

Research report

A pharmacological study of the modulation of neuronal and behavioural nociceptive responses in the rat trigeminal region

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Accepted 6 July 1995

Abstract

Electrical stimulation of the brain, particularly in the periventricular grey areas, caused long-lasting increases in behavioural escape thresholds to heating and mechanical stimuli applied to the facial region of the rat. The brain stimulation selectively suppressed responses to noxious stimuli. Responses to non-noxious stimuli, evoked by low threshold brush, were unaffected. The same animals that were studied in the behavioural tests were then anaesthetized with urethane and the inhibitory effect of the same brain stimulation was studied in single neurones recorded in the caudal trigeminal nucleus. A clear correlation ($r_s = 0.63$) emerged between degree of behavioural antinociception and the amount of inhibition seen in nociceptive neurones. In addition the mean duration of the inhibition (6 min) was similar to the mean duration of the antinociceptive effect (7.3 min). Other classes of non-nociceptive neurones were unaffected by the stimulation. The neurones were also studied using iontophoretically applied monoamine candidates for the inhibitory neurotransmitter, noradrenaline (NA) and 5-hydroxytryptamine (5-HT). The profile of the effects of NA most closely fitted that of the inhibitory neurotransmitter. This profile was expressed in terms of depression and excitation of different classes of neurones, and by the duration of effects. The depressant effects could be antagonized by iontophoretic idazoxan. In addition clonidine induced long-lasting depression of firing. 5-HT was more likely than NA to excite nociceptive neurones and to depress non-nociceptive neurones. Only NA consistently elevated thermal response thresholds in a similar manner to that produced by brain stimulation. These results provide some support for the hypothesis that selective descending inhibition of nociceptive responses in neurones of the rat caudal trigeminal nucleus is mediated by NA, possibly by an action at α_2 -adrenoceptors.

Keywords: Single cell recording; Descending inhibition; Thermal nociception; Microiontophoresis; Monoamine; α -Adrenoceptor; Caudal trigeminal nucleus; Medullary dorsal horn

1. Introduction

Active inhibitory and excitatory mechanisms modulate sensory transmission in the spinal cord and caudal trigeminal nucleus. These effects are mediated through supraspinal centrifugal and local segmental pathways. Hagbarth and Kerr [26] first demonstrated the existence of centrifugal control on somatosensory afferent transmission in the spinal

cord, and subsequent studies have shown similar controls for other sensory systems. The possible clinical importance of this finding has since become apparent by the extensive evidence that there exists an endogenous pain control system in the vertebrate (see reviews, Refs. [35,50]). For example electrical stimulation of mainly midline and periventricular sites of the midbrain and brain stem can abolish behavioural responses to noxious stimuli in a range of different animal species [28,37,40,41,52], including man [1].

The precise mechanisms underlying the modulation of sensory transmission still remain to be fully elucidated. However, at this stage, there is a lack of direct evidence linking the operation of descending inhibition and the display of antinociceptive behaviour. Specifically, it is not known whether the long-lasting antinociceptive effects of

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electrical stimulation of the midbrain are due to the observed inhibitory neuronal events that are confined to the preliminary levels of analysis in the spinal cord and the caudal trigeminal nucleus.

The aim of this report is to provide direct evidence that inhibition of nociceptive responses of caudal trigeminal neurones can account for behavioural antinociception to thermal stimulation that follows electrical stimulation of the periventricular region. In successfully demonstrating a correlation between the degree of long-lasting behavioural antinociception and the degree of inhibition observed in caudal trigeminal neurones, further experiments were directed towards identifying the inhibitory neurotransmitter responsible for such prolonged effects.

2. Materials and methods

2.1. Cranial implantation of pedestal and stimulating electrode

Standard aseptic surgical techniques under pentobarbitone sodium anaesthesia (60 mg/kg intraperitoneal) were used to implant a stimulating electrode and a 2BA plastic nut onto the skull of each of 36 male Wistar derived rats (weighing 300–550 g). The bipolar electrodes were constructed from 0.24 mm diameter teflon-coated stainless steel wires twisted together. Electrode tips were aimed at various rostro-caudal sites in and around the midbrain periventricular grey matter (the area surrounding the 3rd and 4th ventricles). The implanted plastic nut acted as a pedestal for the attachment of a thermal stimulation device during behavioural experiments. Animals were individually caged and seven days were allowed before behavioural testing began. Details of the construction and implantation of the assembly are given by Morris et al. [36].

2.2. Behavioural experiments

A 2-cm² area of the maxillary/mandibular region the face was shaved clean of hair and a 16 mm² contact heating disc was fitted. A feedback circuit was used to drive a 10 Ω heating resistor located in the contact disc, as previously described [36]. Temperature was monitored by an electronic thermometer connected to a copper-constantan thermocouple. The thermocouple was situated in the centre of the disc and in contact with the skin. Thermal ramp stimuli 0.5°C/s were used, with a cut-off temperature of 48°C to avoid tissue damage. Trials typically lasted 16 s and were terminated immediately by the experimenter on observing an escape response (scratching at the device). Each trial was separated by 3 min. Behavioural thermal response thresholds for each trial were stored on a floppy disc for later analysis.

A mechanical stimulation device was also used to apply stimuli to the face of the rat, and this fitted the same pedestal in place of the heating device. The device has been more fully described previously [6] and consisted of a computer controlled pneumatically driven plunger that was in contact with facial skin around the premaxillary or maxillary bones. The tip of the plunger measured 0.4 mm diameter. Each trial consisted of a ramp of 16.3 g/mm² (actual pressure on the skin) per second and a cut-off pressure of 310 g/mm². Trials typically lasted 12 s. The behavioural escape response evoked by the mechanical stimulus was identical to that observed with the thermal device. The stimulus ramp was terminated immediately on the experimenter observing a response, and each threshold (thermal or mechanical) was stored on floppy disc for later analysis. Further details of the testing procedure are more fully described by Cahusac et al. [6].

Animals were given 15 min to habituate to the testing box before behavioural testing began. All animals tolerated the heating and mechanical devices well, and no animals made concerted attempts to remove the devices. During testing animals showed the normal range of behaviours including grooming, and no animals appeared distressed at any time. Since the stimuli were terminated on execution of the escape response, this prevented any suprathreshold nociceptive experience by the animal. These methods allowed animals to be studied for up to 150 min in continuous session, in which up to 50 reliable thresholds could be obtained.

Brain stimulation was applied through a lead attached to the implanted electrode. The stimulation consisted of 0.5 ms duration rectangular pulses delivered at 50 Hz for 20 s from a Grass SD9 isolated stimulator. Current was continuously monitored on an oscilloscope, and ranged from 20–500 μ A. At least two clear control trials in which thresholds varied by less than 1°C preceded the testing of the effect of brain stimulation. On a test trial brain stimulation terminated at the onset of the thermal ramp stimulus. Therefore the effect of the brain stimulation typically had to last at least 10 s to influence the nociceptive behaviour. Test current intensities were usually done in the following sequence: 25, 50, 100, 150 and then increased in 50 μ A steps with an upper maximum of 500 μ A. Test trials were carried out with at least two inter-test control trials where thresholds had returned to baseline values. The degree of antinociception induced was calculated with the widely used formula: $(\text{test} - \text{control}) / (\text{cut-off} - \text{control}) \times 100\%$; where the test value was the temperature at which a response occurred after brain stimulation, and the cut-off was 48°C. A score of 30% or more was taken to represent significant antinociception. Successive test trials were given until significant antinociception was observed or until disturbing motor activation occurred (which consisted variously of circling, explosive jumping and vocalization). To ensure reproducibility of the effects of brain stimulation the test procedure was repeated on several separate ses-

sions (days), and each animal was tested again on the day prior to the electrophysiological experiment.

2.3. Electrophysiology

Fifty-eight naive and the 36 animals that received cranial implants were used in electrophysiological experiments. Animals were anaesthetized with 1.25 g/kg intraperitoneal of urethane, and were prepared for recording as detailed previously [6,32]. The caudal medulla was exposed and the recording electrode penetrations made perpendicular to the surface of the medulla. Penetrations were positioned 1–2.5 mm lateral to the midline and 1–3 mm caudal to the obex.

All micropipettes for single cell recording and microiontophoresis were made using various diameters of fibre-filled borosilicate glass tubing (Clark Electromedical Instruments). Electrodes were filled with a selection of drug solutions that included Na L-glutamate (0.5 M, pH 8.5), γ -amino-N-butyric acid (GABA) (0.5 M, pH 3.5), noradrenaline HCl (0.2 M, pH 4), 5-hydroxytryptamine HCl (0.2 M, pH 3.4), clonidine HCl (0.01 in 0.1 M NaCl, pH 5.3), phenylephrine HCl (0.2 M, pH 4), sotalol HCl (0.2 M, pH 3.9), propranolol HCl (0.2 M, pH 5.1), dibenamine HCl (0.01 in 0.015 M NaCl, pH 3.1), labetalol HCl (0.025 in 0.02 M NaCl, pH 3.1), rauwolscine (0.1 in 0.2 M ascorbic acid, pH 3.7), cinanserin HCl (0.1 M, pH 4.5), methysergide hydrogen-maleate (0.01 M, pH 7), *cis* (*Z*)- and *trans* (*E*)-flupenthixol 2HCl (0.02 M, pH 3.1), piperoxane HCl (0.1 M, pH 4.6), idazoxan HCl (0.2 M, pH 3.3), WB 4101 HCl (0.1 M, pH 3.2) obtained from Ward Blenkinsop, prazosin HCl (0.002 M, pH 6). Agonists were applied using currents up to 100 nA, though on most occasions currents between 20–60 nA were usually effective in producing changes in cell firing. An effect was considered to be significant if an alteration greater than 50% of the initial (control) firing rate occurred following the drug, and the effect observed at least twice in succession. A drug was deemed to have no effect if no alterations in firing occurred following an iontophoretic current of 100 nA for 120 s.

One side-barrel contained Pontamine sky blue (2% in 0.5 M Na acetate) for dye marking. A few single-barrelled electrodes were used and these were filled with Pontamine sky blue dye. The recording barrel of multi-barrelled electrodes was either filled with 3.5 M NaCl or with a carbon fibre and 150 mM NaCl. A further side-barrel was filled with 1 M NaCl for current balancing and for passing control currents.

Conventional single-ended amplification and filtering (100 Hz–3 kHz bandwidth) techniques were used. Gated pulses were fed into a ratemeter and through an interface (502, Cambridge Electronic Design) to an on-line PDP-11 computer. A computer program recorded skin temperature and firing rate simultaneously [31]. Data were stored on floppy disc for detailed post-experiment analysis. Records

of iontophoresis applications, spike ratemeter, blood pressure, heart rate, stimulus skin temperature and other stimulus markers were collected on a multichannel chart recorder, and occasionally on tape recorder (7DS Racal). Mechanical stimulation (brush, prod and pinch) and iontophoresis of glutamate were used to test and search for neuronal activity in the course of making an electrode track.

The thermal stimulus used in the electrophysiological experiments consisted of perfusing heated water over the receptive field of the recorded neurone. This method allowed a large proportion of the neurone's receptive field to be stimulated, and was found to cause less tissue damage than other methods such as the use of radiant heat or the heating disc. The water passed through a 1 m length of plastic tubing and was derived from a hot water reservoir maintained at 65°C. This procedure resulted in an increase of skin temperature at a rate of about 0.7°C/s during each trial. Each stimulus trial was separated by 3 min, as done in the behavioural experiments. For each neurone studied a suitable maximum temperature was selected to evoke a clear response above threshold. This was typically about 48°C and never exceeded 52°C. Stimulus temperature was monitored by a thermocouple placed close to, or in contact with the skin. Thermal thresholds were determined from a plot of firing rate against stimulus temperature through extrapolation from the curve to its intersection with the baseline. The point of intersection was taken as the threshold value. Thresholds were determined after completion of all the experiments, and were carried out blind with respect to the particular experiment and trial number. This ensured there could be no subjective bias in the estimation of thresholds.

Innocuous mechanical stimulation was applied by brushing facial hairs with a camel hair artist brush, and fairly selective activation of hair follicle receptors could be achieved using an air jet playing over the rat's face [32]. Reliable stimulation of low threshold mechanoreceptors also occurred to the initial pulse of water during thermal stimulation trials (see Fig. 5 in Ref. [6]). In this way the possible differential effects of brain stimulation on low threshold mechanical and on thermal nociceptive responses could be studied in multireceptive neurones. Seven different types of neurones were categorized in the following way. *Low threshold mechanoreceptive* neurones responded exclusively to low intensity mechanical stimulation of the receptive field using a brush or 3 g von Frey filament. *Mechano-multireceptive* neurones responded to low and to high intensity mechanical stimulation (noxious prod and pinch using locking plastic forceps), and had a concentric receptive field organization. *Mechano-nociceptive* neurones responded only to high intensity mechanical stimulation. *Thermoreceptive* neurones responded only to cooling stimuli. *Thermo-multireceptive* neurones responded to non-noxious warming stimuli (30–38°C) in addition to low and high intensity mechanical stimulation, and had a con-

centric receptive field. *Noci-multireceptive* neurones responded to noxious heating stimuli (36–51°C) in addition to low and high intensity mechanical stimulation, and had a concentric receptive field. *Nocireceptive* neurones responded to noxious heating stimuli (38–51°C) in addition to high intensity mechanical stimulation, and had a concentric receptive field. For further details see Cahusac et al. [6]. Neurones with receptive fields that extended beyond the trigeminal dermatomes were excluded from study.

In each animal the parameters of brain stimulation (pulse width, duration of train, current intensity) that induced significant behavioural nociception in a given animal were subsequently used in the acute electrophysiological experiment, where the effects of the stimulation were studied on the sensory responses of single cells. The degree of inhibition of evoked responses was calculated using the same formula given above for determining behavioural antinociception.

2.4. Histology

Locations of recording sites were determined in 95% of experiments by iontophoretic Pontamine sky blue ejection from one barrel of the electrode [30] at two positions along each electrode track. In an animal with an implanted stimulating electrode a lesion was made by passing 100 μA DC for 5 s through the electrode. The animal was then perfused with 4% paraformaldehyde in 0.9% NaCl solution through the aorta. Fixed tissue was cut in 100 μm transverse sections using a freezing microtome. Recording positions were reconstructed by interpolation from the positions of dye marks and were plotted on a diagram of a transverse section of medulla adapted from the atlas of

Palkovits and Jacobowitz [38] which corresponded to a level of 1 mm caudal to the obex. The stimulation site was plotted on an appropriate rostro-caudal diagram of a transverse section of the brain adapted from the atlas of Pellegrino et al. [39].

3. Results

3.1. Effects of brain stimulation on behavioural responses to thermal and mechanical stimuli

Of 36 animals studied, 31 animals showed significant antinociception following brain stimulation. An example of thermal antinociception induced by electrical stimulation in one such animal is shown in Fig. 1. The mean antinociceptive effect in the 31 animals was 79% (range 30–100%). The mean duration of antinociception, judged by the time taken for escape thresholds to return to baseline values, was 7.3 min (range 2–20 min). The mean effective current used for brain stimulation was 140 μA (range 20–500 μA). With the exception of one animal, the degree of antinociception observed increased with the current used (the exception showed antinociception at low currents and no antinociception at higher currents). Three of the animals that showed significant thermal antinociception were also tested for mechanical antinociception. Each animal displayed significant mechanical antinociception. The mean antinociception in these three animals was 95%. Tests were carried out on the effect of brain stimulation on other types of somatosensory stimulation, such as the reflex eye-blink responses to low threshold brush stimulation given just below the eye. The blink response depended

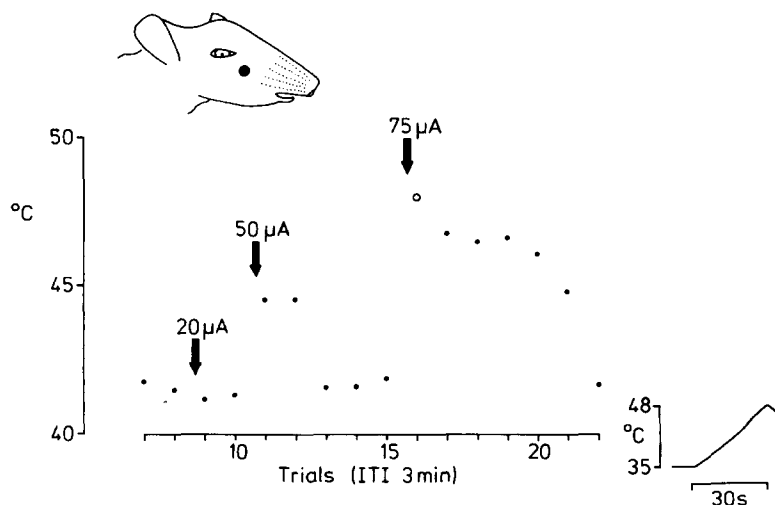


Fig. 1. Antinociceptive effect of brain stimulation on behavioural escape thresholds to a thermal stimulus applied to the rat's face (position indicated by dark circle on the face outline shown above). Trials are plotted on the horizontal axis and the temperature at which escape responses were made (thresholds) plotted on the vertical axis. Electrical stimulation of the brain is indicated by the large downward arrows, and labelled with the current used. Clear current dependence of antinociception was observed in that 20 μA had no effect, while higher currents 50 and 75 μA were increasingly more effective in elevating escape thresholds. The open circle following 75 μA stimulation indicates that the escape threshold exceeded 48°C (100% antinociception). The thermal stimulus ramp used is represented in the inset shown at bottom right. Intertrial intervals of 3 min were used.

upon contact with the guard hairs, and did not occur to the sight of the brush. In these tests, which were given immediately after brain stimulation, the blink response was always intact despite a clear effect upon nociceptive stimuli. Fig. 2 illustrates an example of these tests.

For comparative purposes with other standard tests, 12 animals that displayed trigeminal antinociception to brain stimulation were also tested on the tail immersion test using water at 54°C (a variation on the method described by Janssen et al. [33]). Five of these animals displayed significant antinociception (> 30%) to noxious stimulation of the tail following the same brain stimulation.

Several types of behaviours were evoked by brain stimulation, and their occurrence could not be correlated with antinociceptive effects. The most commonly observed types of evoked behaviour were: exploratory behaviour, sniffing, coordinated circling ipsilateral to the electrode tip position, head movements, uncoordinated jerky movements including ambulation, immobilization, rapid breathing, widening of the eyes, vocalization. These behaviours rarely occurred in isolation. Most of the behaviours were current dependent, so that an animal showing walking/investigative behaviour at low currents showed running and jumping at higher currents.

The effect of the opioid antagonist naloxone (1–10 mg/kg intraperitoneal (i.p.)) were tested on trigeminal thermal antinociception induced by brain stimulation. In only 2 out of 6 animals, antinociception was abolished, suggesting that in many animals the endogenous opioid system was not involved in mediating this form of antinociception.

3.2. Effects of brain stimulation on neuronal responses to thermal and mechanical stimuli

Neurons were classified according to a system described in section 2. The effects of brain stimulation,

previously shown to induce behavioural antinociception, were tested on responses evoked by natural stimulation in four classes of sensory neurone of the caudal trigeminal nucleus. On average, one cell per animal was tested for the effects of brain stimulation. These data are from the 31 animals that displayed behavioural antinociception following brain stimulation.

The responses of all four *low threshold mechanoreceptive* neurones activated by an air jet or light prodding were completely unaffected by the brain stimulation. Even sensory stimulation that followed brain stimulation by a few seconds was unaffected.

The responses of three *mechano-multireceptive* neurones activated by noxious pinch were also unaffected by the brain stimulation; see Fig. 3. The spontaneous activity of an additional neurone of this class was unaffected by the brain stimulation.

Thermal nociceptive responses of 15 out of 17 *nociceptive* neurones were significantly inhibited (> 30% effect) by the brain stimulation. An example of the results from one such experiment is shown in Fig. 4. The effect of the stimulation was to increase the threshold at which the neurones responded to thermal stimulation. Seven of these neurones were spontaneously firing action potentials and in no instance was inhibition of this activity seen. No significant decreases in thermal thresholds were observed. It was of particular interest to study the inhibitory effects of brain stimulation on the low threshold responses that were often evoked by the initial pulse of water (see section 2). Fourteen of the 15 inhibited neurones were also excited by this low threshold mechanical stimulus. In seven cases this response was also inhibited. It should be noted, however, that this stimulus occurred only a few seconds (< 5 s) after the end of the brain stimulation train, whereas the threshold heat intensities were reached 15–25 s following the end of brain stimulation. Therefore, depending on the duration of the effects of brain stimula-

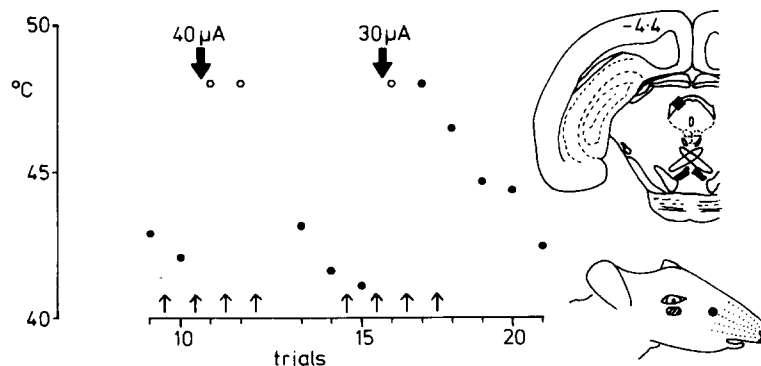


Fig. 2. Selective antinociceptive effect of brain stimulation on noxious stimulation. Brain stimulation (40 μ A and 30 μ A at large downward arrows) resulted in powerful thermal antinociception. At the times indicated by the narrow upward arrows a brush stimulus was applied to a region just below the eye of the rat (shown as a stippled region on the face outline) to test for eye-blink responses. The eye-blink response occurred each time the brush stimulus was applied, and was never affected by the brain stimulation. The filled circle on the face outline indicates where the heating disc was fitted. The tilted square on the transverse section of the midbrain represents the site of the stimulating electrode tip. Trials at which open circles occurred indicates there was no escape response up to the cut-off temperature of 48°C.

tion, the low threshold response may be more susceptible. It is noteworthy therefore that in the remaining seven neurones the low threshold responses were preserved, despite an inhibition of the thermal response.

Five out of eight nociceptive neurones activated by thermal stimuli were significantly inhibited following the brain stimulation.

3.3. Correlation between behavioural and neuronal thermal threshold changes following brain stimulation

For 18 animals, sufficient data were available to allow a correlation to be made between changes in behavioural thermal thresholds with changes in neuronal thermal

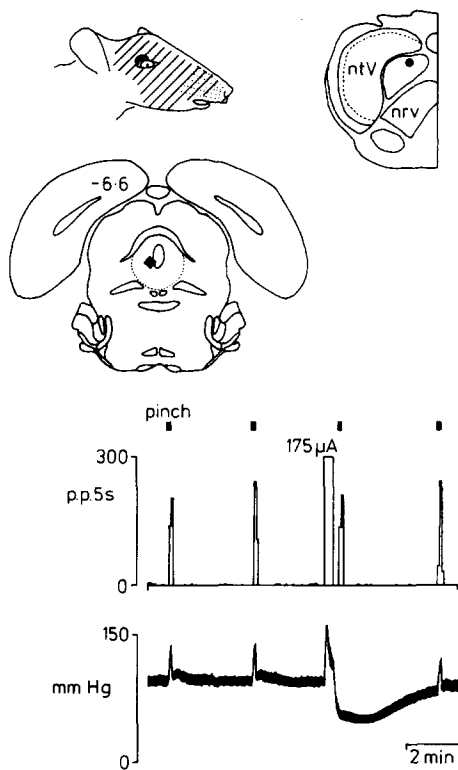


Fig. 3. Lack of effect of periventricular grey matter stimulation on responses of a mechano-multireceptive neurone to a noxious mechanical stimulus. Locking plastic forceps were used to apply the mechanical stimulus (filled area above eye on face outline; shaded area was receptive field). Filled bars above the record show the times at which the stimulus was applied (intertrial interval was 3 min). Brain stimulation at 175 μ A (indicated by large stimulus artefact) preceded the mechanical stimulus by 5 s and no effect on the evoked response was observed. The brisk increases in blood pressure, shown on bottom record, indicate that the mechanical stimulus used was noxious. Note also the pronounced effects of brain stimulation on blood pressure. In behavioural studies this animal had displayed 100% antinociception, and a thermal nociceptive neurone studied shortly after this neurone was powerfully inhibited by the same stimulation. The recording site of the neurone was in laminae IV–V of the medullary dorsal horn (filled circle on diagram of transverse section of medulla); abbreviations used are identified in Fig. 6. The stimulation site is shown by the filled upright square on a transverse section of the mesencephalon.

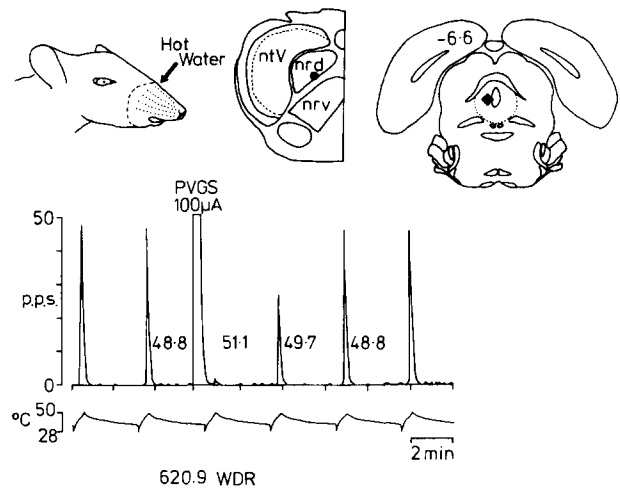


Fig. 4. The inhibitory effect of brain stimulation on a noxious, thermally evoked response of a noci-multireceptive neurone. The thermal stimulus was delivered at regular intervals and consisted of heated water (28–51.2°C) which was applied to a large area of the maxillary and mandibular regions of the face (region rostral to the dotted line on the face outline), and temperature was recorded as shown on the bottom trace. Brain stimulation at 100 μ A (indicated by large stimulus artefact on ratemeter record) inhibited the thermal response. Analysis of the responses using a computer program (see text) produced estimates of thermal firing response thresholds. The respective thresholds (°C) for responses are shown to the right of each response trace. The control threshold was 48.8°C, while the threshold following brain stimulation was elevated to 51.1°C. The thermal thresholds were shown to return to control levels after approximately 6 min. The degree of inhibition was calculated to be 96%. This compares with the degree of behavioural antinociception observed previously in this animal of 71%. Behavioural antinociception lasted approximately 9 min. The recording site of the neurone is shown by the filled circle on the diagram of a transverse section of medulla; abbreviations used are identified in Fig. 6. The stimulation site is shown as an upright filled square on a diagram of the transverse section of the midbrain.

thresholds induced by brain stimulation. Five animals showing no significant behavioural antinociception were included. The electrophysiological data included that gathered from 22 noci-multireceptive and five nociceptive neurones. A previous study had suggested that thermal nociception was primarily mediated by these two classes of neurone in the caudal trigeminal nucleus [6]. The neuronal data from each animal were pooled and expressed as an arithmetic average. A clear correlation was found (see Fig. 5), in which Spearman's rank correlation coefficient $r_s = 0.63$ was statistically significant ($P < 0.01$). The data points appear to be clustered, with the exception of 1 point, into two groups. One group representing animals which displayed strong antinociception and neuronal inhibition, with the second group representing animals with little or no antinociception together with weak neuronal inhibition. The mean antinociceptive effect was 57.5%, which was very similar to the mean of 53.7% for neuronal inhibition.

The mean duration of the inhibition of thermally evoked responses was 6 min (range 3–12 min), this compares

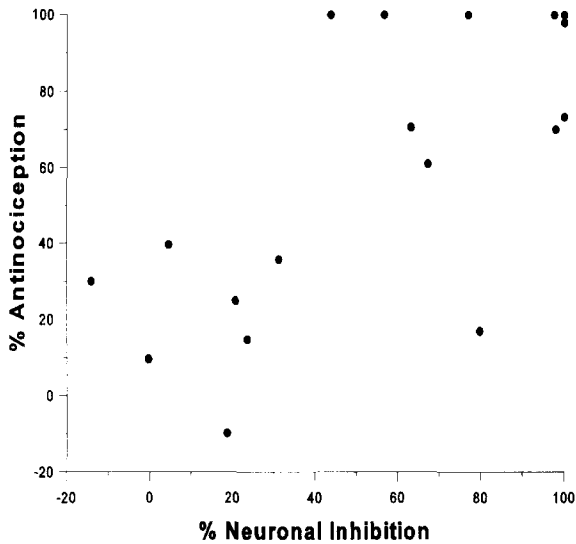


Fig. 5. Plot of percent antinociception against percent neuronal inhibition produced by brain stimulation in 18 animals. Each point represents the degree of behavioural antinociception observed on the previous day plotted against the neuronal data (either from a single neurone or from several neurones pooled and averaged). A significant correlation was observed ($r_s = 0.63$, $P < 0.01$).

favourably with the mean duration of thermal antinociception of 7.3 min.

Recording sites of these neurones were distributed throughout the deeper laminae (IV–VI) as well as in the superficial laminae (I–II) of the medullary dorsal horn (see Fig. 6). Both superficial and deep neurones were similarly affected by antinociceptive brain stimulation. The locations of stimulating electrode tips for 15 of the animals are shown in Fig. 7. Most sites that produced antinociception

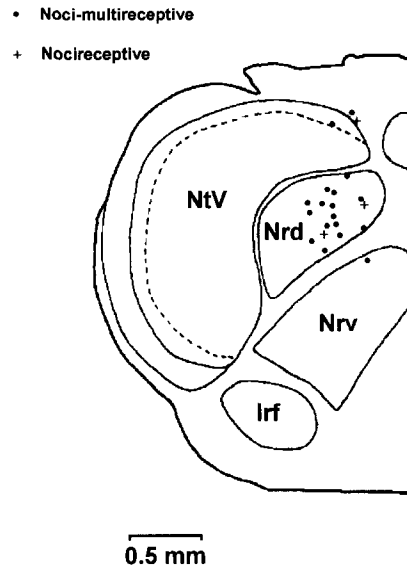


Fig. 6. Recording sites of thermally responsive neurones used to correlate with behavioural antinociception. Filled circles represent noci-multireceptive neurones and crosses represent nocireceptive neurones. Sites were plotted on a diagram of a transverse section of the medulla. NtV, nucleus of the tract of the fifth nerve; Nrd, nucleus reticularis dorsalis; Nrv, nucleus reticularis ventralis; lrf, lateral reticular formation (nucleus). The substantia gelatinosa lies in the outer regions of NtV and is delineated by the dotted lines. The marginal layer and substantia gelatinosa correspond to laminae I and II of the medullary dorsal horn respectively. The magnocellular region (NtV) corresponds to layers III and IV, while the more ventral reticular areas (Nrd and the superficial part of Nrv) correspond laminae V and VI. This system of delineation is consistent with the anatomical criteria given by Gobel et al. [22].

(and inhibition) were within the periventricular grey matter and close to or in the wall of the ventricular cavity. Both dorsal and ventral sites were effective.

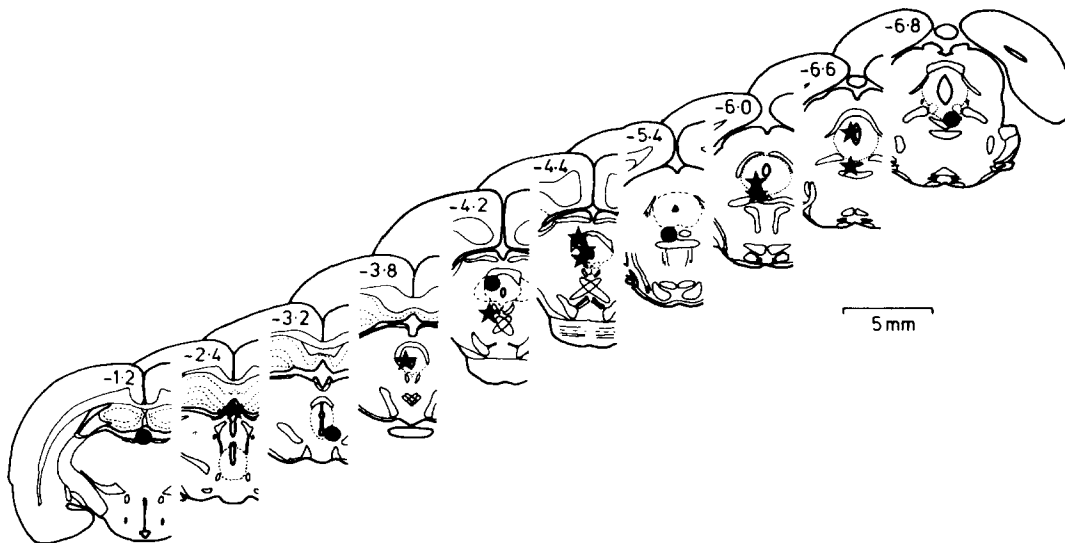


Fig. 7. The loci of stimulating electrode tips plotted on diagrams of transverse sections of the brain. The sections are in rostro-caudal sequence from left to right, and numbers represent positions in mm rostral from the bregma suture. The filled stars represent sites effective at inducing behavioural antinociception, while the filled circles were sites that were ineffective.

3.4. Iontophoretic application of putative monoamine neurotransmitter candidates mediating inhibition of trigeminal neurones

The effects of iontophoretic noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were tested on a variety of evoked and spontaneous activities in classified trigeminal neurones. Non-spontaneous cells were tested by driving their activity with iontophoretic glutamate. The effects of the two monoamines were tested against evoked responses to mechanical and thermal stimuli (as appropriate to the class of cell studied).

The data for noradrenaline are based upon the study of 85 non-thermal nociceptive (non-TN) neurones (43 low threshold mechanoreceptive, 31 mechano-multireceptive, 6 mechano-nociceptive, 4 thermoreceptive, 1 thermo-multireceptive), and 47 thermal nociceptive (TN) neurones (37 noci-multireceptive, 10 nociceptive). Depressant effects of noradrenaline were most notable in TN neurones (85% depression with 15% excitation, biphasic or no effect). The results from one such experiment, where NA produced strong and long-lasting depression, are shown in Fig. 8. The duration of depression was characteristically long (mean = 5.3 min), with mean duration of excitations of 2.3 min. In seven neurones tested with noxious heat, iontophoretic noradrenaline caused a significant increase in thermal thresholds (mean = 62%, range 40–75%). In the

non-TN neurones depression occurred on only 51% of occasions (49% excitation, biphasic or no effect). While the duration of depression was much less than that for TN neurones (mean 1.3 min), and the mean duration of excitation was 2.1 min. All four thermoreceptive neurones studied were unaffected by NA.

The data for 5-HT is based upon the study of 32 non-TN neurones (22 low threshold mechanoreceptive, 7 mechano-multireceptive, 1 mechano-nociceptive, 2 thermoreceptive), and 18 TN neurones (17 noci-multireceptive, 1 nociceptive). The profile of effects of 5-HT on trigeminal neurones was different to that of noradrenaline. 5-HT depressed the activity of TN neurones on 50% of occasions (50% excitation, biphasic or no effect). The mean duration of depression, 2.2 min, was relatively short; while the excitatory effects were long lasting with a mean of 3.9 min. The effects on the activity of non-TN neurones usually consisted of depression, 74% of occasions (26% excitation, biphasic or no effect). The mean duration of depression was 4.2 min, and that for excitation was 1.2 min.

A graphical representation of the profile for the above results obtained using NA and 5-HT on TN and non-TN neurones is given in Fig. 9. Note the contrast between the effects (percentage of neurones affected, and duration of effect) of NA and 5-HT across the two categories of neurones.

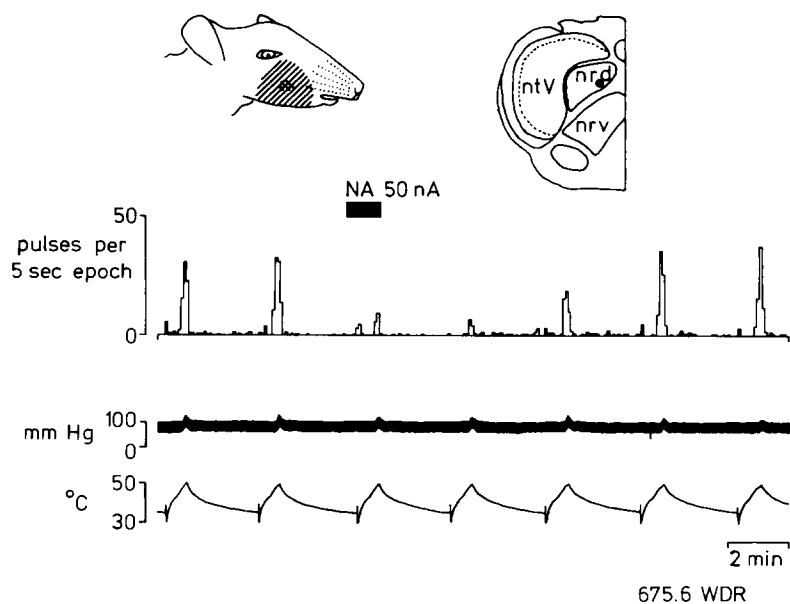


Fig. 8. Ratemeter record illustrating the depressant effect of NA (50 nA, solid bar) on responses evoked by noxious heat of a noci-multireceptive neurone. The thermal stimulus consisted of heated water which was applied as a thermal ramp (see bottom trace for monitored stimulus temperature) to the whole receptive field (shaded region on face outline responsive only to noxious stimuli, and cross-hatched region additionally responsive to low threshold mechanical stimuli). NA depressed the evoked response, elevating the threshold by 47.5%, and the effect was particularly marked in the succeeding trial. The depressant effect lasted approximately 6 min. Thermal responses were also inhibited by periventricular grey stimulation (not shown) producing neuronal inhibition of 98%, and which lasted approximately 9 min. Previously this animal had displayed 70% behavioural antinociception. The deflections of blood pressure (middle trace) indicated that the thermal stimulus was noxious. The neurone was recorded in laminae V–VI of the medullary dorsal horn (filled circle on diagram); abbreviations used are identified in Fig. 6.

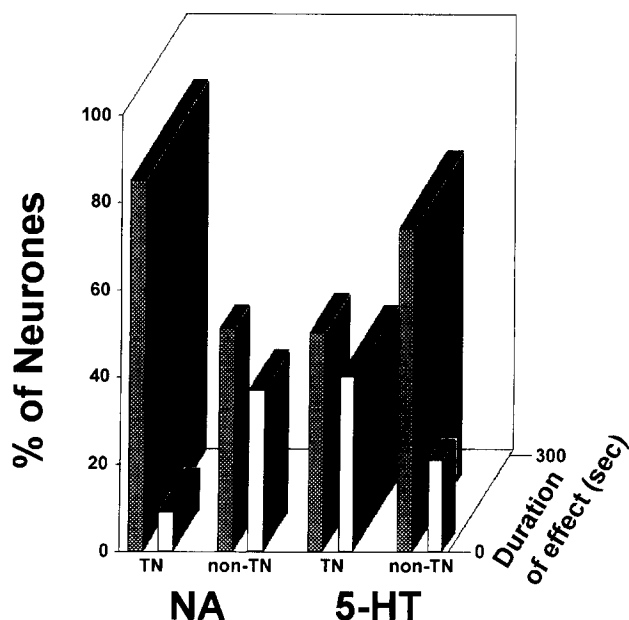


Fig. 9. Three-dimensional bar diagram plot of percentage of neurones depressed (cross-hatched bars) and excited (white bars) by iontophoretic NA and 5-HT depending upon type of neurone studied (TN, thermal nociceptive; non-TN, non-thermal nociceptive). The z-axis shows the mean duration of the depressant and excitatory effects. NA (left half) was shown to depress the highest proportion (85%) of TN neurones, exciting only a few (9%). The depressant effects were also the longest lasting effect observed (mean 5.3 min). NA excited and depressed approximately equal proportions of non-TN neurones. The profile of effects of 5-HT on the different types of neurones contrasted with that seen with NA. 5-HT depressed only 50% of TN neurones, and excited a similar proportion (40%). The mean duration of depression was 2.2 min. A larger proportion of non-TN neurones were depressed by 5-HT (74%), and the mean duration of depression was relatively long-lasting (4.2 min). The diagram does not include the small proportion of neurones that were unaffected by or displayed biphasic responses to NA and 5-HT (and can be obtained by subtraction from 100%).

3.5. Iontophoretic effects of selective agonists and antagonists

Clonidine, the selective α_2 adrenergic agonist, depressed all types of trigeminal neurone activity (2 low threshold neurones, 3 noci-multireceptive, and 1 thermo-multireceptive) using iontophoretic currents between 35–70 nA (mean 50 nA). The time course of the effects were usually longer than those observed with NA. By contrast phenylephrine (mean 55 nA), an α_1 agonist, had different effects on the neurones that were studied. Of 10 low threshold mechanoreceptive neurones, four were depressed, three excited and three no effect. Two mechanomultireceptive neurones were studied, both of which were excited. Four noci-multireceptive neurones were studied of which one was depressed, two excited and in one no effect. In one noci-multireceptive neurone there was no effect. Therefore the most common effect of phenylephrine was that of excitation (40% of responses).

A number of adrenergic receptor antagonists, of varying selectivity for α_1 , α_2 and β receptors, were tested iontophoretically on the depressant or excitatory effects of NA and phenylephrine. Amongst these sotalol (number of cells = 2), propranolol ($n = 3$), dibenamine ($n = 4$), labetalol ($n = 2$) and rauwolscine ($n = 2$) were not successful in reversing agonist effects. Often depressant effects and spike height attenuation effects were observed. Clear selective antagonism of NA depressions was seen using piperoxane (0–9 nA) (obtained in 3 of 5 neurones with control depressions induced by iontophoretic GABA). In one of three neurones tested, NA induced excitations could also be antagonized by piperoxane. The more selective α_2 antagonist, idazoxan [13], proved to be more consistent at antagonizing depressant responses to NA. Currents be-

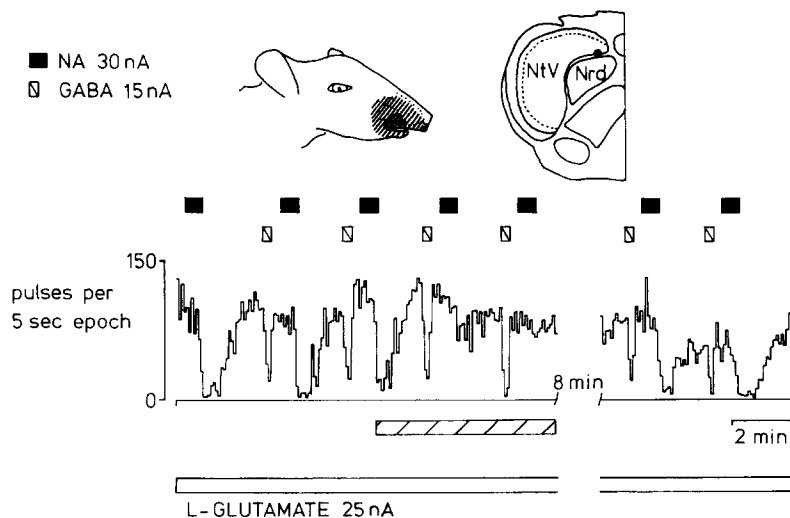


Fig. 10. Antagonism of NA (30 nA, solid bars) induced depressions of a glutamate driven mechano-multireceptive neurone by idazoxan (9 nA, hatched bar). Antagonism was selective against depressions produced by GABA (15 nA, crossed bars). Eight min following the antagonist application recovery of NA depressant responses as observed. The neurone had a concentric surround receptive field typical of this class of neurone (shaded and cross-hatched regions on face outline). The recording site was in laminae IV–V (filled circle on diagram of transverse section of medulla); abbreviations used are identified in Fig. 6.

tween 0–9 nA antagonized NA induced depressions in 10 of 13 cells (see Fig. 10), where the sample included both nociceptive and low threshold cells). Selective antagonism of NA depressions against 5-HT depressions was also seen. As with piperoxane, in one out of three cells idazoxan antagonized NA excitations. The α_1 antagonist, WB4101 (2–10 nA), was able to antagonize phenylephrine induced excitations on all three neurones tested. Prazosin (32 nA), another selective α_1 antagonist, was able to selectively antagonize noradrenaline induced excitations in the one cell studied. The α_1 antagonists were not tested against NA induced depressions, but prazosin was found ineffective against such depressions on neurones in the adjacent ventrolateral regions of the medulla [5]. Ionophoretic cinanserin ($n = 3$), *cis*- and *trans*-flupenthixol ($n = 3$, $n = 4$) and methysergide ($n = 5$) were tested against depressions of firing induced by 5-HT. No instance of convincing antagonism was obtained.

4. Discussion

4.1. Effects of electrical stimulation

The present study is the first to directly correlate, in a quantitative manner, the antinociceptive action of brain stimulation with selective inhibition of thermal nociceptive neurones. Brain stimulation elevated escape response thresholds to thermal stimuli applied to the face of freely moving rats. The second-order neurones in the caudal trigeminal nucleus, believed to mediate thermal nociceptive information [6], were then studied in same animals under anaesthesia. Brain stimulation caused an elevation in the temperature at which thermal responses occurred in thermal nociceptive neurones. The brain stimulation induced a mean inhibition of neuronal responses to noxious thermal stimuli that positively correlated with the degree of facial antinociception previously observed. The mean antinociceptive effect of 57.5% was very close to the mean neuronal inhibition of 53.7%. In addition, the mean time course of the neuronal inhibition and the antinociception were similar. These data provide direct evidence that selective inhibition of thermal nociceptive neurones in the caudal trigeminal nucleus forms the neural basis for the antinociceptive behaviour following brain stimulation. However, the results should be interpreted with some caution since the use of relatively large electrical stimulation currents may have led to the stimulation of brain regions that were differentially involved in the behavioural antinociception and in the neuronal inhibition. As such, this reduces our confidence that discrete regions within the periventricular grey area are causally related to these two phenomena, despite the significant correlation observed between them. A more detailed microstimulation mapping study would be required to determine the exact loci of effective sites. Although electrical stimulation

thresholds and current–effect relationships for individual animals were not obtained, the effects of electrical stimulation across different animals produced a positive relationship between antinociception and neuronal inhibition. The thermal and mechanical antinociceptive effect of brain stimulation could be compared to the antinociceptive effect of low doses of morphine sulphate (2 mg/kg i.p.) and clonidine HCl (0.1–0.15 mg/kg i.p.), which gave 60–100% antinociception using the thermal and mechanical devices [4]. Naloxone, the opioid antagonist, was able to block the induction of antinociception in only 2 of 6 animals tested. This suggests that in many animals the endogenous opioid system was not exclusively involved in mediating the antinociception induced by brain stimulation. However, a role for the opioid system is implicated in the neighbouring region of nucleus reticularis ventralis where neuronal inhibitions evoked by electrical stimulation of the periaqueductal grey matter were shown to be reversed by naloxone [31].

The selective effects of brain stimulation on nociceptive neurones as compared with low threshold mechanoreceptive neurones was a finding that was in general agreement with previous studies [3,35,37,51]. In particular, there was agreement with those studies which used some form of behavioural ‘screening’ for the antinociceptive effects of brain stimulation before electrophysiological experiments were performed [3,28,37]. Relative selectivity of inhibitory effects have been observed [7,44,45], as has no selectivity [11,12,18,24].

A second type of selective inhibition which has previously been observed consists of the inhibition of nociceptive but not innocuous responses on the same neurone [14,37,49]. In the present study, this was found for half of the noci-multireceptive neurones. Two previous studies [3,37] found that in some neurones the response to noxious pinch could be selectively inhibited compared to responses to innocuous brushing. In the present study it was found that responses of mechano-multireceptive neurones to noxious pinching were not inhibited. This latter finding would suggest that the changes in mechanical escape thresholds depend on the inhibition of another class of neurone, or conversely, that these neurones do not subservise mechanical nociception. Candidates for this role could be the mechano-nociceptive, the noci-multireceptive, the thermo-multireceptive or the nociceptive neurone classes; none of which were tested in the present study for the effect of brain stimulation on noxious mechanical stimuli. In this context it is of interest that, in the caudal trigeminal nucleus, noxious mechanical information appears to be transmitted to noci-multireceptive neurones via an excitatory amino acid neurotransmitter, whereas noxious thermal information appears to use another transmitter [42]. It would be of interest to know to what extent this difference is also reflected by differences in descending inhibitory influences on these modalities converging onto this class of neurone.

The reason for disagreement between different investigators over the degree of selectivity of inhibition is not certain. One explanation may be that different conditioning stimulation-to-test response times were used in the different studies. Those studies in which selectivity was not observed used conditioning-to-test intervals of tens of milliseconds, or responses were tested during stimulation. This is in contrast to the majority of those which observed selective inhibition and used conditioning-to-test intervals of seconds or tens of seconds. Another possibility is that selectivity of inhibition may critically depend on the stimulation site in the midbrain. Oliveras et al. [37] found that stimulation sites effective in inhibiting neuronal responses were more widely distributed than those which produced antinociception. Interestingly, Duggan and Morton [14] could only obtain their selective inhibition at sites which were known to produce antinociception.

Supraspinal modulation of spinal and caudal trigeminal neurones by the application of noxious stimuli to regions remote from cutaneous receptive fields has been demonstrated [10,34] (but cf. Gerhart et al. [21]). Such inhibition was primarily observed on noci-multireceptive ('convergent') neurones [34] (but cf. Refs. [21] and [23]). The present study and others have shown that nociceptive neurones were usually affected by brain stimulation, which may suggest a difference between these two types of descending inhibition on neurones.

4.2. Effects of monoamines

The clear predominant effect of the two monoamines studied here, NA and 5-HT, was depression in 5 of the 6 classes of sensory trigeminal neurones. This is consistent with earlier reports of monoamine effects on sensory cells in the dorsal horn [2,29]. Typically the iontophoretic effects had a slow onset of effect, as well as a long duration of effect. Such effects were similar to those initially reported in the spinal cord [16,17,48] and later on dorsal horn neurones [2,29]. Other monoamines, dopamine, tryptamine and adrenaline, also had a predominantly depressant effect on trigeminal neurones [4]. In most respects the effects of NA and 5-HT on low threshold neurones were identical except that lower currents were required and longer depressions were observed with 5-HT. 5-HT depressed most of the responses (71%) of mechano-multireceptive neurones, whereas NA had excitatory effects in almost half (47%) of the sample. By contrast, NA usually depressed activity of noci-multireceptive neurones (90% depressed, 7% excited), whereas 5-HT had a high percentage of excitatory effects (42% excited, 47% depressed). In addition, contrasting durations of excitatory and depressant effects by 5-HT and NA were observed on this class of neurone. With NA, depressions were long-lasting and excitations were shorter, whereas the opposite was true for 5-HT. Such contrasting effects on these two classes of multireceptive neurone strongly suggest the existence of

distinct receptors mediating such responses. In support of this, opposite responses to these substances were observed in the same cell on several occasions. With respect to effects on thermally evoked responses of noci-multireceptive neurones, NA significantly increased response thresholds of all seven neurones tested, whereas 5-HT increased thresholds in two neurones and decreased the threshold in a third neurone. The increase in thermal thresholds induced by NA was qualitatively similar to that produced by brain stimulation.

Both NA and 5-HT had no or little effect on the spontaneous activity of thermoreceptive neurones although glutamate and GABA had their expected effects (excitatory and depressant respectively) on this firing. It may be of significance that previous studies have shown that this class of neurone was not affected by either nucleus raphe magnus or periaqueductal grey stimulation [9,11].

The contrasting effects of the α_1 and α_2 agonists suggest the existence of separate receptors mediating the excitatory and depressant responses respectively. The selective antagonist effects of prazosin, WB4101, piperoxane and idazoxan in this study support this notion. The lack of success with β -adrenergic antagonists suggest that β receptors are not involved in mediating either excitatory or depressant responses. These results are consistent with an iontophoretic study carried out on dorsal horn neurones in which selective depression by NA was antagonized by idazoxan [20]. The lack of success with 5-HT antagonists against responses either indicates a non-specific action of 5-HT or suggests that the antagonists were not selective enough for the class of receptors that exist on these neurones. However, Griensmith and Duggan [25] were able to antagonize 5-HT-produced depressions of dorsal horn neurones with methysergide. Belcher et al. [2] showed that methysergide could antagonize excitatory but not depressant effects of 5-HT on dorsal horn neurones. More recent work has shown that the 5-HT₁ receptor antagonist, cyanopindolol, was able to reverse the depressant effects of nucleus raphe magnus stimulation on noxious pinch responses of dorsal horn neurones, although the antagonist's effectiveness against iontophoretic 5-HT itself was not tested [15]. Todd and Millar [47] found predominantly excitatory effects to iontophoretic 5-HT, and to a lesser extent to NA, on laminae I–III dorsal horn neurones. The effects were of long duration, and similar long duration effects (excitations and inhibitions) following afferent stimulation have been observed in substantia gelatinosa neurones (e.g. Ref. [19]), prompting the suggestion that the iontophoretic effects were simulating synaptically evoked responses. It is possible that deeper laminae neurones could be inhibited by the activity of inhibitory interneurons within the substantia gelatinosa. Therefore in those experiments in which recordings were made from deeper laminae neurones and monoamines applied iontophoretically to lamina II (e.g. Refs. [8] and [29]) it is possible that the inhibitory effects observed may result from disfacilita-

tion. More work on spinal cord and trigeminal neurones must be done in order to resolve these discrepancies with respect to 5-HT effects.

In conclusion, of all the monoamines NA appeared to have actions most consistent with an inhibitory role on nociceptive neurones, and for the following reasons: (1) NA depressed noci-multireceptive neurones most frequently (and consistently depressed those responses to noxious heat by noci-multireceptive and nociceptive neurones); (2) depressant effects of NA were longer lasting in noci-multireceptive neurones; (3) the depressant effects of NA appeared to be mediated specifically through an identified receptor. More conclusive evidence for NA's role as the inhibitory transmitter could be obtained by studying the effects of selective antagonists on antinociception and neuronal inhibition produced by electrical stimulation of the periventricular grey. With respect to the descending NA system, in the rat caudal trigeminal nucleus NA-like terminals most often made axo-dendritic contacts [43]. In the spinal cord, dorsal horn analysis of the fine structure has revealed that NA terminals synapse predominantly on small dendrites and on spines [27]. No contacts were made presynaptically with primary afferents. Selective effects of descending inhibition by NA on nociceptive over non-nociceptive input onto dorsal horn/caudal trigeminal neurones may be mediated by the contacts made on spines and on distal dendrites. The effects of such inhibitory contacts would be expected to be relatively restricted to dendritic subunits [46].

Acknowledgements

The work was supported by Glaxo Group Research via a CASE award from the SERC (BBSRC). We are grateful for the gifts of idazoxan HCl from Reckitt and Colman, prazosin from Pfizer, cinanserin HCl from Squibb, rau-wolscine from Dr J.C. McGrath of the University of Glasgow, sotalol HCl from Bristol-Myers Pharmaceuticals (Squibb), *cis*- and *trans*-flupenthixol from Lundbeck, piperoxane HCl from May and Baker. We would like to thank Dr. Tom Salt for use of his computer program to plot firing rate against temperature.

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