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The effects of intraperitoneal administration of antagonists and development of morphine tolerance on the antinociception induced by stimulating the anterior pretectal nucleus of the rat

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1 The effects of intraperitoneal administration of antagonists to morphine, 5-hydroxytryptamine (5-HT), noradrenaline and dopamine have been studied on the antinociceptive effects of electrical stimulation of the anterior pretectal nucleus (APtN) of the rat.

2 A 15 s period of 35 μA sine wave stimulation of APtN significantly increased the latency of the tail flick reflex to noxious heat for periods up to 1 h.

3 Naloxone (0.25–1.0 mg kg^{-1}) attenuated the effects of APtN stimulation in a dose-dependent manner. In rats made tolerant to morphine by daily administration of morphine, the antinociceptive effects of APtN stimulation were significantly reduced.

4 The 5-HT receptor antagonists methysergide (5 mg kg^{-1}) and ketanserin (1 mg kg^{-1}), the dopamine receptor antagonist haloperidol (1 mg kg^{-1}) and the β-adrenoceptor antagonist propranolol (1 mg kg^{-1}) had little effect on the antinociceptive effects of stimulating the APtN.

5 α-Adrenoceptor antagonists caused a dose-dependent antagonism of the response. The order of potency was; idazoxan > prazosin > phenoxybenzamine, the respective ED_{50} for each drug being 0.08: 0.45: 1.5 mg kg^{-1}.

6 It is concluded that antagonism at opioid receptors and α-adrenoceptors but not β-adrenoceptors, dopamine or 5-HT receptors reduces the antinociceptive effects of APtN stimulation. This differs from the reported effects of these antagonists on the antinociception caused by stimulating other sites in the brain.

Introduction

The observation that electrical stimulation of the periaqueductal grey (PAG) causes antinociception in the rat (Reynolds, 1969) provoked intensive research which has culminated in the use of this stimulation in man for the relief of clinically intractable pain (Hosobuchi *et al.*, 1977). It is believed that PAG stimulation activates opioid and monoamine systems in the brain because naloxone and monoamine antagonists potently reduce the antinociceptive effects of PAG stimulation (Basbaum & Fields, 1978). Many surgeons however, implant stimulating electrodes into the brainstem well anterior to the PAG and report greater pain relief with fewer aversive side effects (J. Miles-personal communication). It is not clear if the mechanisms activated by these anterior electrodes are identical to those activated by PAG stimulation.

Prado & Roberts (1985) conducted a survey of sites in the forebrain of rats which caused inhibition of the tail flick response to noxious heat and reported that stimulation of the dorsal brainstem on the mesencephalic – diencephalic border potently inhibited this response and was not accompanied by escape behaviour. Roberts & Rees (1986) subsequently reported that the most sensitive sites lay in the anterior pretectal nucleus (APtN) and that the inhibition of the tail flick was not accompanied by motor or general sensory deficits. APtN stimulation activates a descending pathway which runs in the dorsolateral funiculus to inhibit multireceptive (Lamina V-type) spinothalamic neurones (Rees & Roberts, 1987). This is

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similar to the effects of PAG stimulation but APtN stimulation differs in that marginal spinothalamic high threshold neurones are not inhibited as they are by PAG stimulation (Rees & Roberts, 1987). Thus there are important differences between the effects of the two stimulation sites.

The present study examines the effects of intraperitoneally administered antagonists on the antinociception caused by APtN stimulation to determine whether antagonists of opioids, 5-hydroxytryptamine (5-HT) and catecholamines are effective, as they are known to be against PAG-induced antinociception. It is shown here that pretectally-evoked antinociception resembles PAG-evoked antinociception in that naloxone is an effective antagonist and cross-tolerance is shown between stimulation and systemically administered morphine. However, 5-HT antagonists do not reduce the effects of APtN stimulation which seems therefore to involve different mechanisms from those activated by PAG stimulation. α-Adrenoceptor antagonists are effective but a β-adrenoceptor antagonist and haloperidol are not.

Methods

All experiments were conducted upon male albino Wistar rats weighing between 200–220 g. Changes in the latency of the tail flick escape from noxious heating of the skin was used to assess the antinociceptive effect of electrical stimulation of the APtN. Drugs were administered intraperitoneally.

Implantation of guide cannulae

Rats were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1} i.p.) the head was shaved and placed into a Kopf stereotaxic frame. The skull was exposed and a hole drilled with a number 4 round dental burr directly over the APtN. A 12 mm length of 23 ga. stainless steel hypodermic needle tubing (the guide cannula) was lowered into the hole until the tip of the guide cannula lay 4 mm above the APtN. The stereotaxic coordinates of APtN were A +3.9; L 1.1; H + 1.0 using the zero planes and incisor bar positions described by Konig & Klippel (1963).

Three more holes were drilled into the skull and 10 B.A. steel screws inserted. Dental acrylic was then poured over the screw heads and around the guide cannula. The animal received intramuscular penicillin (Mylipen, 75 mg kg^{-1}), topical terramycin and lignocaine gel. The animal was removed from the stereotaxic frame and housed separately for one week before further experimentation. At the end of each experiment the location of the stimulation site was determined histologically to lie in the APtN as defined by Paxinos & Watson (1982).

Electrical stimulation

Threshold levels of electrical stimulation of the APtN have been determined previously (Prado & Roberts, 1985) and therefore for these studies a 15 s period of a 2 times threshold stimulation of 35 μA r.m.s. 50 Hz sine wave was applied on every occasion. This was applied to a monopolar insulated steel electrode of 16 mm length inserted into the guide cannula just before the stimulation period and removed immediately after. It has been found that this technique allows the repeated stimulation of the anterior pretectal nucleus over a period of a few weeks without severe decrement of the response. Permanently implanted electrodes often show such decrements due to granulation and tissue reactions. Nevertheless, where experiments required repeated stimulation they were carefully designed to eliminate effects due to progressive changes in effectiveness of the stimulation (see below).

Tail flick latency

Rats were placed in a ventilated glass tube and the tail flick recorded at 5 min intervals for periods of up to one hour. The tail was laid across a small wire coil which was at room temperature (20°C). A timer initiated the passage of an electric current through the wire coil such that its temperature rose at 9°C s^{-1}. Normal rats flicked the tail away from the heat source after 2.5 to 3.5 s by which time the coil temperature was between 42.5 and 51.5°C. The latency of this response was recorded. The heat stimulus was always applied to the tail between 4 and 6 cm from the tip. At least three tail flick latencies were taken at 5 min intervals for each animal before a drug was given or the brain stimulated. To enable the pooling of data from a group of animals, the tail flick latencies (TFLs) were normalised according to the following formula:

$$\text{IA} = \frac{\text{TFL} - \text{baseline TFL}}{6 - \text{baseline TFL}}$$

Where IA is the 'Index of Analgesia' which is a term used previously (Azami *et al.*, 1982) and for consistency will continue to be used here. The baseline TFL for each animal was the average of the first three latencies recorded. Six seconds was the maximum TFL recorded because after this period, if the animal had not moved the tail, it was removed by the experimenter to prevent damage by the heat which otherwise leads to changes in sensitivity of the tissues during subsequent tests. This formula gives a value of 0.0 if there were no change from baseline values and 1.0 if the maximal inhibition of the tail flick was seen.

Results are presented as graphs of averaged IA values against time for a group of animals and the variation is indicated by the standard error of the mean. However, non parametric tests for significance

(Mann-Whitney U test) have had to be used as the data are not normally distributed.

Drugs

A range of antagonists was administered intraperitoneally. Naloxone hydrochloride (Endo) 1 mg kg^{-1} was given in the first experiment. In subsequent experiments a dose-response curve was constructed with doses of 0.25, 0.5, 0.75 and 1.0 mg kg^{-1}. The 5-HT antagonists methysergide bimaleate (Sandoz) and ketanserin tartrate (Janssen) were administered in doses of 5 mg kg^{-1} and 1 mg kg^{-1} respectively. As little effect of these very high doses was observed, dose-response curves were not constructed. Similarly the dopamine antagonist haloperidol (free base, Janssen) was given in only one dose of 1 mg kg^{-1}. The adrenoceptor antagonists prazosin hydrochloride (Pfizer, 2 mg kg^{-1}), idazoxan hydrochloride (Reckitt & Colman, 0.5 mg kg^{-1}), phenoxybenzamine hydrochloride (Smith, Kline & French, 2 mg kg^{-1}) and propranolol hydrochloride (I.C.I, 1 mg kg^{-1}) were administered and dose-response curves to prazosin (0.25, 0.5, 1.0 and 2.0 mg kg^{-1}), idazoxan (0.05, 0.075, 0.1 and 0.5 mg kg^{-1}) and phenoxybenzamine (1.0, 2.0 and 4.0 mg kg^{-1}) were made subsequently.

All antagonists were administered 15 min before stimulation of the APtN with the exception of phenoxybenzamine which was given 180 min before stimulation. This was done because the central effects of phenoxybenzamine develop slowly (Azami *et al.*, 1982).

Tolerance to morphine

Further detail of the opioid nature of the response to APtN stimulation was sought by examining the response to APtN stimulation of animals which had developed tolerance to morphine. The change in tail flick latency caused by a high s.c. dose of morphine (5 mg kg^{-1}) was determined for a group of 16 rats. For the next week all the rats received a daily injection; half were given 0.9% NaCl and half morphine s.c. The morphine doses were increased daily in the following sequence: 5, 6, 7, 8, 10, 12 and 15 mg kg^{-1}. On the 8th day they all received 5 mg kg^{-1} morphine for the second time and the effect of this on the tail flick latency was examined.

Another group of 10 animals was subjected to an identical experimental design but instead of the 5 mg kg^{-1} test doses of morphine, effects of APtN stimulation were studied.

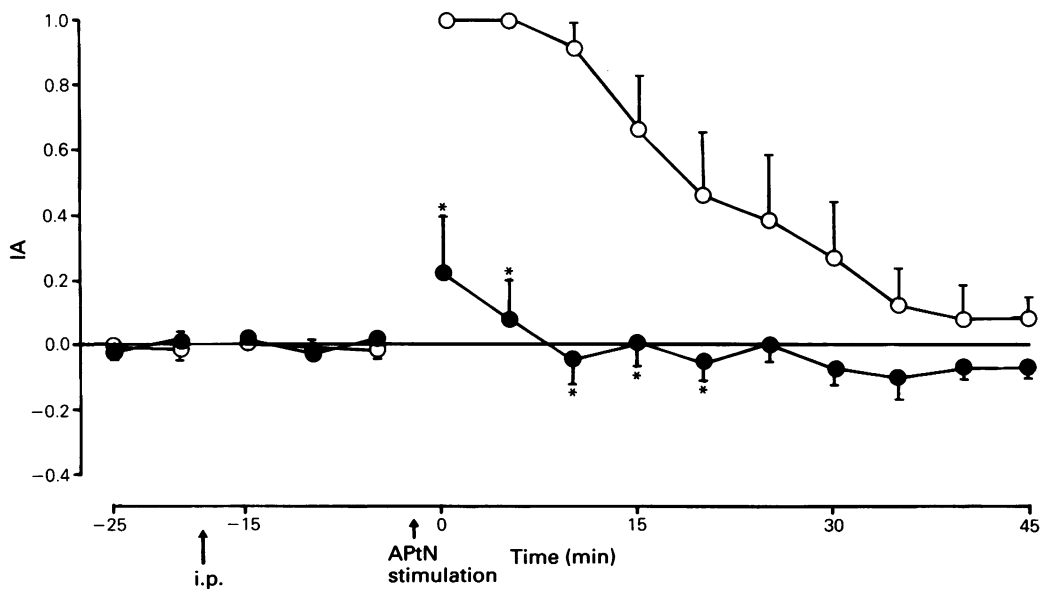

Figure 1 The effects of electrical stimulation of the anterior pretectal nucleus (APtN) on the normalized tail flick latency (IA) of a group of 5 rats. The increase in latency caused by stimulation in animals pretreated with 0.9% NaCl (○, 1.0 ml kg^{-1}) was much reduced in animals pretreated with naloxone (●, 1 mg kg^{-1}). The calculation of 'index of analgesia' (IA) is described in the text. The APtN was stimulated with 35 μA r.m.s. sine wave current for 15 s at time 0. The baseline tail flick latency was established with tests every 5 min during the preceeding 25 min. The i.p. injections were given just before time − 15 min. *Significant difference from control value ($P < 0.05$).

Results

The APtN was stimulated in 65 rats. The stimulation site was confirmed by microscopic examination of brain sections for each of these. With all these animals, stimulation at 35 μA r.m.s. for 15 s usually prevented the tail flick occurring within the 6 s period for 5–10 min after the stimulation. Subsequently the tail flick latency returned to control levels, taking between 30–45 min (see 0.9% NaCl controls in Figure 1, 3b, 4, 5 and 7). As reported previously, the stimulated animals showed no grossly abnormal behaviour. They walked normally but tended to be less active although they responded to touch or noise. Many of the animals however displayed a Straub tail for some minutes after the stimulation.

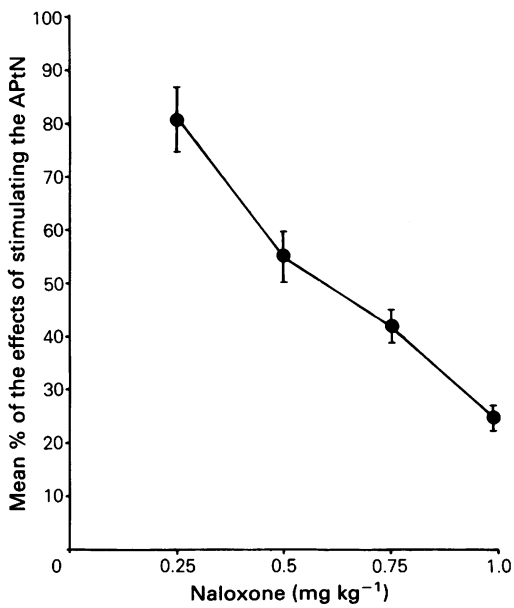

Figure 2 The effects of different doses of naloxone on the increase in tail flick latency caused by APtN stimulation. All animals received i.p. 0.9% NaCl and the response to APtN stimulation established by calculating the 'area under the curve' (e.g. Figure 1). Thus both the magnitude of the tail flick inhibition and the time for which it was maintained was taken into account. The area under the control curve was taken as 100% for each animal. The effect of various i.p. doses of naloxone are shown as a percentage of the response in 0.9% NaCl pretreated animals. Pretreatment with 0.25 mg kg^{-1} naloxone reduced the effects of stimulation to 82.1% of the control. Increasing doses caused increasing antagonism.

Effects of naloxone

The first experiment examined the effect of naloxone on the inhibition of the tail flick reflex by APtN stimulation. A group of 5 animals received i.p. injection of 0.9% NaCl (0.2 ml) or naloxone (1 mg kg^{-1}) 15 min before stimulation of the APtN. On a second occasion 5 days later the same animals again received either 0.9% NaCl or naloxone and were stimulated again. In this and all the other experiments described here the order in which the animals received control or drug injections was varied to ensure that decrements in the effect of stimulation could not be confused with effects of the drug. The time course of the changes in tail flick latency are shown in Figure 1. Stimulation of the APtN failed to inhibit the tail flick reflex significantly following this dose of naloxone.

The effect of different doses of naloxone were studied in a group of 9 animals. Each animal was stimulated on 3 occasions at weekly intervals, receiving pretreatment with 0.9% NaCl, 0.25, 0.5, 0.75 or 1.0 mg kg^{-1} naloxone i.p. in a random order.

The full time course of the effects of APtN stimulation were studied after each injection. The 0.9% NaCl control response to APtN stimulation was not significantly different from the 0.9% NaCl control of the previous experiment. Figure 2 shows the effect of APtN stimulation following each dose. For this calculation both the IA scores and the period for which they were elevated were used (area under the curve of the data as plotted in Figure 1). Naloxone 0.25 mg kg^{-1} i.p. reduced the tail flick inhibition by APtN stimulation to 80%. Increasing doses caused increasing antagonism which was not maximal at 1.0 mg kg^{-1}.

Cross-tolerance between morphine and APtN stimulation

Daily administration of morphine for 1 week markedly attenuated the inhibition of the tail flick reflex by a test dose of morphine. Figure 3a shows that the early phase of the response to morphine was depressed to a small extent but the later parts of the response were absent in the tolerant rats. Similar injections of 0.9% NaCl did not change the response to morphine from that seen before the daily injections were given.

An identical experimental design was used to determine the effects of morphine tolerance on the response to APtN stimulation. The results are shown in Figure 3b. APtN stimulation increased the tail flick latency for between 30 and 40 min as shown previously. Daily injections of 0.9% NaCl for one week had no significant effect on this response but daily injections of morphine profoundly attenuated the effect of APtN stimulation.

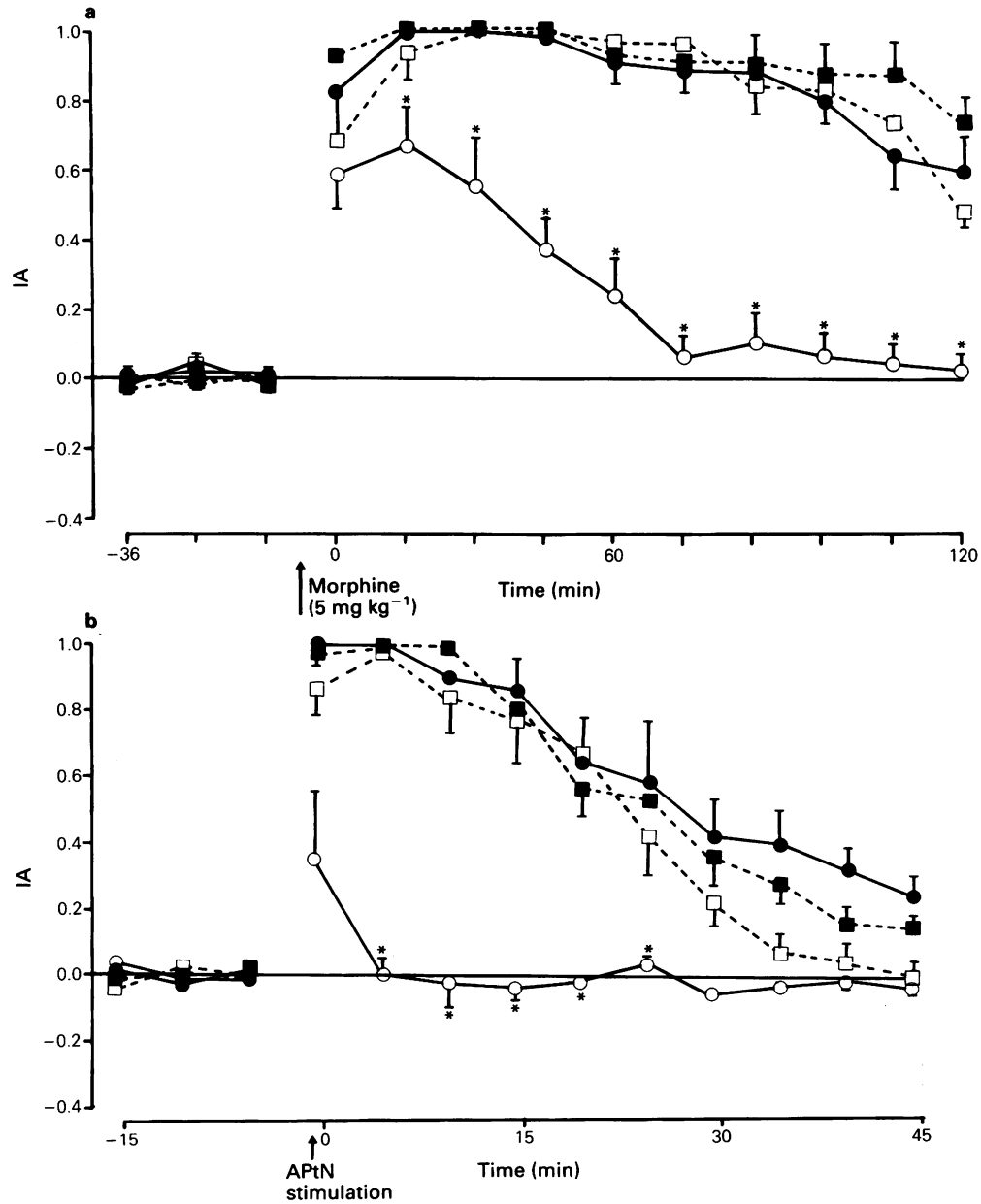

Figure 3 The effects of morphine tolerance on the increase in tail flick latency produced by 5 mg kg^{-1} morphine (a) and APtN stimulation (b). The graph (a) shows the time course of the effects of 5 mg kg^{-1} morphine s.c. on the tail flick reflex. Prior to treatment with 0.9% NaCl (■, $n = 8$) a long lasting increase in tail flick latency was recorded. Following one week of 0.9% NaCl treatment no decrement in the response to morphine was seen (□). This same increase in tail flick latency was seen in the second group of animals prior to morphine treatment (●, $n = 8$). However, following daily morphine administration for one week the effects of 5 mg kg^{-1} morphine (○) were severely attenuated. Section (b) shows the results of an identical experiment except that the test dose of morphine was replaced by APtN stimulation to study the development of cross tolerance between morphine and APtN stimulation. The effect of APtN stimulation was established before 0.9% NaCl administration (■, $n = 6$), and before daily morphine administration (●, $n = 4$). The effects of APtN stimulation were attenuated in animals made tolerant to the effects of morphine (○). The 0.9% NaCl-treated animals (□) were not affected. *Significant difference from control value ($P < 0.05$).

Antagonists of 5-hydroxytryptamine

Very high doses of the 5-HT receptor antagonists methysergide (5 mg kg^{-1}, $n = 8$) and ketanserin (1 mg kg^{-1}, $n = 5$) were administered i.p. 15 min before stimulation of the APtN (Figure 4). Neither antagonist altered the baseline tail flick latencies nor significantly changed the effect of APtN stimulation.

Adrenoceptor antagonists

Prazosin (2 mg kg^{-1}, $n = 5$), idazoxan (0.5 mg kg^{-1}, $n = 6$), phenoxybenzamine (4 mg kg^{-1}, $n = 7$) and propranolol (1 mg kg^{-1}, $n = 6$) were administered intraperitoneally to 4 groups of rats. Each rat also received an injection of 0.9% NaCl solution on a different occasion. Prazosin, idazoxan and propranolol were administered 15 min before stimulation of APtN but phenoxybenzamine was given 3 h previously. The effects of these treatments are shown in Figure 5. It can be seen that the drugs alone did not alter tail flick latencies and that the β-adrenoceptor antagonist propranolol did not alter the response to APtN stimulation. However, the α-adrenoceptor antagonists phenoxybenzamine, idazoxan and prazosin significantly reduced the effects of APtN stimulation.

The relationship between dose and response was studied for prazosin (0.25, 0.5, 1.0 and 2.0 mg kg^{-1} i.p.), idazoxan (0.05, 0.075, 0.1 and 0.5 mg kg^{-1} i.p.) and phenoxybenzamine (1, 2 and 4 mg kg^{-1} i.p.). Figure 6 shows the effects of APtN stimulation following each dose. On the ordinate scale, 100% indicates no change from 0.9% NaCl pretreatment. The percentage reduction was calculated as before from the area under the IA curves (e.g. Figure 5) taking into account both the increase in tail flick latency and the time for which the increase was recorded.

It can be seen that the α-adrenoceptor antagonist prazosin was more potent than phenoxybenzamine. The highest dose of each drug almost abolished the response to APtN stimulation. The response to APtN stimulation was extremely sensitive to the effects of the α_2-adrenoceptor antagonist idazoxan. The dose-response curve was extremely steep, 0.05 mg kg^{-1} having no effect and 0.1 mg kg^{-1} completely abolishing the effects of APtN stimulation.

Effects of haloperidol

The dopamine receptor antagonist haloperidol was administered i.p. 15 min before APtN stimulation in a single dose of 1 mg kg^{-1}. As no effect of this dose was observed (Figure 7) no study of lower doses was made.

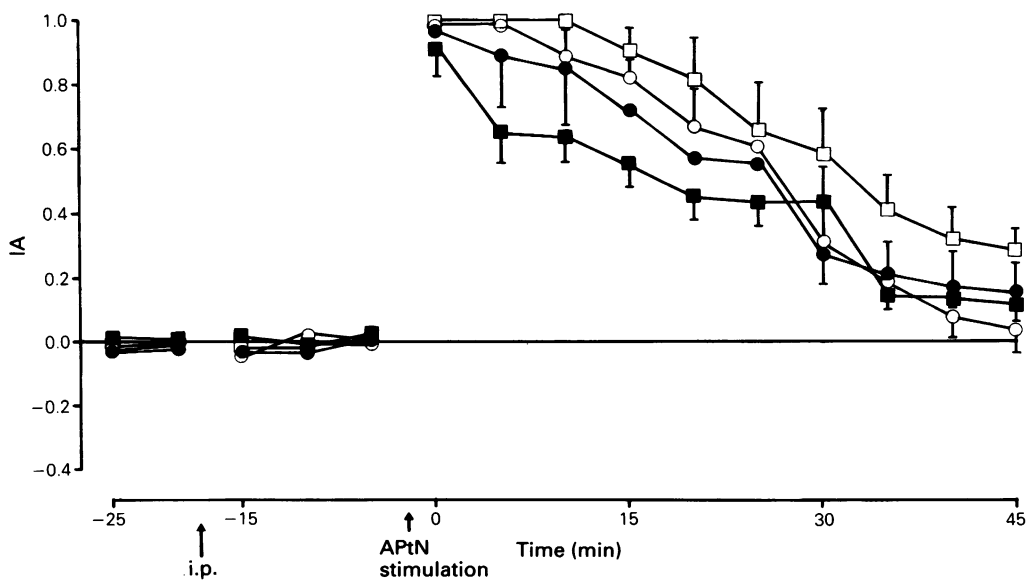

Figure 4 The insignificant effects of the 5-HT antagonists methysergide and ketanserin (i.p.) on the increase in tail flick latency caused by APtN stimulation. Administration of high doses of either methysergide (■, 5 mg kg^{-1}, $n = 8$) or ketanserin (●, 1 mg kg^{-1}, $n = 5$) had no significant effect on the magnitude and time course of the increase in tail flick latency following APtN stimulation, compared with their respective 0.9% NaCl controls (□, ○).

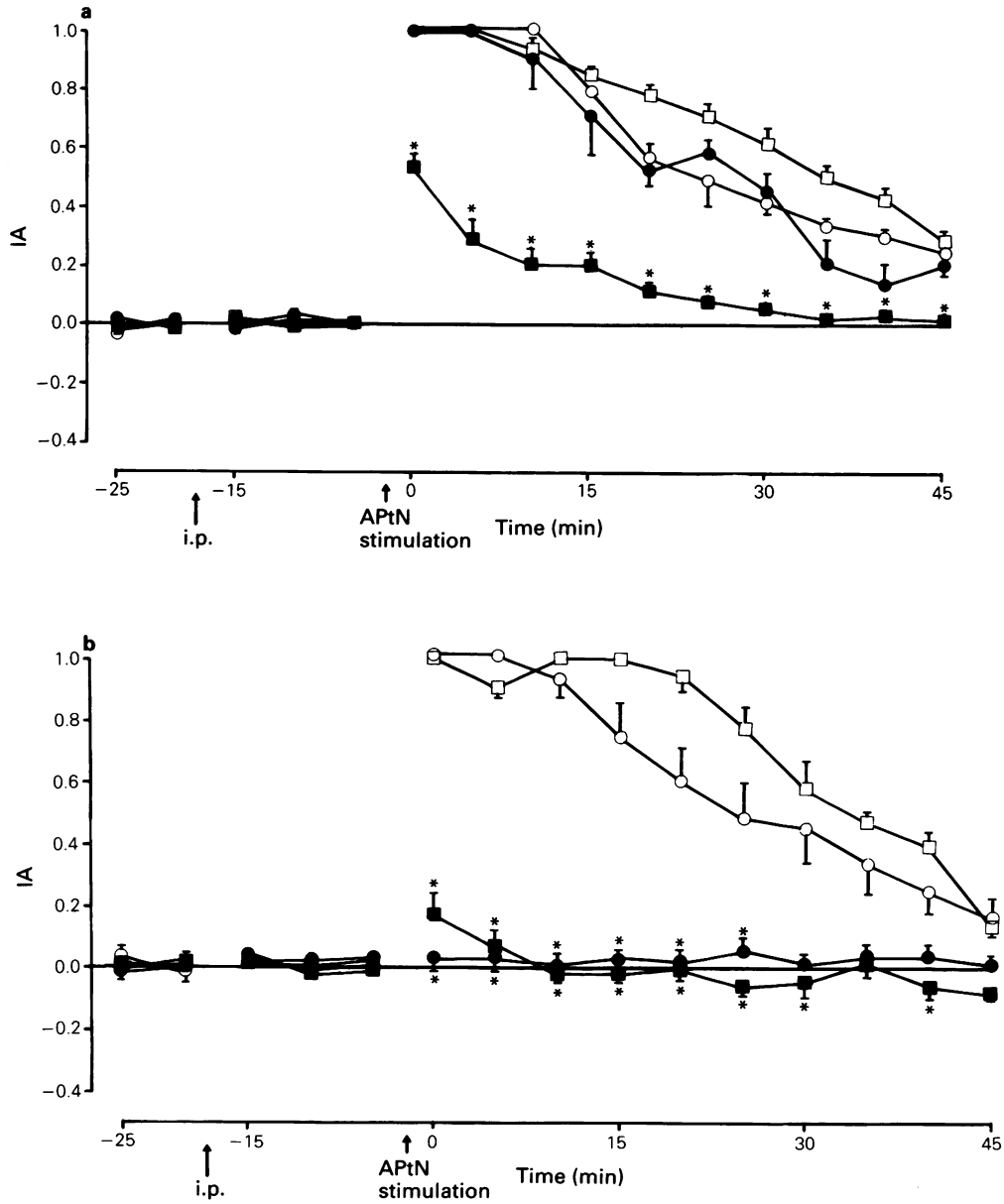

Figure 5 The effect of i.p. administration of antagonists of noradrenaline on the effects of APtN stimulation: (a) shows the effects of the β-adrenoceptor antagonist propranolol (●, 1 mg kg^{-1}, $n = 6$), its 0.9% NaCl control (○, 1 ml kg^{-1}) and the α-adrenoceptor antagonist phenoxybenzamine (■, 4 mg kg^{-1}, $n = 7$) and its 0.9% NaCl control (□). (Phenoxybenzamine, unlike all other drugs used in this study, was administered 180 min before stimulation of the APtN, allowing time for the drug to cross the blood brain barrier). In (b) are shown the effects of the selective α_1-adrenoceptor antagonist prazosin (●, 2 mg kg^{-1} $n = 5$), the α_2-adrenoceptor antagonist idazoxan (■, 0.5 mg kg^{-1} $n = 6$), and their respective 0.9% NaCl controls (○, □). The β-adrenoceptor antagonist was ineffective but phenoxybenzamine reduced the effects of APtN stimulation. Both the α_1- and the α_2-adrenoceptor antagonists nearly abolished the effects of APtN stimulation, possibly because high doses of both were studied (however, see Figure 6). *Significant differences from control value ($P < 0.05$).

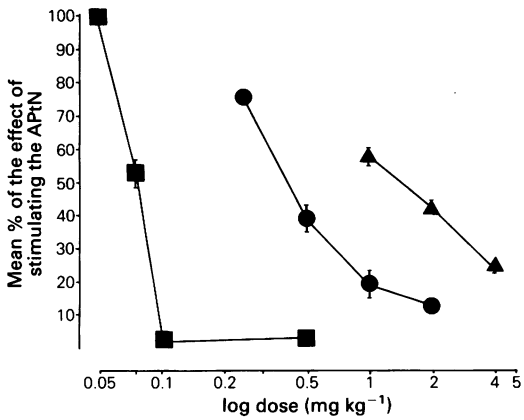

Figure 6 Log dose-response relationships for the α-adrenoceptor antagonists (given i.p.) phenoxybenzamine (▲), prazosin (●) and idazoxan (■). On the ordinate scale, 100% represents no change from 0.9% NaCl pretreatment. As in Figure 2, the magnitude of the inhibition of the tail flick reflex and the period for which it was maintained, were taken into account. It can be seen that idazoxan was very much more potent than either prazosin or phenoxybenzamine.

Discussion

Brief stimulation of the APtN with low currents inhibited the tail flick reflex to noxious heat for periods up to 45 min. As reported previously (Prado & Roberts, 1985), the animals made no attempt to escape this stimulation and seemed placid and docile. They responded to innocuous stimuli and moved easily around the home cage. These observations are similar to an earlier quantitative report which showed that stimulation had little effect on locomotor and startle tests (Roberts & Rees, 1986). Many of the animals developed a Straub tail for some minutes after the stimulation which has been reported to be one of the effects of opioid drugs (Blumberg & Slovak, 1981). The potent reduction by naloxone of the inhibition of the tail flick by APtN stimulation further suggests that this stimulation activates opioid mechanisms. This was confirmed by the demonstration of cross-tolerance between morphine and APtN stimulation.

It is well known that the antinociceptive effects of PAG stimulation are reduced by naloxone (Akil *et al.*, 1976) and exhibit cross-tolerance with chronically administered morphine (Mayer & Hayes, 1975). The effects of APtN stimulation are very potently affected by these treatments. Naloxone almost abolished the response, and chronic morphine administration for just one week with doses that are low compared with the doses used by Mayer and Hayes (1975) (who gave

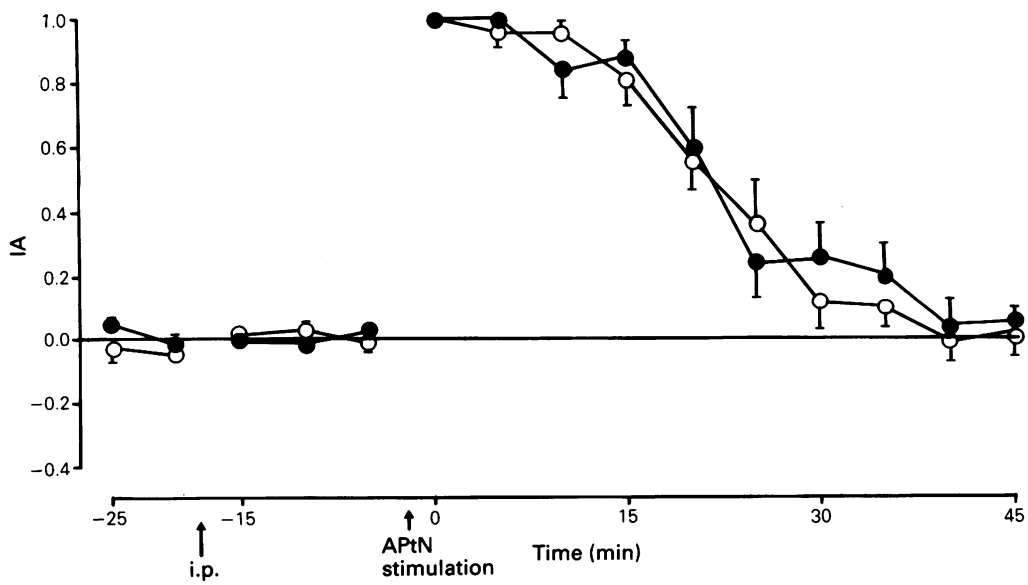

Figure 7 The effects of the dopamine receptor antagonist haloperidol (●, 1 mg kg^{-1} i.p., $n = 5$). No effect was observed at this dose when comparison was made with the 0.9% NaCl control (○).

up to 600 mg kg^{-1} daily for 21 days) caused only partial effects on the antinociception caused by morphine itself and yet almost abolished the response to APtN stimulation. It is concluded that the effects of APtN stimulation are extremely sensitive to manipulation of opioid mechanisms. Confirmation that an opioid mechanism mediates these effects requires only the demonstration that stimulation causes release of an opioid and that the inactive isomer of naloxone is without effect (Hayes *et al.*, 1977b).

It has frequently been demonstrated that the antinociception evoked by stimulating the brainstem in the midline is potently reduced by antagonists of 5-HT Hayes *et al.*, 1977a; Azami *et al.*, 1982) and also by depletion of 5-HT with *p*-chlorophenylalanine (Rivot *et al.*, 1980). It has been assumed that the 5-HT synapses lie in the dorsal horn of the spinal cord and there is considerable evidence for this (Roberts, 1984). However, Llewelyn *et al.* (1983, 1984) have postulated an additional 5-HT synapse between PAG and nucleus raphe magnus (NRM). These two synapses appear to differ in the nature of their 5-HT receptors. The responses of NRM cells to 5-HT are easily blocked by 5-HT antagonists but the antinociceptive actions of 5-HT applied to dorsal horn neurones are resistant to antagonism (Belcher *et al.*, 1978; Griersmith *et al.*, 1981). Central inhibition of nociceptive spinal reflexes may therefore be mediated by either of two 5-HT receptor types. The functional receptor types for 5-HT have recently been classified by Bradley *et al.* (1986) as 5-HT$_1$-like, 5-HT$_2$ and 5-HT$_3$. Evidence for 5-HT$_3$ receptors in the CNS is sparse. Methysergide is the least selective of the 5-HT antagonists as it binds to both 5-HT$_1$ and 5-HT$_2$ binding sites in the brain (Leysen *et al.*, 1981). Ketanserin, on the other hand, is amongst the most selective of 5-HT antagonists (Leysen *et al.*, 1981), binding with very high affinity to the 5-HT$_2$ site. Schmauss *et al.* (1983) have reported that intrathecal methysergide antagonizes the effects of intrathecal 5-HT and that ketanserin is much less potent. They suggest therefore, that receptors which mediate the effects of 5-HT are of the 5-HT$_1$ subtype. This is supported by the observations of Monroe & Smith (1980) who were able to identify only 5-HT$_1$ binding sites in the spinal cord. The effects of APtN stimulation however are resistant to high doses of either methysergide or ketanserin and it is concluded that it is unlikely that APtN stimulation causes inhibition of the tail flick by activating 5-HT systems which contain either 5-HT$_1$ or 5-HT$_2$ receptor types. Carstens *et al.* (1981) have shown that the effects of PAG stimulation are abolished by doses of methysergide between 0.07 and 1 mg kg^{-1}. There is therefore a fundamental difference between the effects of stimulating PAG and APtN.

The failure of 5-HT antagonists to block the antinociceptive effects of stimulating certain sites in the brain has been reported by others. Stimulation of the lateral mesencephalic reticular formation inhibits the responses of dorsal horn cells to noxious stimuli but this inhibition is not blocked by doses of methysergide, lysergic acid diethylamide or *p*-chlorophenylalanine which are effective against PAG stimulation (Guilbaud *et al.*, 1973, Carstens *et al.*, 1981). APtN stimulation inhibits spinal nociceptive reflexes and also inhibits the responses of spinal neurones to noxious stimuli via a pathway which runs in the dorsolateral funiculus (Rees & Roberts, 1987). There is no direct pathway to the spinal cord from APtN but there is a dense projection to the lateral mesencephalic reticular formation (Berkley & Mash, 1978). Direct reticulo-spinal projections run in the ventrolateral funiculus (Kuypers & Maisky, 1977; Basbaum & Tohyama *et al.*, 1979) but indirect projections to the dorsolateral funiculus are via the locus coeruleus (Nygren & Olsen, 1977; Commissiong *et al.*, 1978). It has been suggested that noradrenergic fibres mediate some of the effects of lateral reticular stimulation because such effects are blocked by antagonists of noradrenaline (Carstens *et al.*, 1981). Studies were made therefore of the effects of catecholamine antagonists on the response to APtN stimulation.

The dopamine antagonist haloperidol and the β-adrenoceptor antagonist propranolol had no effect on APtN stimulation but the non-selective α-adrenoceptor antagonist phenoxybenzamine and the α_1-adrenoceptor antagonist prazosin and the α_2-adrenoceptor antagonist idazoxan were potently active.

It has long been known that noradrenaline mediates some of the inhibition of sensory input to dorsal horn neurones (Mayer & Price, 1976) and it has been conclusively demonstrated with many techniques that α-adrenoceptor agonists mimic this inhibition and α-adrenoceptor antagonists potently block it (Kuraishi *et al.*, 1977; Reddy & Yaksh, 1980). These studies have shown that dopamine and β-adrenoceptor antagonists were much less effective. More recent studies have concluded that PAG and mid-line medullary stimulation have effects mediated in part by α_2-adrenoceptors (Camarata & Yaksh, 1985; Fleetwood-Walker *et al.*, 1985; Barbaro *et al.*, 1985). However Camarata & Yaksh (1985) compared the effects of α_1- and α_2-adrenoceptor antagonists on the response to PAG stimulation and found them to be equipotent. They argued that as the affinity of prazosin for α_1-adrenoceptors was much greater than the affinity of yohimbine for α_2-adrenoceptors, the likelihood is that the response was mediated by α_2-adrenoceptors. They stressed however that an involvement of α_1-adrenoceptors could not be excluded. The present studies of the effects of α_1- and α_2-adrenoceptor antagonists on APtN stimulation suggest that similar conclusions may be made. Although the α_2-adrenoceptor antagonist was very much more potent than the α_1-adrenocep-

tor antagonist prazosin this may be due to differences in the penetration into brain tissue or metabolism of the two drugs. It is possible that PAG and APtN stimulation may activate similar α-adrenergic mechanisms.

In conclusion it seems that electrical stimulation of the APtN causes inhibition of the tail flick reflex in the rat by mechanisms which in many ways resemble those which are activated by PAG stimulation but in other respects are strikingly different. Similarities include the effectiveness of opioid and α-adrenoceptor antagonists. Differences include the stronger and longer lasting antinociception from APtN which is not blocked by 5-HT receptor antagonists. It has been reported elsewhere that APtN stimulation is less behaviourally aversive (Prado & Roberts, 1985) and physiologically more selective than PAG stimulation as it inhibits the response of multireceptive lamina V-type spinal neurones without affecting superficial marginal cells in the spinal cord (Rees & Roberts, 1987). These studies have shown that intraperitoneally administered antagonists affect responses to electrical stimulation of the APtN differently from responses to PAG stimulation. This may imply differences in the physiological mechanisms activated by the two sites.

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