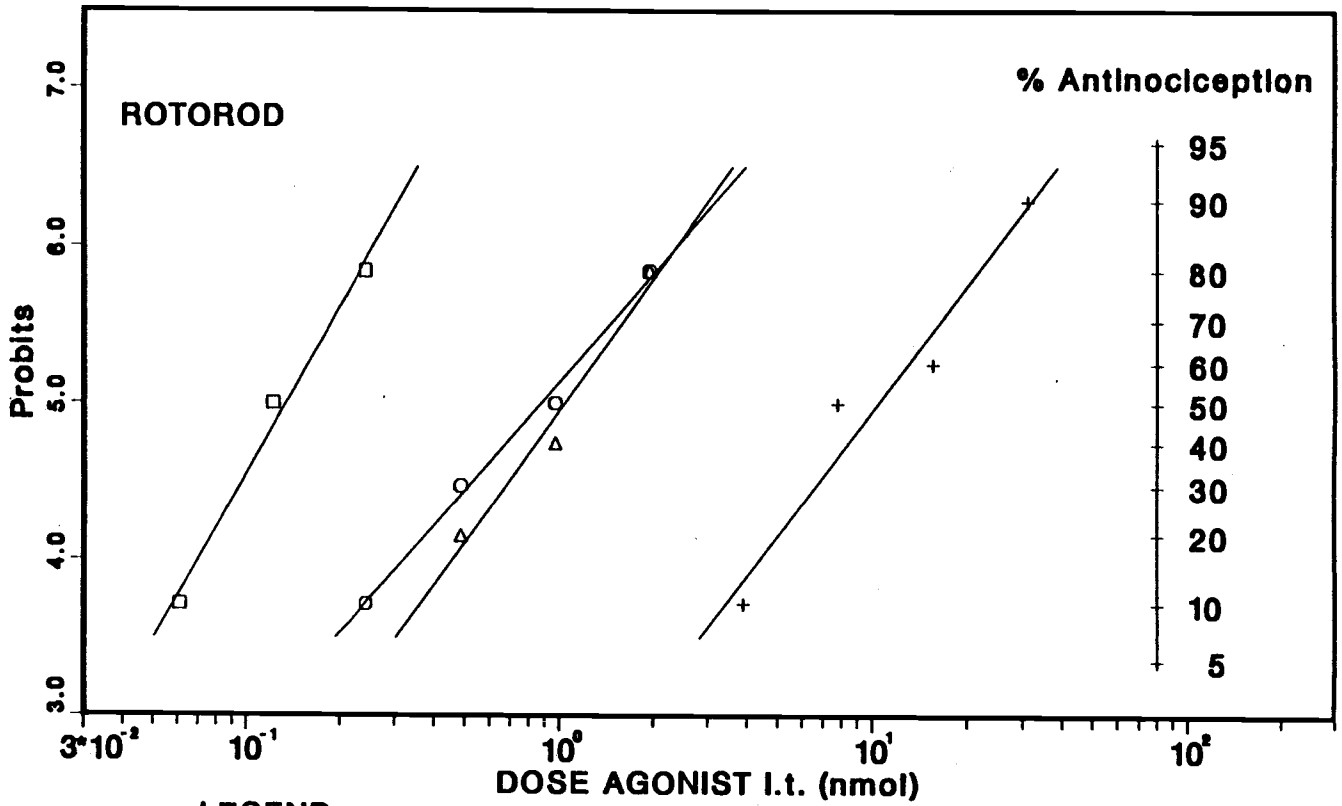

ROTOROD
% Antinociception
Probits
7.0
6.0
5.0
4.0
3.0
95
90
80
70
60
50
40
30
20
10
5
3*10⁻²
10⁻¹
10⁰
10¹
10²
DOSE AGONIST I.t. (nmol)
LEGEND
□ = NECA I.t.
○ = R-PIA I.t.
△ = CADO I.t.
+ = S-PIA I.t.

Table 1. ED50 values for adenosine agonists (i.t.)-induced antinociception or motor impairment in tail flick, hot plate and rototrod assays.

AGONIST	TAIL FLICK nmol	HOT PLATE nmol	ROTOROD nmol
NECA	0.11 (0.074 to 0.17)a	0.14 (0.095 to 0.21)	0.13 (0.09 to 0.20)
R-PIA	0.41 (0.24 to 0.70)	0.91b (0.60 to 1.4)	0.88b (0.53 to 1.5)
CADO	0.86 (0.47 to 1.6)	2.0b (1.1 to 3.6)	1.0 (0.69 to 1.6)
S-PIA	1.8 (1.2 to 2.7)	15b (9.6 to 22)	10b (6.1 to 18)
ADENOSINE	32 (20 to 51)	126b (79 to 203)	126b (79 to 202)

a Numbers in parentheses indicate 95% confidence interval

b Significantly different from tail flick ED50 value ($p < 0.05$)

assay, but adenosine was still less potent than adenosine agonists. A similar rank order potency was observed for potentiation of morphine (i.c.v.)-induced antinociception by 20% antinociceptive doses of adenosine agonists administered i.t. as measured by the tail flick assay (table 2).

Absolute potencies among adenosine agonists differ markedly between the tail flick assay and hot plate and rotorod assays. Adenosine, R-PIA and S-PIA were significantly less potent in hot plate and rotorod assays. Potency ratios for adenosine agonists obtained from the rotorod assay were similar to the potency ratios observed on the hot plate assay (table 3).

Effect Of Intrathecal Administration Of Theophylline On Adenosine Agonist (i.t.)-Induced Antinociception And Motor Impairment

Effects induced by adenosine agonists (i.t.) were dose-dependently antagonized by theophylline in all assays. Dose-response curves for theophylline antagonism of NECA (figures 9, 10 and 11) were typical of results observed with other adenosine agonists. A six to eight fold shift to the right of NECA (i.t.) dose-response curves occurred with the highest dose of theophylline (i.t.) on tail flick, hot plate and rotorod assays. Theophylline

Table 2. ED50 values for morphine (i.c.v.)-induced antinociception as potentiated by adenosine agonists (i.t.) or NBI (i.t.) in tail flick assay

i.t. Drug Treatment nmol	TAIL FLICK ug	
saline	1.2	(0.679 to 2.12)a
NECA 0.030c	0.310b	(0.203 to 0.473)
NECA 0.061d	0.134b	(0.0897 to 0.201)
R-PIA 0.122d	0.142b	(0.0948 to 0.212)
CADO 0.486d	0.142b	(0.0948 to 0.212)
S-PIA 0.973d	0.142b	(0.0948 to 0.212)
NBI 25.0e	0.625b	(0.413 to 0.946)

a Numbers in paretheses indicate 95% confidece interval

b Significantly less than saline control ED50 value ($p < 0.05$)

c 0.030 nmol NECA does not typically induce antinociception

d These doses typically induce 20% antinociception

e 25 nmols of NBI has no behavioral affects on the animals

Table 3. Potency ratios for adenosine agonists(i.t.)-induced antinociception or motor impairment in tail flick, hot plate or rotorod assays

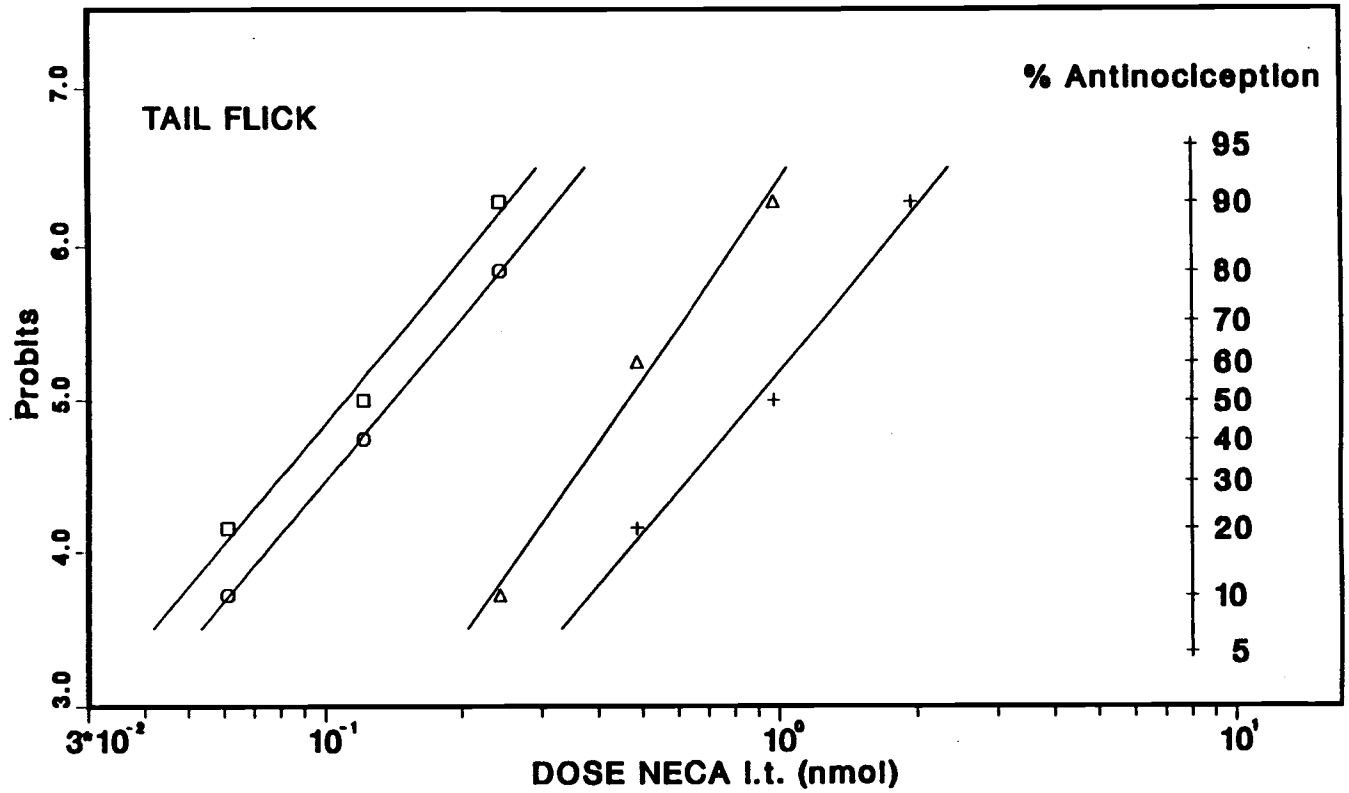
DRUG	TAIL FLICK	HOT PLATE	ROTOROD
S-PIA	1.00	1.00	1.00
CADO	1.89 (1.33 to 2.75)a	8.14b (6.79t to 9.69)	9.96b (6.99 to 14.1)
R-PIA	4.40 (3.04 to 6.28)	15.0b (12.4 to 18.0)	12.3b (8.80 to 16.9)
NECA	17.0 (11.6 to 25.0)	98.9b (81.6 to 119)	75.4b (52.8 to 107)

a Numbers in parentheses indicate 95% interval

b Significantly different than tail flick potency ratio ($p < 0.05$)

Figure 9. Antagonism of NECA (i.t.)-induced antinociception by theophylline injected i.t. as measured by the tail flick assay. Mice were injected i.t. with various doses of NECA (□) or NECA and theophylline, 55.5 nmol (O), 111 nmol (△) or 222 nmol (+) 10 minutes before testing for antinociception in the tail flick assay.

Figure 9

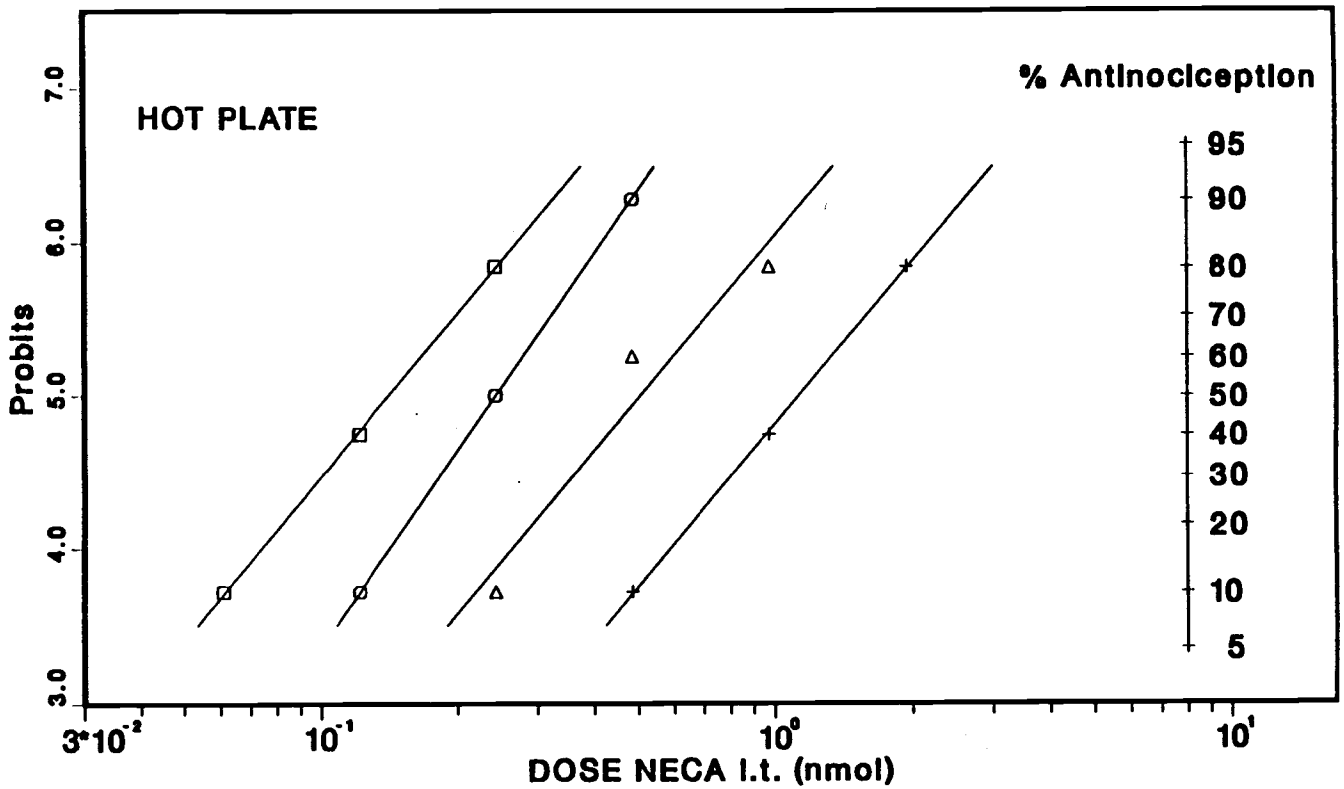


LEGEND

□ = NECA I.t.
○ = NECA + 55.5 nmol THEOPHYLLINE I.t.
△ = NECA + 111.0 nmol THEOPHYLLINE I.t.
\+ = NECA + 222.0 nmol THEOPHYLLINE I.t.

Figure 10. Antagonism of NECA (i.t.)-induced antinociception by theophylline injected i.t. as measured by the hot plate assay. Mice were injected i.t. with various doses of NECA (□) or NECA and theophylline, 55.5 nmol (O), 111 nmol (Δ) or 222 nmol (+) 10 minutes before testing for antinociception in the hot plate assay.

Figure 10

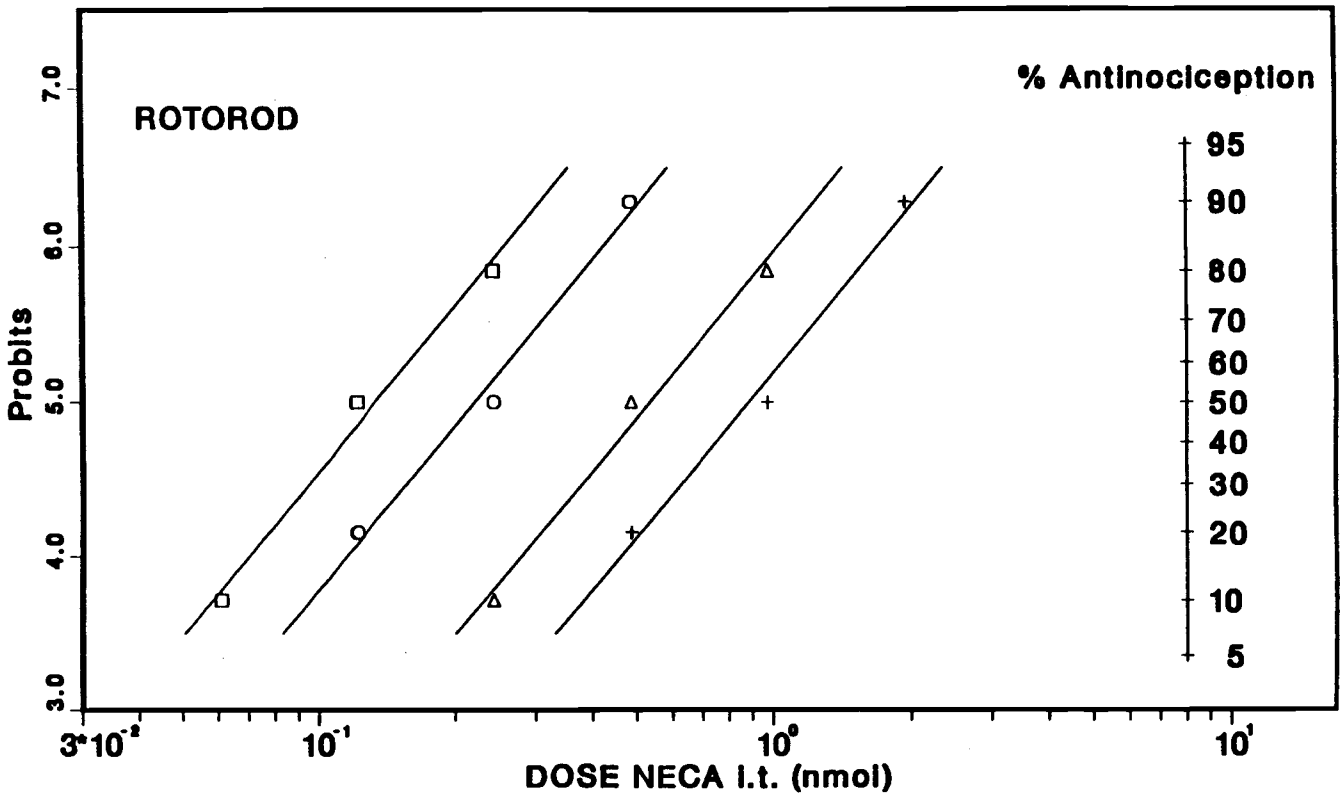


LEGEND

□ = NECA I.t.
○ = NECA + 55.5 nmol THEOPHYLLINE I.t.
△ = NECA + 111.0 nmol THEOPHYLLINE I.t.
\+ = NECA + 222.0 nmol THEOPHYLLINE I.t.

Figure 11. Antagonism of NECA (i.t.)-induced hindlimb paralysis by theophylline injected i.t. as measured by the rotorod assay. Mice were injected i.t. with various doses of NECA (□) or NECA and theophylline, 55.5 nmol (O),111 nmol (△) or 222 nmol (+) 10 minutes before testing for motor impairment in the rotorod assay.

Figure 11



LEGEND

□ = NECA I.t.
○ = NECA + 55.5 nmol THEOPHYLLINE I.t.
△ = NECA + 111.0 nmol THEOPHYLLINE I.t.
\+ = NECA + 222.0 nmol THEOPHYLLINE I.t.

(i.t.) antagonism of R-PIA and CADO were similar to NECA on all assays (tables 4, 5 and 6). Theophylline (i.t.), however, was significantly more potent in its ability to antagonize S-PIA (i.t.)-induced antinociception or motor impairment. pA2 analysis of theophylline/adenosine interactions gave values of approximately 7.2 with slopes of -1 for all combinations in all assays, supporting a competitive, receptor mediated interaction (data not shown). Peak time for theophylline (i.t.) antagonism occurred at five minutes and persisted for additional five minutes (data not shown). Higher doses of theophylline were not used due to solubility limitations.

Effects Of Intrathecal Administration Of Adenosine Agonists Or NBI On Morphine (i.c.v.)-induced Antinociception

Interactions of adenosine with descending opioid stimulated pathways were confirmed by potentiation of morphine (i.c.v.)-induced antinociception by adenosine agonists administered i.t. A subantinociceptive dose of NECA (0.030 nmol) (i.t.) potentiated the morphine (i.c.v.) dose-response curve (fig. 12), 3.9 fold to the left, which is significantly greater than the theoretical ED50 value expected for an additive effect (0.892 ug with a corresponding 95% confidence interval of 0.472 to 1.69). ED50 values and corresponding 95% confidence limits

Table 4. ED50 values for adenosine agonist (i.t.)-induced antinociception in the presence of various doses of theophylline (i.t.) in tail flick assay

Drug	Theophylline nmol			
	Control	55.5	111	222
NECA	0.111a (0.0741-0.165)b	0.142 (0.0948-0.211)	0.465c (0.290-0.744)	0.884c (0.591-1.32)
R-PIA	0.412 (0.244- 0.700)	1.23c (0.733-2.07)	1.67c (1.12-2.50)	3.89c (2.43-6.23)
CADO	0.862 (0.467-1.59)	2.36 (1.38-4.02)	7.57c (4.48-12.8)	9.86c (5.86-16.6)
S-PIA	1.82 (1.20-2.75)	9.06c	d (6.05-13.6)	d

a ED50 values in nmols

b Numbers in parentheses indicate 95% confidence interval

c Significantly different from control ED50 value ($p < 0.05$)

d Due to solubility limitations of S-PIA this dose was not determined

e S-PIA (i.t.) dose-respone curve was shifted to the right by 27.8 nmol and 41.6 nmol of theophylline (i.t.) gave rise to ED50 values of 4.07 nmol and 7.79 nmol and corresponding 95% confidence interval of 2.55 to 6.52 and 4.86 to 12.5, respectively

Table 5. ED50 values for adenosine agonists (i.t.)-induced antinociception in the presence of various doses of theophylline (i.t.) in hot plate assays

Drug	Theophylline nmol			
	Control	55.5	111	222
NECA	0.142a (0.0948-0.211)b	0.243c (0.152-0.389)	0.506c (0.287-0.895)	1.13c (0.757-1.69)
R-PIA	0.908 (0.599-1.38)	1.95c (1.22-3.12)	2.26c (1.51-3.38)	7.79c (4.86-12.5)
CADO	2.00 (1.11-3.60)	3.42c (2.14-5.49)	8.26c (4.78-14.3)	9.83c (5.64-17.1)
S-PIA	14.5 (9.57-21.7)	d	d	d

a ED50 values in nmols

b Numbers in parentheses indicate 95% confidence interval

c Significantly different from control ED50 value ($p < 0.05$)

d Not determined, ED50 values not obtained due to solubility limitations of S-PIA

Table 6. ED 50 values for adenosine agonists (i.t.)-induced motor impairment in the presence of various doses of theophylline (i.t.) in rotorod assay

Drug	Theophylline nmol			
	Control	55.5	111	222
NECA	0.134 (0.0898-0.200)b	0.221c (0.148-0.330)	0.535c (0.358-0.800)	0.884c (0.590-1.32)
R-PIA	0.877 (0.529-1.46)	1.77c (1.18-2.64)	3.35c (2.24-5.00)	7.07c (4.73-10.6)
CADO	1.04 (0.689-1.58)	2.91c (1.70-4.98)	7.44c (4.64-11.9)	13.4c (8.96-20.0)
S-PIA	10.4	d	d	d

a ED50 values in nmols

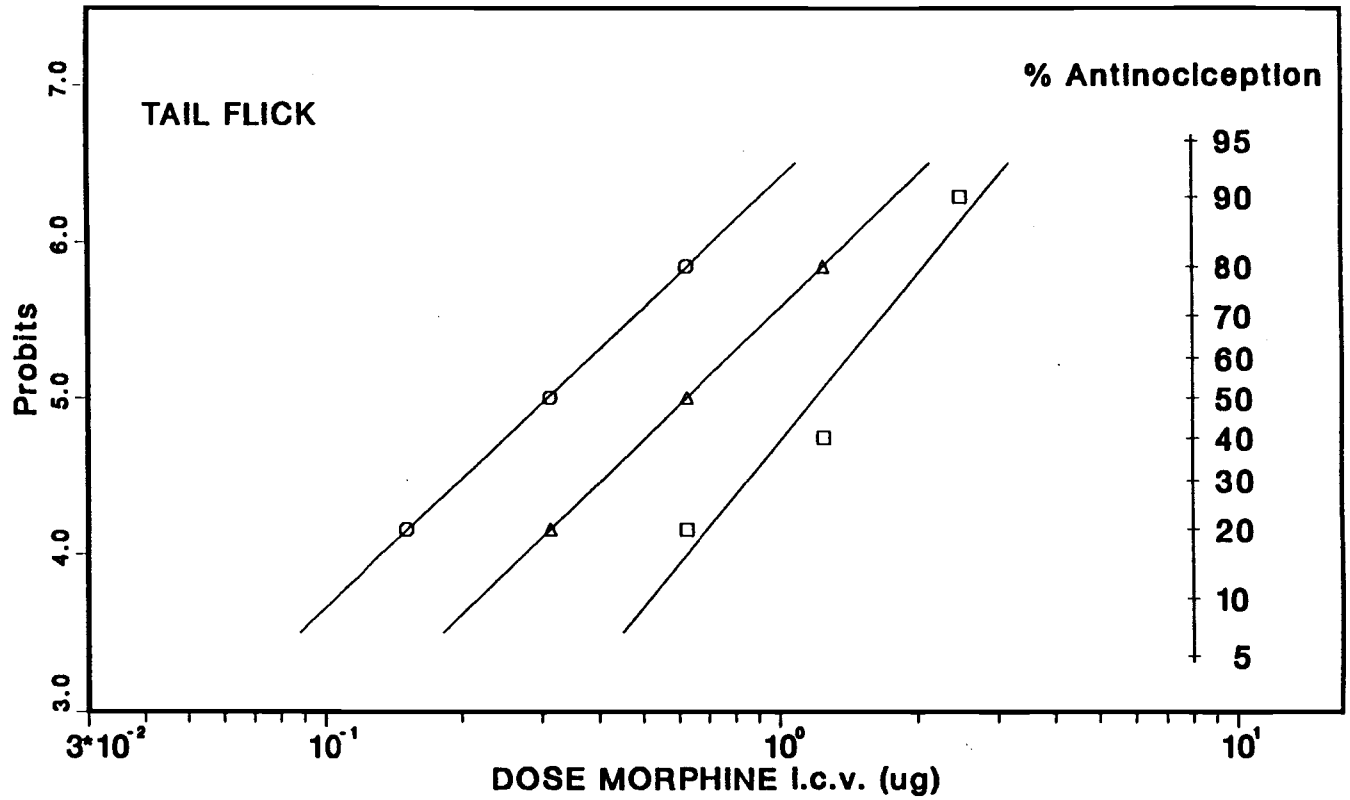
b Numbers in parentheses indicate 95% interval

c Significantly different from control ED50 value ($p < 0.05$)

d Not determined, ED50 values not obtained due to solubility limitations of S-PIA

Figure 12. Potentiation of morphine (i.c.v.)-induced antinociception by NECA or NBI injected i.t. Mice were injected i.c.v. with various doses of morphine 15 minutes before testing for antinociception in the tail flick assay. Ten minutes before testing, mice were administered i.t. injections of 0.030 nmol NECA (O) or 25 nmol of NBI (Δ).

Figure 12



LEGEND

□ = MORPHINE I.c.v.
○ = MORPHINE I.c.v. + 0.030 nmol NECA I.t.
△ = MORPHINE I.c.v. + 25.0 nmol NBI I.t.

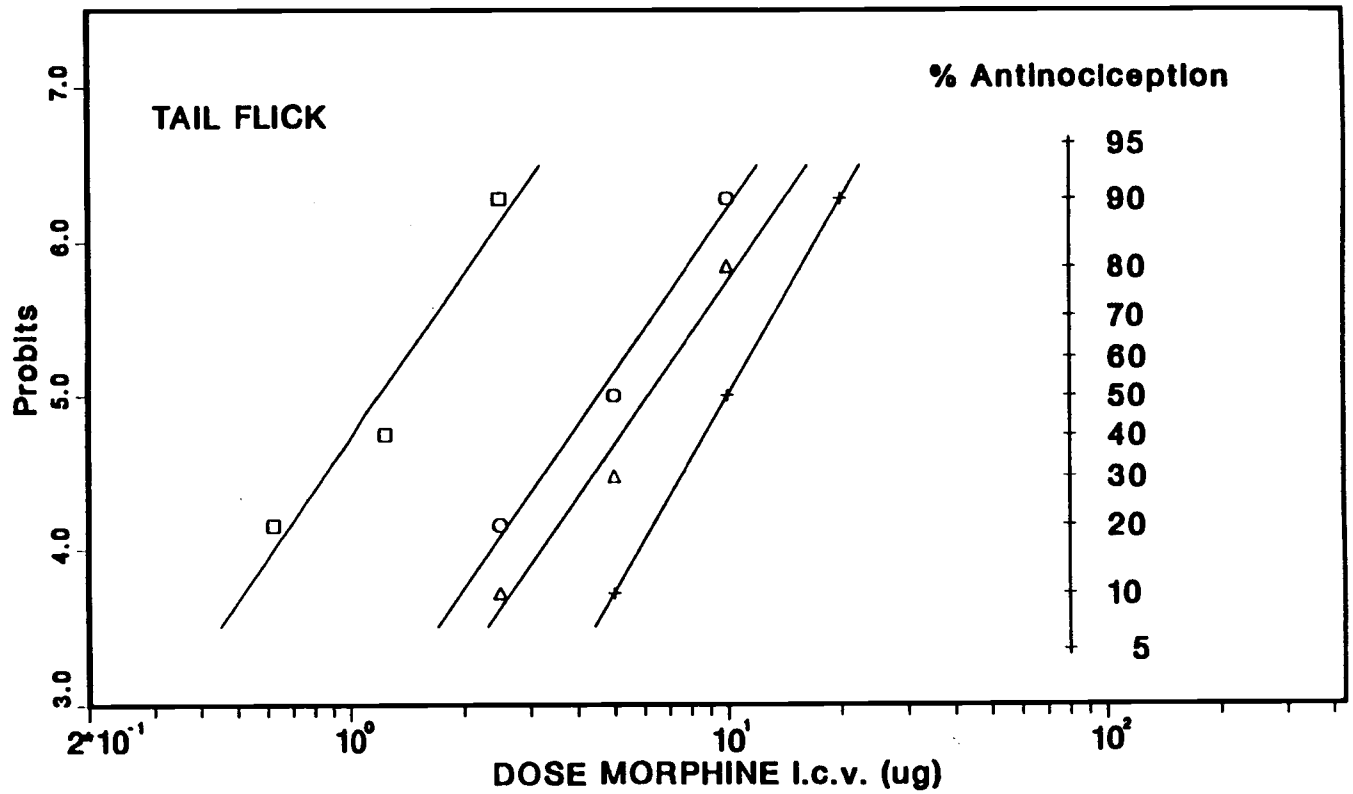
are shown in table 2. A similar potentiation of the morphine (i.c.v.) dose-response curve occurred with 20% antinociceptive (i.t.) doses of each adenosine agonist (table 2). Twenty-five nmol of the adenosine reuptake inhibitor NBI, administered i.t., also potentiated morphine (i.c.v.)-induced antinociception (fig. 12) (table 2). NBI was administered in a 50% (v/v) solution of DMSO. DMSO vehicle controls did not alter tail flick or hot plate control latencies, however, most animals scratched the injection site immediately after injections.

Effects Of Intrathecal Administration Of Theophylline, Phentolamine Or Methysergide On Morphine (i.c.v.)-Induced Antinociception

Significant antagonism of morphine (i.c.v.)-induced antinociception occurred with 1 microgram of phentolamine administered i.t. A 3.8 fold and 2.9 fold shift of the morphine (i.c.v.) dose-response curves to the right was observed in the tail flick and hot plate assays, respectively (figures 13 and 14). Two micrograms phentolamine administered i.t. caused an even greater shift of the morphine (i.c.v.) dose-response curve (table 7). Antagonism by 1 ug phentolamine (i.t.) was similar to that observed with 111 nmol of theophylline (i.t.) (table 7). Antagonism of the morphine (i.c.v.) dose-response curve by

Figure 13. Antagonism of morphine (i.c.v.)-induced antinociception by theophylline, phentolamine or a combination of theophylline and phentolamine injected i.t., as measured by the tail flick assay. Mice were injected i.c.v. with various doses of morphine 15 minutes before testing for antinociception in the tail flick assay. Five minutes before testing, mice were administered i.t. injections of saline (□), 111 nmol of theophylline (△), 1 ug of phentolamine (O) or 111 nmol of theophylline and 1 ug of phentolamine (+).

Figure 13

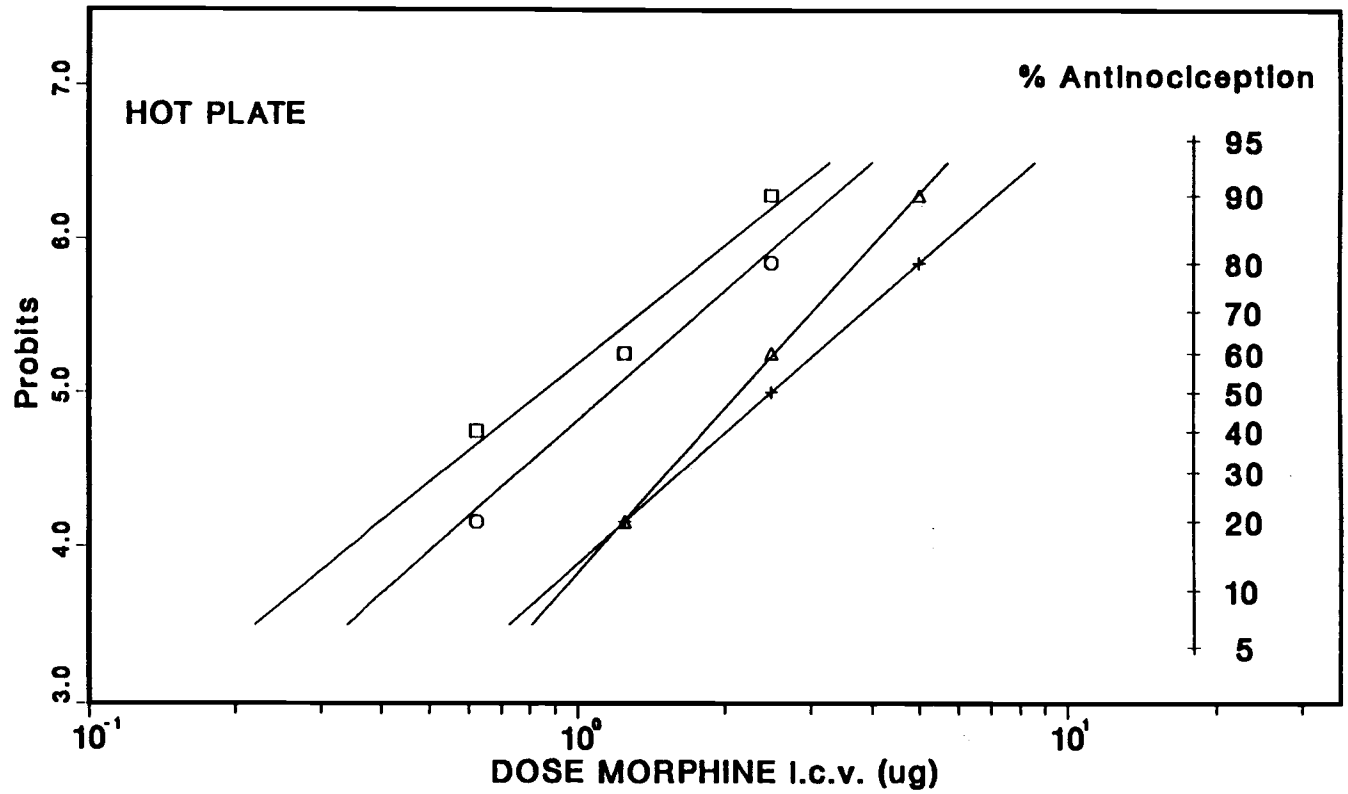


LEGEND

□ = MORPHINE I.c.v.
○ = MORPHINE I.c.v. + 1 ug PHENTOLAMINE I.t.
△ = MORPHINE I.c.v. + 111 nmol THEOPHYLLINE I.t.
\+ = MORPHINE I.c.v. + 1 ug PHENTOLAMINE and 111 nmol THEOPHYLLINE

Figure 14. Antagonism of morphine (i.c.v.)-induced antinociception by theophylline, phentolamine or a combination of theophylline and phentolammine injected i.t., as measured by the hot plate assay. Mice were injected i.c.v. with various doses of morphine 15 minutes before testing for antinociception in the hot plate assay. 5 minutes before testing, mice were administered i.t. injections of saline (□), 111 nmol of theophylline (O), 1 ug of phentolamine (+) or 111 nmol of theophylline and 1 ug of phentolamine (Δ).

Figure 14



LEGEND

□ = MORPHINE I.c.v.
○ = MORPHINE I.c.v. + 111 nmol THEOPHYLLINE I.t.
△ = MORPHINE I.c.v. + 1 ug PHENTOLAMINE and 111 nmol THEOPHYLLINE
\+ = MORPHINE I.c.v. + 1 ug PHENTOLAMINE I.t.

Table 7. ED50 values for the antagonism of morphine (i.c.v.)-induced antinociception in the presence of purinergic or monoaminergic antagonists (i.t.) in tail flick or hot plate assays

i.t. Drug Treatment	TAIL FLICK ug	HOT PLATE ug
Saline	1.2 (0.679 to 2.12)a	0.850 (0.487 to 1.48)
Theophylline 111 nmol	6.17b (4.13 to 9.22)	1.17 (0.771 to 1.76)
Phentolamine 1 ug	4.54b (3.04 to 6.79)	2.27b (1.52 to 3.40)
Phentolamine 2 ug	9.09b (6.08 to 13.6)	1.93b (1.20 to 3.10)
Methysergide 1 ug	5.0b (3.30 to 7.57)	1.08 (0.719 to 1.61)
Methysergide 2 ug	5.0b (3.12 to 8.01)	1.25 (0.826 to 1.89)
Phentolamine 1 ug + Theophylline 111 nmol	10.0b (6.24 to 16.0)	2.15b (1.44 to 3.21)
Phentolamine 1ug + Methysergide 1ug	9.09b (6.08 to 13.6)	2.15b (1.44 to 3.21)
Methysergide 1ug + Theophylline 111 nmol	5.50b (3.68 to 8.23)	1.03 (0.642 to 1.65)

a Numbers in parentheses indicate 95% confidence interval

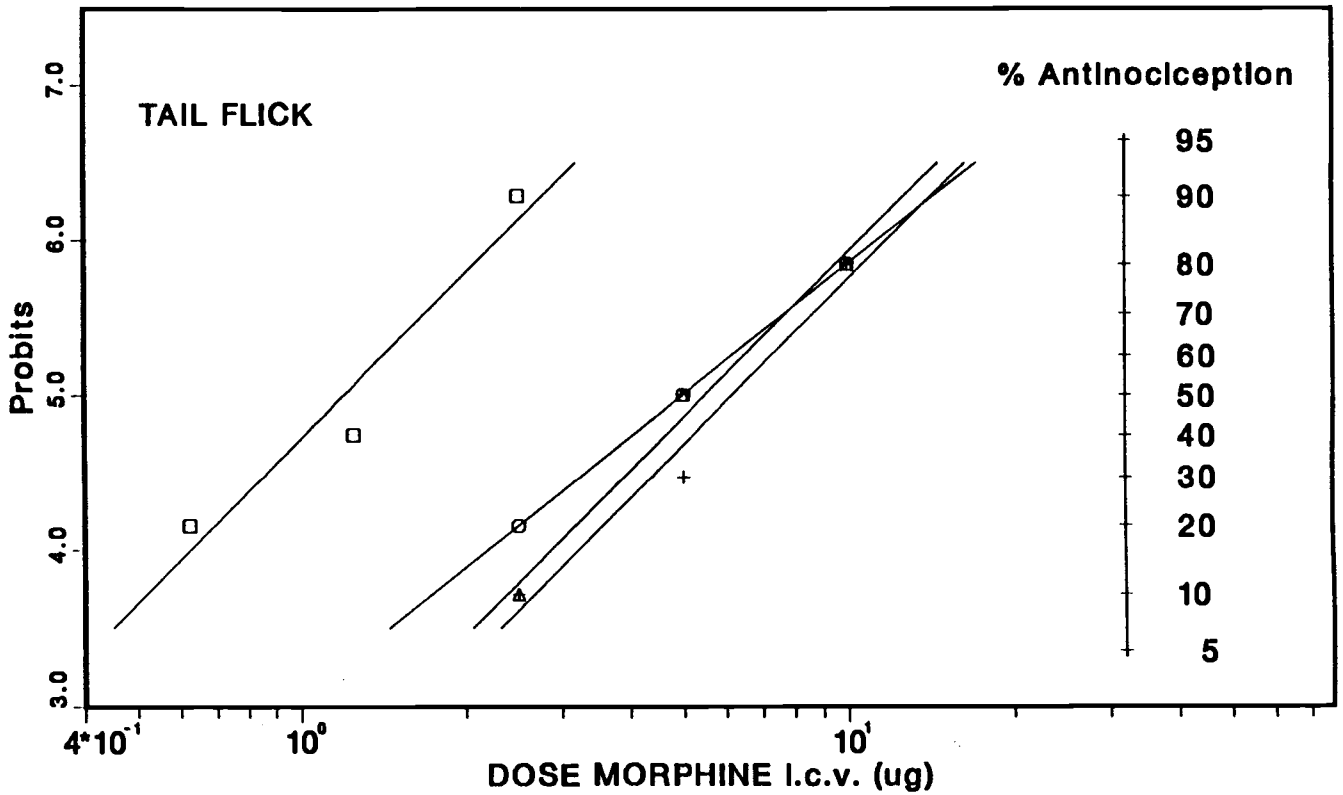
b Significantly greater than saline control ED50 value ($p < 0.05$)

a combination of one microgram phentolamine and 111 nmol of theophylline was equal to the arithmetic sum of the change induced by each antagonist administered alone, as measured by the tail flick assay (fig. 13) (table 7). Co-administration of phentolamine and theophylline, however, did not significantly shift the morphine (i.c.v.) dose-response curve further to the right as compared to either phentolamine or theophylline alone in the hot plate assay (fig. 14).

Significant antagonism of morphine (i.c.v.)-induced antinociceptive dose-response curve also occurred with 1 microgram of methysergide administered i.t. as measured by the tail flick assay (fig. 15). A 4.6 fold shift of the morphine (i.c.v.) dose-response curve to the right was observed, (table 7). The relative degree of methysergide (i.t.) antagonism in the tail flick assay was similar to that observed with either 111 nmol of theophylline or one microgram phentolamine (i.t.). Two micrograms methysergide failed to shift morphine (i.c.v.) dose-response curve further to the right. Neither one or two micrograms of methysergide administered i.t. was able to significantly shift the morphine (i.c.v.) dose-response curve to the right as measured by the hot plate assay (table 7). A combination of 1 microgram of methysergide and 111.0 nmol of theophylline did not shift the morphine (i.c.v.) dose-response curve any further to the right

Figure 15. Antagonism of morphine (i.c.v.)-induced antinociception by theophylline, methysergide or a combination of theophylline and methysergide injected i.t. Mice were injected i.c.v. with various doses of morphine 15 minutes before testing for antinociception as measured by the tail flick assay. Five minutes before testing, mice were administered i.t. injections of saline (□), 111 nmol theophylline (+), 1 ug of methysergide (O) or 111 nmol theophylline and 1 ug of methysergide (△).

Figure 15



LEGEND

□ = MORPHINE I.c.v.
○ = MORPHINE I.c.v. + 1 ug METHYSERGIDE I.t.
△ = MORPHINE I.c.v. + 1 ug METHYSERGIDE and 111 nmol THEOPHYLLINE
\+ = MORPHINE I.c.v. + 111 nmol THEOPHYLLINE I.t.

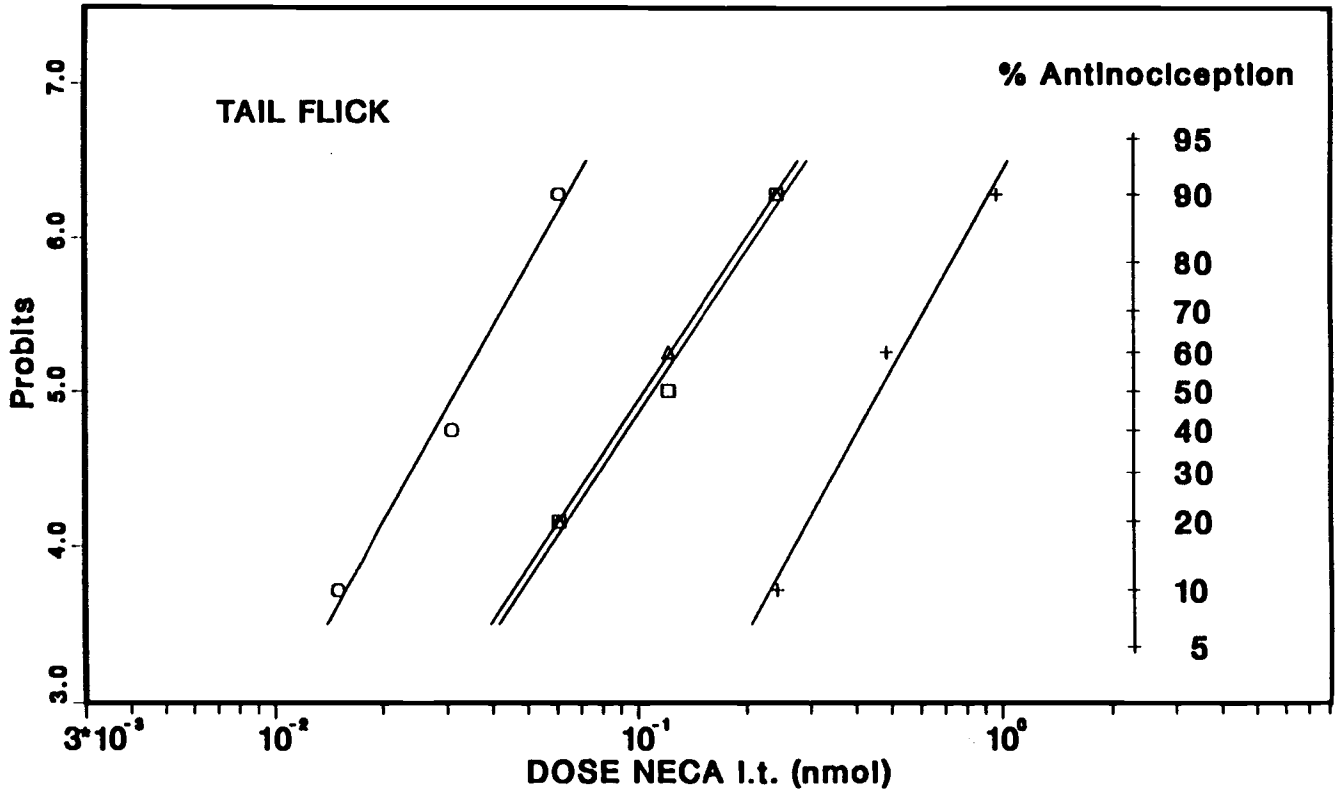
as compared to either methysergide or theophylline alone in the tail flick assay (fig. 15). A combination of one microgram methysergide and one microgram of phentolamine co-administered i.t., however, produced a similar degree of antagonism in the tail flick assay as did the combination of 111 nmol of theophylline and one microgram of phentolamine (table 7).

Effects Of Intrathecal Administration Of Norepinephrine, Serotonin, Theophylline, Phentolamine Or Methysergide On NECA (i.t.)-Induced Antinociception

Experiments were designed to examine spinal adenosine involvement with specific neurotransmitters involved in antinociception. A subantinociceptive dose of norepinephrine (1.22 nmol) administered i.t. significantly shifted the NECA (i.t.) dose-response curve to the left on the tail flick assay, (fig. 16). A subantinociceptive dose of serotonin (58.8 nmol) administered i.t., however, failed to potentiate antinociception induced by NECA (i.t.). A dose of 111 nmol of theophylline administered i.t. significantly antagonized NECA (i.t.) induced antinociception on the tail flick assay, as reported earlier (fig. 16), but neither 1 microgram of methysergide or phentolamine (i.t.) had effects (table 8).

Figure 16. Potentiation of NECA (i.t.)-induced antinociception by i.t. administration of norepinephrine. Mice were injected i.t. with various of NECA (□) 10 minutes before testing for antinociception in the tail flick assay. Ten minutes before testing, mice were also administered i.t. injections of 1.22 nmol of norepinephrine (O), 58.8 nmol of serotonin (△) or 111 nmol of theophylline (+).

Figure 16



LEGEND

□ = NECA I.t.
○ = NECA I.t. + 1.22 nmol NOREPINEPHRINE I.t.
△ = NECA I.t. + 58.8 nmol SEROTONIN I.t.
\+ = NECA I.t. + 111.0 nmol THEOPHYLLINE I.t.

Table 8. ED50 values for NECA (i.t.)-induced antinociception in the presence of purinergic or monoaminergic antagonists (i.t.) or monoaminergic agonists (i.t.) in tail flick or hot plate assay.

i.t. Drug Treatment	TAIL FLICK nmol	HOT PLATE nmol
NECA	0.111 (0.0741 to 0.165)a	0.142 (0.0948 to 0.211)
NECA + 111.0 nmol Theophylline	0.465b (0.290 to 0.744)	0.501b (0.287 to 0.895)
NECA + 1 ug Phentolamine	0.111 (0.0741 to 0.165)	0.128 (0.0797 to 0.240)
NECA + 1 ug Methysergide	0.0941 (0.0587 to 0.151)	0.114 (0.0752 to 0.172)
NECA + 1.22 nmol Norepinephrine	0.0319c (0.0198 to 0.0515)	d
NECA + 58.8 nmol Serotonin	0.105 (0.0702 to 0.157)	d

a Numbers in parentheses indicate 95% confidence interval

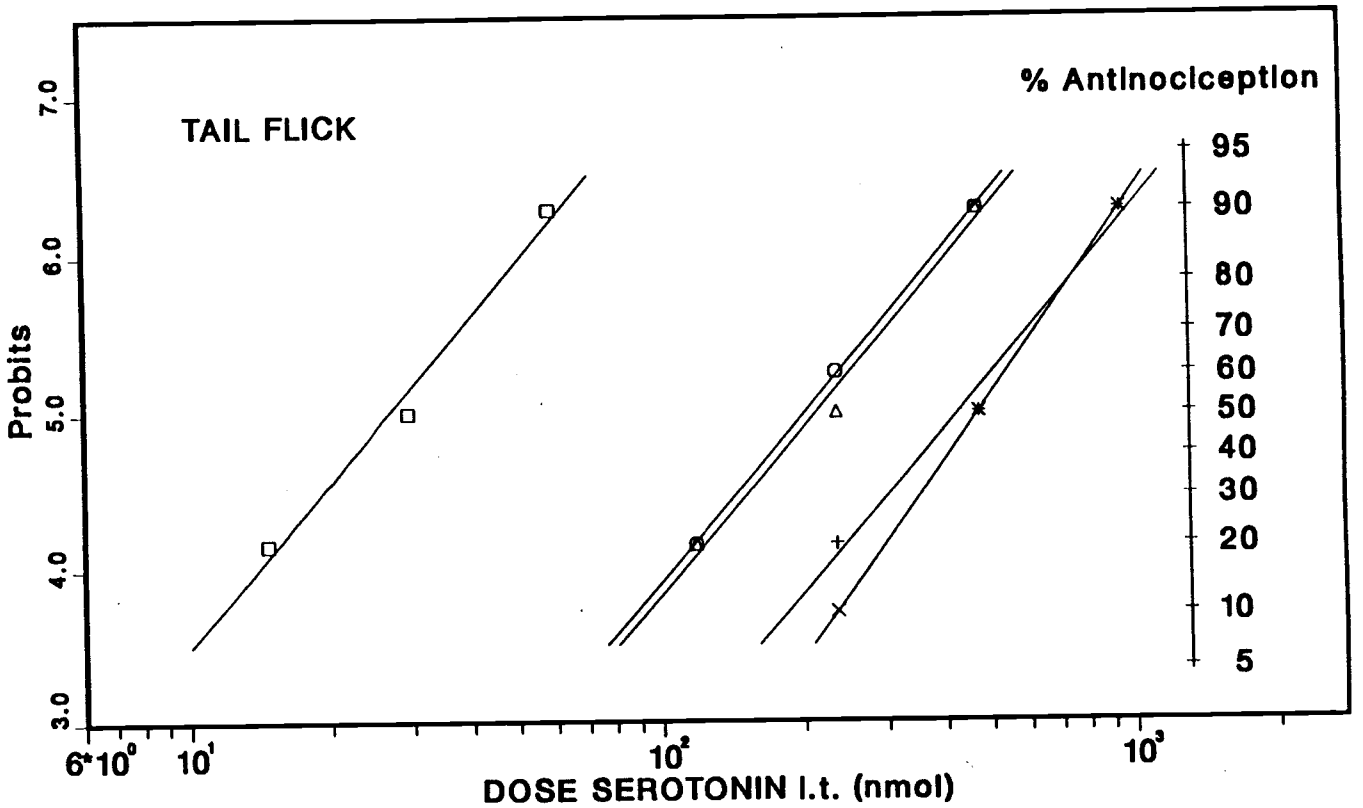
b Significantly greater than NECA (i.t.) ED50 value ($p < 0.05$)

c Significantly less than NECA (i.t.) ED50 value ($p < 0.05$)

d Not determined

Figure 17. Potentiation of serotonin (i.t.)-induced antinociception by i.t. administration of norepinephrine. Mice were injected i.t. with various doses of serotonin (O) 5 minutes before testing for antinociception in the tail flick assay. Five minutes before testing mice were also administered i.t. injections of 1.22 nmol of norepinephrine (□), 0.030 nmol of NECA (△), 111 nmol of theophylline (+) or 1 ug of methysergide (X).

Figure 17



LEGEND

□ = SEROTONIN I.t. + 1.22 nmol NOREPINEPHRINE I.t.
○ = SEROTONIN I.t.
△ = SEROTONIN I.t. + 0.030 nmol NECA I.t.
\+ = SEROTONIN I.t. + 111.0 nmol THEOPHYLLINE I.t.
× = SEROTONIN I.t. + 1.0 ug METHYSERGIDE I.t.

Effects Of Intrathecal Administration Of Norepinephrine, NECA, Theophylline, Phentolamine Or Methysergide On Serotonin (i.t.)-Induced Antinociception

A subantinociceptive dose of norepinephrine, but not NECA, administered i.t. significantly shifted the serotonin (i.t.) dose-response curve to the left, as measured by the tail flick assay, (fig. 17). One microgram of methysergide (i.t.) significantly shifted the serotonin (i.t.) dose-response curve to the right as measured by the tail flick assay (fig. 17). Similar antagonism of the serotonin (i.t.) dose-response curve occurred with 111 nmol theophylline (i.t.). A dose of 1 microgram phemtolamine administered i.t. had no effect on serotonin (i.t.) dose-response curve (table 9).

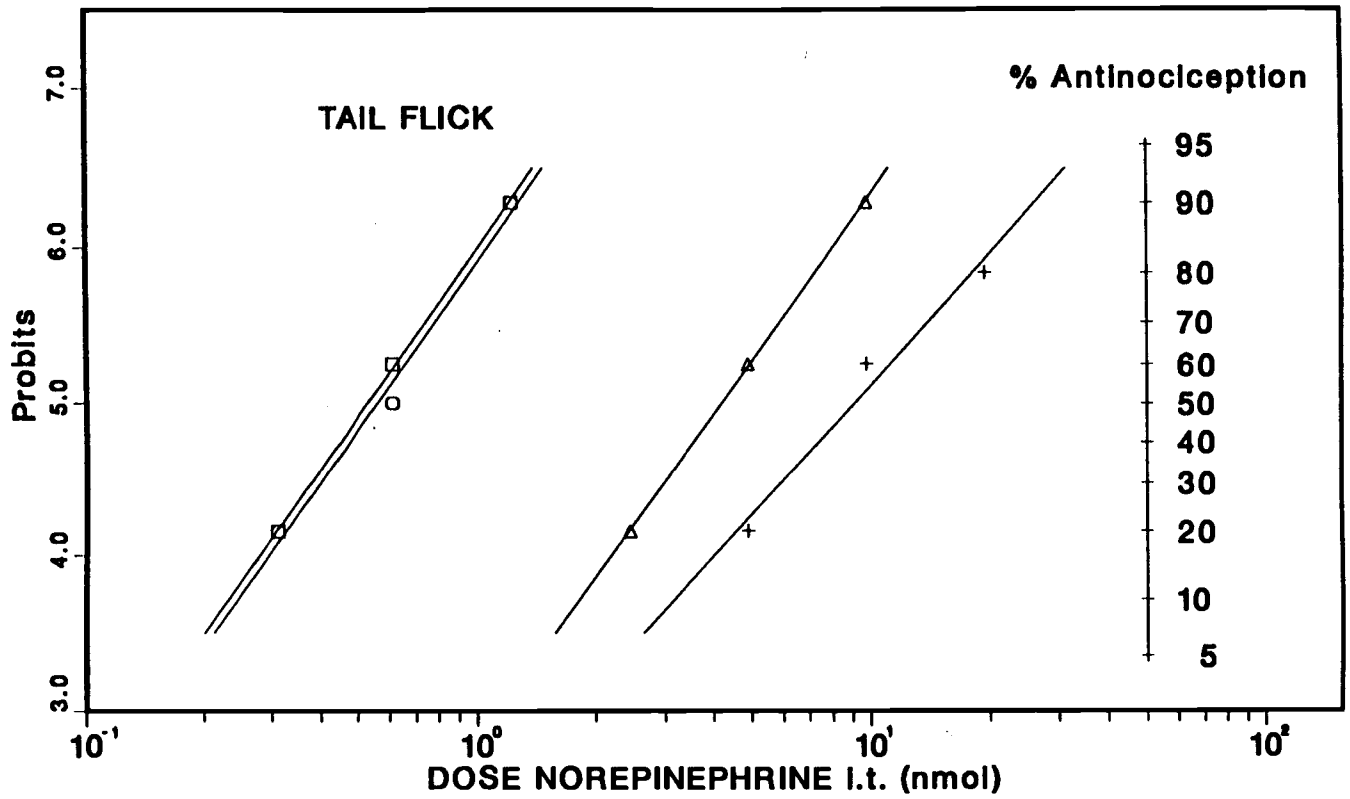
Effects Of Intrathecal Administration Of NECA, Serotonin, Theophylline, Phentolamine Or Methysergide On Norepinephrine (i.t.)- Or Clonidine (i.t.)-Induced Antinociception

Subantinociceptive doses of NECA (0.030 nmol) or serotonin (58.8 nmol) administered i.t. shifted norepinephrine and clonidine (i.t.) dose-response curves significantly to the left as measured by the tail flick assay (figures 18 and 19). One microgram of phentolamine

administered i.t. shifted the norepinephrine (i.t.) dose-response curve 2.2 fold and the clonidine (i.t.) dose-response curve 2.3 fold to the right (figures 18 and 19). In contrast, 1 microgram of methysergide or 111 nmol of theophylline (i.t.) had no effect on norepinephrine (i.t.)- clonidine (i.t.)-induced antinociception on the tail flick assay (table 9).

Figure 18. Potentiation of norepinephrine (i.t.)-induced antinociception by i.t. administration of NECA or serotonin. Mice were injected i.t. with various doses of norepinephrine (△) 5 minutes before testing for antinociception in the tail flick assay. Five minutes before testing mice were also administered i.t. injections of 0.030 nmol of NECA (□), 58.8 nmol of serotonin (O) or 1 ug of phentolamine (+).

Figure 18

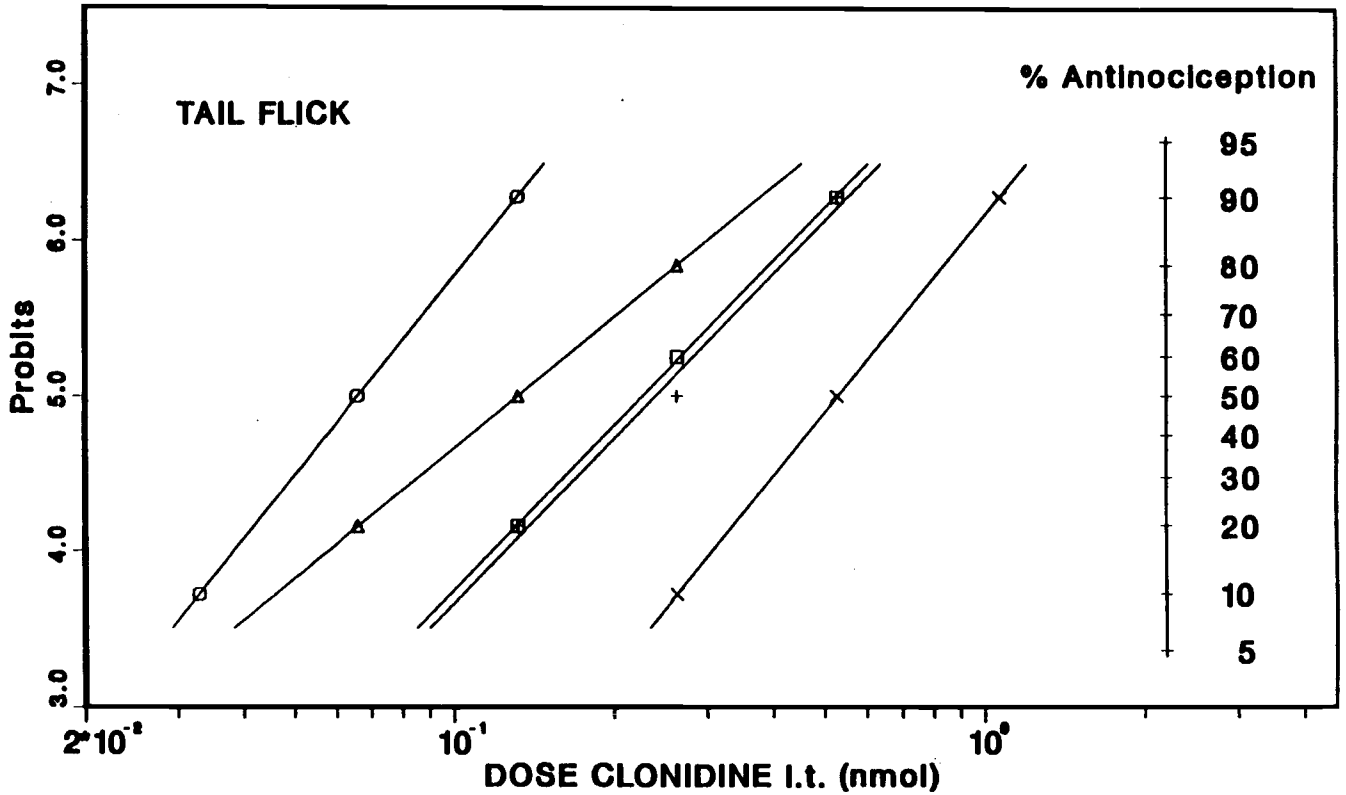


LEGEND

□ = NOREPINEPHRINE + 0.030 nmol NECA I.t.
○ = NOREPINEPHRINE I.t. + 58.8 nmol SEROTONIN I.t.
△ = NOREPINEPHRINE I.t.
\+ = NOREPINEPHRINE I.t. + 1.0 ug PHENTOLAMINE I.t.

Figure 19. Potentiation of clonidine (i.t.)-induced antinociception by i.t. administration of NECA or serotonin. Mice were injected i.t. with various doses of clonidine (□) 5 minutes before testing for antinociception in the tail flick assay. Five minutes before testing mice were also administered i.t. injections of 0.030 nmol of NECA (O), 58.8 nmol of serotonin (Δ), 1.22 nmol of norepinephrine (+) or 1 ug of phentolamine (X).

Figure 19



LEGEND

□ = CLONIDINE I.t.
○ = CLONIDINE I.t. + 0.030 nmol NECA I.t.
△ = CLONIDINE I.t. + 58.8 nmol SEROTONIN I.t.
\+ = CLONIDINE I.t. + 1.22 nmol NOREPINEPHRINE I.t.
× = CLONIDINE I.t. + 1.0 ug PHENTOLAMINE I.t.

Table 9. ED50 values for monoaminergic agonist (i.t.)-induced antinociception in the presence of purinergic or monoaminergic antagonists (i.t.) or agonists (i.t.) in tail flick assay

i.t. Drug Treatment	Serotonin	Norepinephrine	Clonidine
Control	203a (136 to 302)b	4.02 (2.81 to 6.27)	0.227 (0.152 to0.340)
1 ug Methysergide	470c (293 to 753)c	4.68 (2.65 to 8.27)	0.264 (0.165 to 0.423)
1 ug Phentolamine	246 (154 to 394)	9.10c (6.02 to 13.8)	0.530c (0.330 to 0.850)
111 nmol Theophylline	427c (286 to 639)	4.43 (2.97 to 6.63)	0.291 (0.194 to 0.434)
0.030 nmol NECA	214 (143 to 319)	0.528d (0.355 to 0.786)	0.0660d (0.0412 to 0.106)
58.8 nmol Serotonin	e	0.558d (0.375 to 0.830)	0.132d (0.0872 to 0.200)
1.22 nmol Norepinephrine	26.7 (17.9 to 39.9)	e	e

a ED50 values in nmols

b Numbers in parentheses indicate 95% confidence interval

c Significantly greater than control ED50 value ($p < 0.05$)

d Significantly less than contol ED50 value ($p < 0.05$)

e Not determined

DISCUSSION

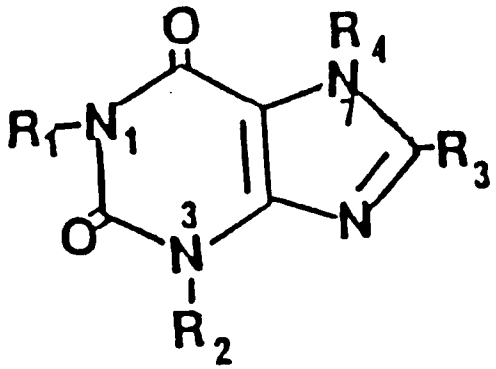
Endogenous spinal adenosine has been implicated in antinociceptive actions of morphine administered i.c.v. or i.t. (DeLander and Hopkins, 1986.). In addition, several adenosine agonists administered i.c.v. will induce antinociception (Yarbough amd McGuffin-Clinschmidt, 1981). Preliminary investigations have similarly suggested adenosine agonists administered i.t. induce antinociception in rats (Holmgren et al., 1984; Post, 1984) and in mice (DeLander and Hopkins, 1986). In the present investigations, dose-related antinociceptive effects were confirmed and characterized for adenosine and four adenosine agonists, CADO, NECA, R-PIA and S-PIA, administered i.t. We have demonstrated several lines of evidence for adenosine agonist (i.t.)-induced antinociception being mediated by interactions with adenosine receptors. First, R-PIA was significantly more potent than S-PIA in both antinociceptive assays used supporting stereospecific receptor interactions. Secondly, antinociception induced by adenosine agonists was dose-dependently and competitively antagonized by theophylline, an adenosine receptor antagonist. pA2 values for theophylline/adenosine agonist interactions were not significantly different for all combinations in all assays and had slopes of negative one to support

competitive, receptor mediated interactions.

It should be noted that, theophylline inhibits the phosphodiesterase enzymes in addition to its ability to block adenosine receptors. The possibility that theophylline-mediated antagonism of antinociception-induced by adenosine agonists is due to inhibition of phosphodiesterase has been addressed in previous investigations (DeLander and Hopkins, 1986). Isobutylmethylxanthine (IBMX) is an alkylxanthine with similar structure (table 10) and potency as theophylline and caffeine in antagonizing adenosine receptors (Daly, 1983), but is several fold more potent as an inhibitor of phosphodiesterase enzymes (Smellie et al., 1979). DeLander and Hopkins (1986) saw similar antagonism of morphine (i.c.v.)-induced antinociception when IBMX was injected i.t. as that observed following i.t. administration of theophylline or caffeine in equimolar doses. If phosphodiesterase inhibition was responsible for the antagonism observed with theophylline, caffeine or IBMX, then IBMX injections should have shifted the morphine (i.c.v.) dose-response curves several fold farther to the right than was observed for either theophylline or caffeine. Therefore, antagonism of antinociception induced by i.c.v. administration of morphine by methylxanthines is likely due to interactions at spinal adenosine receptors

Table 10. Structure of substituted alkylxanthines

Alkylxanthines



Compound	R_1	R_2	R_3	R_4
Caffeine	CH_3	CH_3	CH_3	H
Isobutylmethyl-xanthine (IBMX)	CH_3	$CH_2CH(CH_3)_2$	H	H
Theophylline	CH_3	CH_3	H	H

rather than inhibition of phosphodiesterase enzymes. Due to the specificity of adenosine antagonist and agonists at spinal adenosine receptors, it can be concluded that antinciception induced by adenosine agonists was due to interactions with adenosine receptors.

Extracellular adenosine receptors were initially subdivided into A1 or A2 receptors based on the ability of adenosine or adenosine agonists to inhibit (A1) or stimulate (A2) adenylate cyclase activity (van Calker et al., 1979; Londos et al., 1980). Rank order potencies determined with adenosine agonists in tail flick and hot plate assays in these investigations resemble the rank order potency characteristically observed during drug interactions with A2 adenosine receptors. The rank order potency for antinociception induced by adenosine agonists, however, did not exactly match the rank order potency that has been previously reported for A2 adenosine receptor mediated events (Williams, 1984). The variation in our studies, from established rank order potency was that R-PIA was found to be more potent than CADO in both antinociceptive assays. Since the rank order potencies for adenosine agonists have historically been established using in vitro assays, it is not surprising that rank order potencies differ slightly for adenosine agonist (i.t.)-induced antinociception measured by in vivo assays. Moreover, it is not uncommon to find

slight discrepencies in rank order potencies among other *in vitro* assays (Geiger *et al.*, 1985). In the present investigations, the greater potency of NECA as compared to R-PIA or adenosine was the distinguishing characteristic and allowed for us to suggest that spinal antinociception induced by adenosine agonists is due to interactions at A2 adenosine receptors.

As mentioned previously, CADO, R-PIA and S-PIA are significantly less potent on the hot plate assay as compared to the tail flick assay. Previous studies have revealed similar differences. Theophylline administered i.t. was found to inhibit morphine (i.c.v.)-induced antinociception more effectively in the tail flick assay than in the hot plate assay (DeLander and Hopkins, 1986). Decreased potency of adenosine agonists and antagonists in the hot plate assay, suggests that adenosine is more effective in inhibiting the polysynaptic spinal reflex arc stimulated by primary afferents than in inhibiting the ascending transmission of nociceptive information. Jurna (1981; 1984) has used a combination of pharmacologic and electrophysiologic methods to reach a similar conclusion based on inhibition of morphine effects by aminophylline, another adenosine receptor antagonist.

Delayed hind limb paralysis was observed in several animals treated with moderate to high doses of adenosine

agonists. Motor impairment has been reported by other investigators (Vapaatalo et al., 1975; Crawley et al., 1981; Yarbough and McGuffin-Clineschmidt, 1981; Phillis et al., 1986.), but never characterized. Motor impairment induced by adenosine agonists could easily lead to misinterpretation of antinociception assays, therefore, investigations were performed to differentiate adenosine agonist induced antinociception from motor impairment. Hind limb paralysis induced by adenosine agonists (i.t.) was dose-dependent and antagonized by theophylline. Rank order potency for adenosine agonist (i.t.)-induced motor impairment was the same as the rank order potency observed for adenosine agonist (i.t.)-induced antinociception. Spinal A2 adenosine receptors, therefore, also appeared to be involved in motor function.

Although rank order potencies for adenosine agonists are identical for the tail flick, hot plate and rotorod assays, R-PIA and S-PIA are less potent in the hot plate and rotorod assays than in the tail flick assay.

Peak time for drug action for R- and S-PIA isomers in the rotorod assay was at least two fold longer than the time of peak effect in either antinociception assays. Potency and time course differences were less obvious for effects induced by i.t. administration of NECA or CADO. The time required for adenosine to reach peak effect did

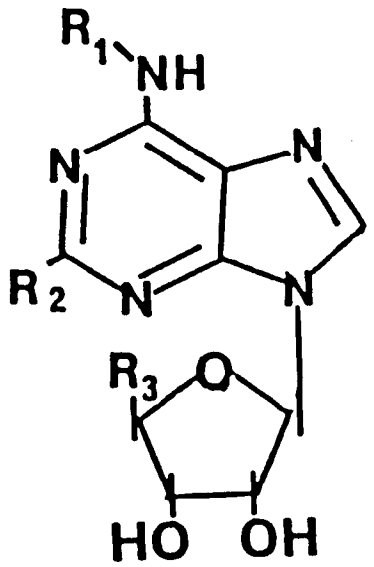
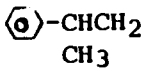
not change among the three assays, but adenosine was four fold less potent on hot plate and rotorod assays. ED50 values for adenosine on hot plate and rotorod assays are in the low micromole range which supports the involvement of adenosine A2 receptors, however, adenosine was found to have nanomole affinity in the tail flick assay. Daly et al. (1983) subdivided adenosine A2 receptors into two classes, based upon observations by Premont et al. (1979) and Londos et al. (1982) that some A2 receptors have EC50 values for adenosine in the high nanomolar range rather than the characteristic micromolar range. The proposed high affinity A2 receptors have been found in the striatum and nucleus accumbens, whereas the low affinity receptors exist in almost all areas of the brain (Bruns et al., 1986). Different A2 adenosine receptors is one possible explanation for potency differences observed for adenosine between the tail flick assay and the hot plate and rotorod assays. From these preliminary investigations, using this model, it can be hypothesized that high affinity A2 receptor sites appear to be involved in pain pathways which utilize polysynaptic spinal reflex arc. Low affinity A2 receptor sites would appear to have a wider distribution then the high affinity A2 receptor site in the spinal cord, similar to the distribution ratio found in the brain (Burns et al., 1986). Low affinity A2 receptor sites appear to be

involved in pain pathways which require supraspinal processing and neuronal tracts mediating motor function.

No significant difference among pA2 values were observed for theophylline/adenosine agonists interactions on all assays, providing evidence against multiple adenosine A2 receptor subtypes. Theophylline, however, has a similar affinity for A1 and A2 adenosine receptors (Snyder, 1985). If theophylline also has similar affinity for both the high and low affinity A2 receptor subtypes, one would expect to find no significant difference among pA2 values. Thus, theophylline may not be able to differentiate A2 receptor subtypes and would be a poor choice as an antagonist to use in a pA2 analysis to differentiate adenosine A2 receptor subtypes. Definitive proof of adenosine A2 receptor subtypes will have to await the development of specific high or low affinity adenosine A2 receptor agonists or antagonists.

An alternative explanation for the differences in potency and time courses for R-PIA and S-PIA is explained by their solubility. The structure of PIA includes a N-phenylisopropyl side chain at N5, causing PIA to be less water soluble than either NECA or CADO (table 11). Decreased drug solubility would impair transport and drug action mechanisms. The decrease in potency and peak time for drug action for R-PIA and S-PIA may be explained

Table 11. Structure of substituted adenosine agonists

Compound	R_1	R_2	R_3
Adenosine Analogs			
			
Adenosine	H	H	CH_2OH
2-Chloroadenosine (CADO)	H	Cl	CH_2OH
5'-N^6-Ethylcarboxamido-adenosine (NECA)	H	H	$CONHCH_2CH_3$
N^6-(2-Phenylisopropyl)-adenosine R(-)-isomer, (R-PIA)		H	CH_2OH
S(+)-isomer, (S-PIA)			

by transport and diffusion limitations due to a water insoluble drug. This difference in time course and potency of R-PIA and S-PIA suggest that antinociception and hind limb paralysis are mediated by different populations of A2 adenosine receptors. Goodman and Snyder (1982) have found high levels of adenosine receptors in the substantia gelatinosa in rats. The substantia gelatinosa is composed of laminae II and III of the dorsal horn of the spinal cord and is a major site for synapses of both primary sensory afferents and descending spinal neurons. Adenosine receptors responsible for mediating motor impairment effects induced by adenosine agonists, however, may lie in laminae IV or V or even deeper within the spinal cord, thus accounting for the time and potency discrepancies observed between antinociception- and hind limb paralysis-induced by i.t. administration of adenosine agonists.

If A2 adenosine receptors which mediate antinociception are more superficial than A2 adenosine receptors associated with neurons innervating hind limb musculature, why did adenosine have the same peak time for drug action on all assays, but was four fold less potent on the hot plate and rotorod assays? The answer to this question may involve mechanisms responsible for the inactivation of adenosine. Adenosine deaminase

degrades adenosine to inosine (Snyder, 1985), and Zetterstorm et al. (1982) have used adenosine deaminase inhibitors to demonstrate that adenosine deaminase contributes to physiological inactivation of adenosine. Further, like most biogenic amines and amino acid neurotransmitters, high affinity, sodium dependent uptake systems are responsible for the removal of adenosine from the synaptic cleft (Bender et al., 1981). Therefore, the decreased potency of adenosine in hot plate and rotorod assays may be a function of adenosine inactivation via uptake and catabolism mechanisms. Stable adenosine analogs are not substrates for adenosine reuptake pump or adenosine deaminase, accounting for their long duration of action.

After characterization of adenosine agonist (i.t.)-induced antinociception, we were interested in characterizing endogenous spinal adenosine involvement with descending neuronal pathways stimulated by morphine. Accumulation of data supports spinal adenosine involvement in morphine (i.c.v.)-induced antinociception coraborating the indirect opioid-induced release of adenosine hypothesis.

There is remarkable capacity to modulate the perception of pain in the CNS. Indirect modulation of nociception by opioids involves the supraspinal stimulation of descending antinociceptive pathways which

inhibit transmission of painful stimuli at the spinal level.

Theophylline administered i.t. (DeLander and Hopkins, 1986) antagonized morphine (i.c.v.)-induced antinociception, suggesting that endogenous adenosine facilitates opioid-stimulated descending antinociceptive pathways. Support for spinal adenosine involvement in opioid-stimulated pathways in these investigations is provided by potentiation of morphine (i.c.v.)-induced antinociception with NECA (i.t.). NECA administered i.t. in a subantinociceptive dose (0.030 nmol) shifted the morphine (i.c.v.) dose-response curve 3.9 fold to the left. Similar results have been used to indicate multiplicative interactions exist between supraspinal and spinal sites (Yeung and Rudy, 1980a; b).

Potentiation of morphine (i.c.v.)-induced antinociception by twenty percent antinociceptive doses of adenosine agonists was also demonstrated. Rank order potency for adenosine agonist (i.t.)-induced potentiation (NECA > R-PIA > CADO> S-PIA) is consistent with the rank order potency observed for adenosine agonist (i.t.)-induced antinociception and once again supports the involvement of spinal A2 adenosine receptors. These results suggest that the adenosine A2 recptors responsible for mediating the potentiation of morphine (i.c.v.)-induced antinociception are the same adenosine

A2 receptors responsible for adenosine agonist (i.t.)-induced antinociception.

Twenty-five nmol of NBI, adenosine uptake inhibitor, administered intrathecally after morphine i.c.v. injections, significantly shifted the morphine (i.c.v.) dose-response curve to the left. This provides more evidence that endogenous spinal adenosine is involved in opioid-stimulated descending antinociceptive pathways. Twenty-five nmol of NBI administered intrathecally alone did not produce any affect. This is consistent with results reported by DeLander and Hopkins (1986) that theophylline (i.t.) does not produce hyperalgesia, suggesting that although endogenous spinal adenosine is involved in morphine mediated antinociception, it may not be involved in the tonic modulation of the sensitivity to nociceptive stimuli.

Morphine (i.c.v.)-induced antinociception, as measured by the tail flick and hot plate assays, was also antagonized by intrathecal injections of phentolamine, a relatively non-selective alpha antagonist. These results are in agreement with similar studies by Yaksh (1979), where he administered microinjections of morphine into the periaqueductal gray area to produce antinociception in rats and found that this antinociception was antagonized by intrathecal injections of phentolamine. Additional evidence for descending noradrenergic neurons

comes from antagonism of antinociception induced by subcutaneous injections of morphine (Proudfit and Hammond, 1981) or electrical stimulation of the nucleus reticularis paragigantocellularis (Hammond and Yaksh, 1984) by intrathecal injections of phentolamine. In the present investigations phentolamine (i.t.) was observed to be less potent in its ability to antagonize morphine (i.c.v.)-induced antinociception in the hot plate assay than the tail flick assay.

Antagonism of morphine (i.c.v.)-induced antinociception with 1 ug of phentolamine (i.t.) was similar to that observed with 111 nmol of theophylline (i.t.). The combination of 111 nmol theophylline and one microgram of phentolamine shifted the morphine (i.c.v.) dose-response curve to the right equal to the arithmetic sum of change induced by each antagonist alone. Theoretical conclusions regarding drug action can be made from the effect a drug has upon a dose-response curve. If a drugs have additive effects on the dose-response curve, then the two drugs are thought to act via the same mechanism. However, if a drug potentiates the dose- response curve of another drug, then the two drugs are considered to act via different mechanisms. The above results suggest mechanistically, that endogenous spinal norepinephrine and adenosine modulate descending antinociceptive pathways in a similar manner.

As for phentolamine and theophylline, intrathecal injections of methysergide, a serotonergic receptor antagonist, antagonized morphine (i.c.v.)-induced antinociception as measured by the tail flick assay. These results are in agreement with results from studies using rats (Yaksh, 1979; Proudfit and Hammond, 1981; Hammond and Yaksh, 1984; Barbaro et al., 1985) and cats (Foong et al., 1985). The combination of 1 ug methysergide and 111 nmol theophylline did not shift the morphine (i.c.v.) dose-response curve further to the right than either antagonist did alone as measured by the tail flick assay. The lack of increased antagonism observed with the combination of methysergide and theophylline may be due to the fact that one microgram of methysergide produced maximal antagonism of morphine (i.c.v.)-induced antinociception. Based on this data, one would expect methysergide and theophylline to act by similar mechanisms since potentiation would be expected if both drugs acted by different mechanisms, even at maximal methysergide dose. Theophylline and methysergide administered i.t. in combination or alone, failed to antagonize the morphine (i.c.v.)-induced antinociception as measured by the hot plate assay.

Differential antagonism of morphine (i.c.v.)-induced antinociception by intrathecal administration of methysergide or phentolamine in tail flick and hot plate

assays also has been reported in rats. Camarata and Yaksh (1985) propose several possibilities to explain these observations. First, descending pathways may interact directly or indirectly through other spinal receptor systems, which do not involve monoamine and adenosine receptors. A second alternative is the descending monoamine pathways may have an insignificant role in ascending information processing, or that the antinociceptive threshold for this pathway is not significantly influenced by these drugs. Finally, different types of responses between tail flick and hot plate assays also offers a possible explanation. The tail flick assay induces a spinally-mediated reflex, whereas the hot plate assay uses a longer stimulus duration and requires responses which are not primarily based on muscle reflexes. Therefore, drugs which influence motor rather than sensory components of nociceptive pathways would have a greater effect on the tail flick assay.

Studies by Hammond and Yaksh (1984) suggest that monoamine antagonists administered intrathecally do not affect the motor component. They used noxious pinch assay, which utilizes only sensory components, to complement results obtained using the tail flick assay. Intrathecal administration of monoamine antagonists inhibits responses to noxious pinch, which

are in agreement with the results found using the tail flick assay. Thus, phentolamine and methysergide antagonistic effects on morphine (i.c.v.)-induced or stimulation-produced antinociception are not a result of an action of these drugs on motor function.

Experiments using adenosine, noradrenergic, and serotonergic antagonists to inhibit morphine (i.c.v.)-induced antinociception provided preliminary suggestions concerning the neurocircuitry of spinal antinociceptive pathways. Results were not consistently compatible with previous reports, however, including our inability to potentiate the antagonistic actions of phentolamine with methysergide, a compound thought to act by an entirely separate mechanism. Given these observations and the multiple possibilities discussed by Camarata and Yaksh (1984) with respect to the complexity of descending systems, we felt more accurate information could be determined if interactions between the neurotransmitters were investigated when co-administered i.t.

NECA (i.t.)-induced antinociception was potentiated by a subantinociceptive dose of norepinephrine (1.22 nmol) administered i.t., but no potentiation was observed with a subantinociceptive dose (58.8 nmol) of serotonin. Furthermore, no antagonism of the NECA (i.t.)-induced antinociception was observed with the serotonergic

antagonist, methysergide or the noradrenergic antagonist, phentolamine. These results suggested that the mechanism of action for NECA (i.t.)- or serotonin (i.t.)-induced antinociception is different than the mechanism for norepinephrine (i.t.)-induced antinociception. This conclusion is supported by results from experiments using norepinephrine to induce antinociception.

Intrathecal injections of norepinephrine produced dose-dependent antinociception and this effect was antagonized by phentolamine. These results are supported by earlier studies where i.t. injections of norepinephrine induced antinociception in rats (Kuraishi et al., 1977; Reddy et al., 1980). A subantinociceptive dose of NECA (0.030 nmol) or serotonin (58.8 nmol) administered i.t. potentiated norepinephrine (i.t.)-induced antinociception. Theophylline or methysergide caused no antagonism of norepinephrine (i.t.)-induced antinociception.

Previous studies using intrathecal injections of selective alpha 1 and alpha 2 receptor agonists (Howe et al., 1983) and antagonists (Reddy and Yaksh, 1980), show that antinociceptive effects of noradrenergic agonists are mediated by interactions with alpha 2 receptors. Moreover, Camarata and Yaksh (1985) find that the alpha 2 adrenergic receptors which mediate noradrenergic agonists (i.t.)-induced antinociception, are the same alpha 2

adrenergic receptors involved in mediating the spinal effects produced by microinjections of morphine into the periaqueductal gray. Alpha 2 adrenergic receptor involvement in spinal antinociceptive pathways was investigated in the present study with the use of clonidine, a relatively selective alpha 2 adrenergic receptor agonist. Experiments with clonidine yielded similar results as experiments using norepinephrine.

To confirm that serotonin and adenosine functioned in the same pathway separate from norepinephrine, similar experiments were performed as above using serotonin (i.t.) to induce antinociception. In the current studies, i.t. administration of serotonin produced antinociception, as has been demonstrated in other studies using rats (Yaksh and Wilson, 1979; Schmauss et al., 1983) and cats and rabbits (Yaksh and Wilson, 1979). Theophylline (i.t.) but not phentolamine antagonized the serotonin (i.t.) dose-response curve, suggesting that serotonin (i.t.)-induced antinociception may involve release of adenosine. Phentolamine (i.t.) had no affect on antinociception-induced by serotonin (i.t.), providing further evidence that the two descending monoaminergic antinociceptive pathways function via different mechanisms of action. In support of this conclusion, a subantinociceptive dose of norepinephrine (i.t.) potentiated the serotonin

(i.t.) dose-response curve. As expected, the serotonin (i.t.) dose-response curve was not significantly affected by a subantinociceptive dose of NECA (i.t.).

Radioligand binding assays suggest possible involvement of 5-HT 1 receptors in antinociception in rats (Zemlan et al., 1983). Schmauss et al. (1983) used rank order potency of relatively non-selective serotonin receptor antagonists in rats to also suggest a role for 5-HT 1 receptors in mediating the spinal effects of serotonin in the tail flick assay. Although no attempt was made to characterize which spinal serotonin receptors are involved in antinociception in these experiments, it appears that spinal 5-HT 1 receptors in mice may be responsible for mediating the antinociceptive effects of i.t. administered serotonin. Our conclusion is based on similar doses of methysergide used by Schmauss et al. (1983) which allowed them to differentiate between 5-HT 1 and 5-HT 2 receptors.

These results support the hypothesis that bulbospinal noradrenergic and serotonergic pathways, exert effects through their respective spinal receptors to modulate spinal nociceptive processing. Moreover, these results demonstrate that the two descending antinociceptive pathways utilize different mechanisms of action to regulate spinal nociceptive processing. Spinal adenosine appears to function within the same

antinociceptive pathway as serotonin.

These conclusions are based upon data obtained from monoamine and adenosine agonist (i.t.)-induced spinal antinociception but was not well substantiated by purinergic, noradrenergic or serotonergic antagonism of the supraspinal morphine (i.c.v.)-induced antinociception. If spinal serotonin and adenosine induce antinociception via a common mechanism of action or pathway which differs from the mechanism of action which norepinephrine utilizes to induce antinociception, then a multiplicative antagonism would be expected when phentolamine (i.t.) was used in combination with either theophylline (i.t.) or methysergide (i.t.) to antagonize morphine (i.c.v.)-induced antinociception. The combination of phentolamine and theophylline or phentolamine and methysergide administered i.t., however, produced an additive, not multiplicative, antagonism. An explanation for this discrepancy between supraspinal- and spinal-induced antinociception may be that adenosine and norepinephrine do in fact function within a common spinal pathway, an additional pathway that differs from the bulbospinal pathway. One might hypothesize that the spinal pathway is an extension of the bulbospinal pathway and functions to stimulate other endogenous spinal pathways which are not sensitive to monoamine or purinergic receptor antagonists. A

neurocircuit map may be described as two descending pathways (i.e. noradrenergic and serotonergic) which stimulate an array of spinal pathways.

The pain pathway is an evolutionary development which functions as a protective mechanism. Animals receive continuous stimuli from the environment which must be modulated to distinguish pain. Tonically active noradrenergic and serotonergic neuronal pathways modulate sensitivity to nociceptive stimuli at the spinal cord level by establishing a nociceptive threshold (Proudfit and Hammond, 1981). Therefore, stimuli which exceeds this predispositioned nociceptive threhold is preceived as painful and the body reacts accordingly. Situations arise, however, when an elevated nociceptive threshold is desired. Endogenous spinal adenosine interacting with bulbospinal norepinephrine pathways may function in this regard. Adenosine release may be induced by serotonin and serve to elevate the existing nociceptive threshold in occasions of acute or chronic pain by potentiating the actions of the tonically active norepinephrine antinociceptive pathway.

Adenosine may also be involved in the development of tolerance to morphine. Roerig et al. (1984) hypothesizes the mechanism for development of tolerance to opiates involves a decrease in multiplicative interactions between supraspinal and spinal sites. Results from this

thesis clearly implicate endogenous spinal adenosine in this interaction. Roerig et al. conclusions are based on observations that mice tolerant to the effects of morphine had a change in descending monoaminergic pathways with no accompanying alterations at either the supraspinal or spinal sites to morphine. These results suggest the direct action of morphine at spinal sites does not involve the descending monoaminergic pathways. This conclusion is supported by a recent study by Hu et al. (1984), which found no involvement of the monoaminergic pathway in the direct spinal action of morphine.

These investigations have led to several basic discoveries in adenosine and opioid pharmacology. These studies demonstrated antinociception and motor impairment induced by intrathecal administration of adenosine agonists was mediated by A2 adenosine receptors and that separate populations of A2 adenosine receptors is a probable explanation for differences observed between assays. In addition, spinal A2 adenosine receptors responsible for mediating the antinociceptive effects of adenosine agonists (i.t.), appear to be the same receptors responsible for mediating antagonism of morphine (i.c.v.)-induced antinociception by theophylline. Morphine (i.c.v.)-induced antinociception was potentiated by i.t. administration of adenosine

agonists. This multiplicative interaction between supraspinal and spinal sites involve adenosine interactions with the descending antinociceptive noradrenergic pathway and not the serotonergic pathway. Serotonin may, however, be involved in the activation of adenosine release.

Adenosine (i.t.) may have potential clinical use to potentiate the analgesic actions of intrathecal administration of morphine or norepinephrine.

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