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HETEROTOPIC ACTIVATION OF Aδ AND C FIBRES TRIGGERS INHIBITION OF TRIGEMINAL AND SPINAL CONVERGENT NEURONES IN THE RAT

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SUMMARY

1. Extracelluar recordings were made from fourteen non-noxious only and nineteen convergent neurones in trigeminal nucleus caudalis of halothane-anaesthetized rats. All the neurones studied were excited by the continuous micro-electrophoretic ejection of an excitatory amino acid, DL-homocysteic acid (DLH), with mean currents of 38·0 ± 7·2 and 39·8 + 6·5 nA producing steady discharges of 35·0 ± 3·3 and 31·8 ± 1·3 spikes/s from the non-noxious only and convergent neurones respectively.

2. The repeated percutaneous application (100 trials; 0·66 Hz) of single square-wave stimuli (10 mA; 2 ms) to the tail always induced a biphasic depression of the activity of the convergent, but never of the non-noxious only, neurones. Both the early and late components of this inhibition occurred at shorter latencies when the base rather than the tip of the tail was stimulated. Differences in latencies from the two sites of stimulation (100 mm apart) were used to estimate the conduction velocities of the peripheral fibres which were triggering the inhibitions.

3. The cumulated results showed that, for the onset of the earlier component of the inhibition, the mean difference between the latencies from the two sites of stimulation was 13·6 ± 1·9 ms, corresponding to a peripheral conduction velocity of 7·3 ± 0·3 m/s, which is in the Aδ-fibre range. For the onset of the late component of inhibition, the mean difference was 147·7 ± 14·9 ms, corresponding to a peripheral conduction velocity of 0·68 ± 0·07 m/s, which is in the C-fibre range.

4. When currents of different intensities were applied percutaneously to the two stimulation sites, the thresholds for obtaining the Aδ component were in the range 0·25–0·5 mA whereas the C component appeared with currents 1–2 mA. A clear relationship between current intensity and magnitude of inhibition was observed in the 0·25–2 mA range for the Aδ component and in the 1–5 mA range for the C component.

5. In an additional series of experiments recordings were made from eleven convergent neurones in the dorsal horn of the lumbar spinal cord. By using essentially the same experimental procedure the effects of repetitive application (100 trials, 0·66 Hz) of percutaneous electrical stimuli (1 or 10 mA, 2 ms) applied to the muzzle, were studied on the steady discharges (42·3 ± 5·4 spikes/s) induced by DLH. The application of the 10 mA stimuli induced a biphasic depression of activity, whereas only an early component was observed following 1 mA stimuli.

6. These results demonstrate that extrasegmental post-synaptic inhibitory processes acting on convergent neurones are specifically triggered by the activation of Aδ and C fibres. The participation of these phenomena in the diffuse noxious inhibitory controls (d.n.i.c.) system and in procedures commonly employed to induce analgesia by transcutaneous electrical stimulation is proposed.

INTRODUCTION

There is a great deal of literature to support the idea that convergent neurones in the dorsal horn of the spinal cord and its homologue for the face, trigeminal nucleus caudalis, play an important role in the transmission of nociceptive messages towards the higher centres of the brain (Melzack & Wall, 1965; Wall, 1978; Willis & Coggeshall, 1978; Dubner & Bennett, 1983; Le Bars, Dickenson, Besson & Villanueva, 1986).

We have previously observed that both propriospinal- and supraspinal-mediated mechanisms which depress the activity of convergent neurones can be triggered by noxious stimulation of widespread areas of the body (Le Bars, Dickenson & Besson, 1979*a*,*b*; Cadden, Villanueva, Chitour & Le Bars, 1983). The most powerful inhibitions, both in terms of magnitude and duration, were observed in intact as opposed to spinal rats, thus involving supraspinal structures. These inhibitions affect all kinds of activities of convergent neurones, including those evoked by the microelectrophoretic application of excitatory amino acids (Villanueva, Cadden & Le Bars, 1984*a*,*b*).

These phenomena have been termed diffuse noxious inhibitory controls (d.n.i.c.) by our group (Le Bars *et al.* 1979*a*,*b*) and we have proposed that these mechanisms may both play a role in pain signalling processes (for review see Le Bars *et al.* 1986) and provide a neurophysiological basis for the success of procedures which employ strong stimuli to alleviate some forms of pain, i.e. counter-stimulation or counter-irritation procedures (for review see Le Bars, Calvino, Villanueva & Cadden, 1984).

The aim of the present study was to investigate the mechanisms underlying the inhibitions triggered by extrasegmental percutaneous electrical stimulation, and by so doing to clarify some points regarding the peripheral part of the loop which subserves d.n.i.c.; such experiments might give additional information regarding the mechanisms of analgesia produced by peripheral electrical stimulation.

For this purpose, we took advantage of the fact that trigeminal and spinal dorsal horn cells respond with relatively steady discharges to the application of the excitatory amino acid DL-homocysteate (DLH) when adequate electrophoretic currents are applied; as will be shown, the application of single extrasegmental electrical stimuli induced inhibitions of such discharges. This method was chosen as an alternative to the technically difficult procedure of recording intracellularly from the trigeminal nuclei and spinal dorsal horn for a long period. On the basis that DLH excites cells by a direct, post-synaptic effect on their membranes, this technique or variations of it using other excitatory amino acids have previously been used to obtain evidence for post-synaptic inhibition of dorsal horn neurones resulting from natural stimulation of the segmental inhibitory field (Besson, Catchlove, Feltz &

Le Bars, 1974) or of heterotopic areas (Villanueva *et al.* 1984*a*), from electrical stimulation of the raphé (Belcher, Ryall & Shaffner, 1978; Jordan, Kenshalo, Martin, Haber & Willis, 1978) and from the electrophoretic application of other drugs (Belcher *et al.* 1978). A preliminary report of this work has already appeared in abstract from (Bouhassira, Le Bars & Villanueva, 1986).

METHODS

Animal preparation

Experiments were performed on twenty-one male rats weighing 220–300 g. Following an intraperitoneal injection of 100 μg atropine sulphate, the animals were deeply anaesthetized with 2% halothane in a nitrous oxide:oxygen mixture (2:1). A tracheal cannula was inserted, the jugular vein cannulated and the animals were paralysed by intravenous injection of gallamine triethiodide (Flaxedil) and artificially ventilated; the rate (70–80 strokes/min) and volume of ventilation were adjusted to maintain a normal acid–base equilibrium (Freminet, Bursaux & Poyart, 1972). Heart rate was monitored continuously and core temperature maintained at 37 ± 0·5 °C by means of a homeothermic blanket system.

During the main series of experiments, recordings were made from neurones in trigeminal nucleus caudalis. In these experiments the animals were mounted in a stereotaxic frame with the head fixed in a ventroflexed position by means of a metallic bar cemented to the skull. The caudal medulla was then exposed by removing the overlying musculature, atlanto-occipital membrane and dura mater. In a second series of experiments in which recordings were made from lumbar dorsal horn neurones, the vertebral column was mounted in a rigid frame, and the spinal cord was exposed between the T12 and L1 vertebrae.

After surgery the level of halothane was reduced to 0·5% to achieve a level of anaesthesia which was adequate for ethical purposes but did not excessively depress neuronal responses to noxious stimuli. In this regard, we have previously reported (Weil-Fugazza, Godefroy & Le Bars, 1984) that this anaesthetic regime allows a very stable level of anaesthesia under which neither e.e.g. arousal nor cardiovascular reactions are observed during the application of strong stimuli. This point has been further discussed by Benoist, Kayser, Gautron & Guilbaud (1984).

Recordings

Unitary extracelluar recordings were made with glass micropipettes (10–15 MΩ) filled with a mixture of 5% NaCl and Pontamine Sky Blue dye. This electrode was sealed with dental composite resin to a seven-barrelled micropipette which was used for the electrophoresis of drugs; the electrodes were arranged so that the tip of the electrophoresis pipette was in contact with the recording pipette 10 μm from its tip. The outer barrels of the electrophoresis pipette contained a solution of DLH (0·2 M, pH 6·0), which was ejected as anion. Retaining currents of opposite polarity were used to prevent leakage and a central barrel filled with NaCl (165 mM) was used for current compensation.

Single-unit activity was amplified and fed into a window discriminator, the output of which was connected to a tape-recorder and a multichannel analyser (Tracor TN 1710) to allow further processing of the data.

When recordings were being made from trigeminal nucleus caudalis the micropipettes were inserted on the left side, 1·5–2·0 mm caudal to the obex and 1·5–2·5 mm lateral to the mid line. Stability for the recordings was achieved by placing a glass frame over the surface of the medulla; this was held in position with a micromanipulator and 2% Ringer solution–agar gel. Recordings from the lumbar dorsal horn were made in the area of the cord which exhibited the maximal dorsum potential evoked by percutaneous stimulation of the extremity of the hind paw. In both cases non-noxious search stimuli were used to help to isolate unitary activity, and neurones were classified as being 'convergent' or 'non-noxious only' on the basis of their characteristic responses to both mechanical and electrical stimuli applied to their peripheral receptive fields (Menétrey, Giesler & Besson, 1977). Once a cell had been identified the extent of its receptive field was determined.

Only cells showing no serious alterations in spike amplitude or wave form during the complete experimental procedure were considered.

Experimental design

The experimental procedure involved the use of continuous electrophoretic ejection of DLH to produce a stable level of evoked activity from the neurone under study.

First series of experiments. The general procedure used in the first series of experiments is illustrated in Fig. 1. During the steady discharges induced by electrophoretic application of DLH, electrical stimuli were delievered through pairs of stainless-steel stimulating electrodes inserted

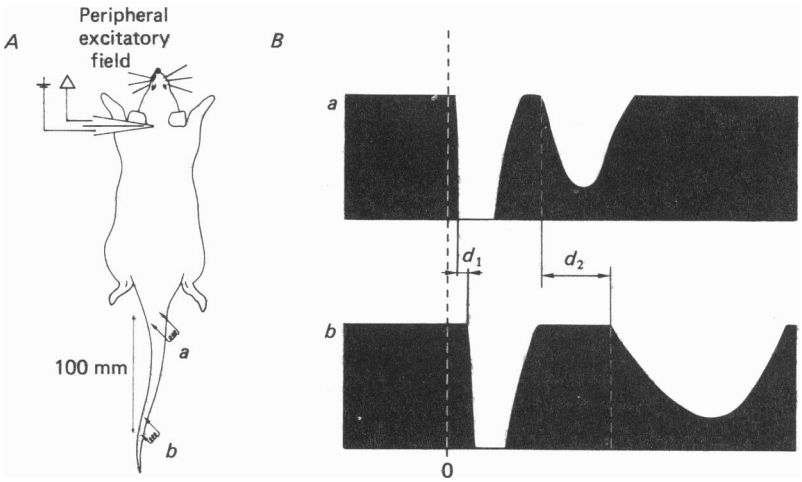

Fig. 1. Schematic representation of the experimental design. Non-noxious only and convergent neurones with receptive fields located ipsilaterally on the muzzle were recorded in trigeminal nucleus caudalis (*A*). The continuous electrophoretic application of an excitatory amino acid, DL-homocysteic acid (DLH) induced a steady discharge from the neurone under study (black area, *B*). The repetitive application of single percutaneous electrical stimuli of adequate intensities induced biphasic depressions of activity (*B*) which exclusively affected convergent neurones. The two components of this inhibition occurred earlier when the base (*a*) as opposed to the tip (*b*) of the tail was stimulated. The differences in latencies between data obtained following stimulation of the base (*a*) and those obtained following stimulation of the tip (*b*) were calculated for both the earlier (d_1) and the later component (d_2). Since the sites of stimulation were 100 mm apart it was possible to determine the conduction velocities of the peripheral fibres which triggered these phenomena.

subcutaneously into the base (Fig. 1*Aa*) and the tip (Fig. 1*Ab*) of the tail, 100 mm apart. The effects of the repeated application (100 trials; 0·66 Hz) of single square-wave stimuli of different intensities (0·25, 0·5, 1, 2, 5 and 10 mA; 2 ms duration), to the tip or the base of the tail were analysed using peri-stimulus histograms (p.s.h.s) built using the multichannel analyser.

When percutaneous electrical stimuli of adequate intensities were applied to *a* or *b*, the continuous firing of the convergent neurones was inhibited in a biphasic manner (Fig. 1*B*).

Second series of experiments. In the second series of experiments, during which recordings were made at the lumbar level, the experimental procedure was essentially the same except that electrical stimulation (10 mA or 1 mA) was delivered to a single area of the muzzle.

Date analysis

For each sequence of stimulation, the number of spikes recorded during the 200 ms period preceding the percutaneous electrical stimuli was taken as the control level of firing of the neurone.

The detailed analysis of data during the first series of experiments is explained below. Analysis of data from the second series of experiments was similar, but less complex, and is explained in the Results section.

Latency measurement. The latencies of each component of inhibition were determined to an accuracy limited by the bin width of the p.s.h.s which were 1 ms for the earlier and 5 ms for the later component of inhibition (e.g. see Fig. 2). The difference in the latencies (d_1) of the earlier component triggered from the tip (Fig. 1 *Bb*) and from the base (Fig. 1 *Ba*) of the tail was calculated; an identical calculation was made for the late component (d_2). Similar types of calculation were made with respect to the latencies of the end of each inhibitory period.

Percentage of inhibition. For each sequence, the percentage of inhibition was calculated for both the earlier and later components of inhibition in the following fashion. For a given cell, a window corresponding to the time course of each inhibitory period triggered by a 10 mA stimulus was determined on the p.s.h. Thereafter the percentage inhibition was calculated for those cells which were tested with several intensities of stimulation, by using an identical window on the p.s.h.s corresponding to the lower stimulus intensities.

Histological controls

At the conclusion of the experiments the recording sites were marked by electrophoretic deposition of Pontamine Sky Blue and the medulla or the lumbar spinal cord was removed and fixed in a 10% formalin solution. The tissue was frozen, cut into serial 100 μm-thick sections and Nissl-stained with cresyl violet or carmine. Recording sites were then determined by histological examination and camera lucida drawings were made.

RESULTS

General properties of units recorded in trigeminal nucleus caudalis

A total of nineteen convergent and fourteen non-noxious only neurones were studied. They were located within the magnocellular layer of nucleus caudalis and in the adjacent reticular formation, as determined by histological recovery of the dye spots made at the end of the experiments. All the neurones gave responses characteristic of their class types when natural or electrical stimuli were applied to their excitatory receptive fields on the ipisilateral part of the muzzle. Non-noxious only neurones responded exclusively to innocuous stimulation (movements of vibrissae, stroking or light pressure) and gave responses of short duration, i.e. rapidly adapting, when pinches were applied on their receptive fields. Convergent neurones responded to identical innocuous stimuli, except the movement of vibrissae, but a tonic discharge always followed the phasic one produced by noxious stimulation (pinch) of the receptive field on the ipsilateral muzzle. All the convergent units gave responses corresponding to A- and C-fibre activation following percutaneous electrical stimulation of their receptive fields.

All the neurones were excited by the electrophoretic application of DLH: statistically stable responses with mean levels of $35{\cdot}0 \pm 3{\cdot}3$ spikes/s and $31{\cdot}8 \pm 1{\cdot}3$ spikes/s were observed with mean electrophoretic currents of $38{\cdot}0 \pm 7{\cdot}2$ nA and $39{\cdot}8 \pm 6{\cdot}5$ nA for non-noxious only and convergent neurones respectively.

In every case, heterotopic nociceptive stimulation of widespread areas of the body (e.g. noxious pinch of the paws or the tail) depressed all kinds of activity of the convergent neurones, whether evoked by natural or electrical stimulation, or by the electrophoretic application of DLH. The same procedure did not affect any of the non-noxious only neurones. These are the phenomena which have been studied in

some depth by our group and have been labelled d.n.i.c. (Le Bars *et al.* 1979*a*,*b*; Dickenson, Le Bars & Besson, 1980).

Effects of high-intensity percutaneous electrical stimuli, applied to the tail, on DLH-evoked activities of trigeminal convergent neurones

As illustrated by an individual example in Fig. 2, the repeated application (100 trials; 0·66 Hz) of percutaneous electrical stimuli (10 mA; 2 ms duration) to the tail

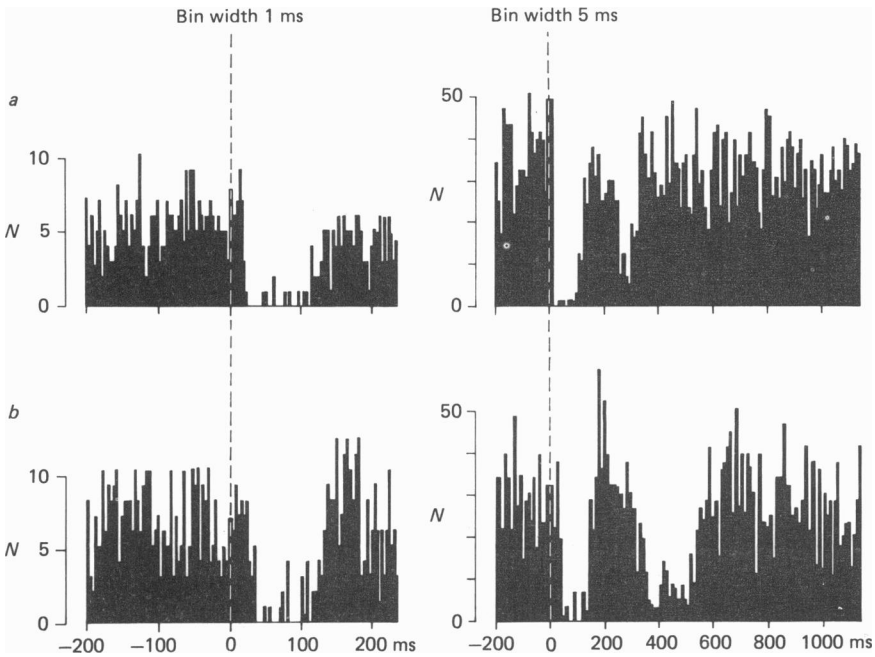

Fig. 2. Individual example of the biphasic inhibitory processes triggered by repetitive single percutaneous electrical stimuli (10 mA; 0·66 Hz; 2 ms duration; 200 ms delay) applied to the base (*a*) or the tip (*b*) of the tail, on the discharge of a trigeminal convergent neurone evoked by the continuous electrophoretic application of DLH (17 nA). Peri-stimulus histograms (bin width 1 ms, left; 5 ms, right,) were built, as in subsequent Figures, from 100 trials. The earlier component is detailed in the left part of the Figure, while the whole biphasic inhibition is shown on the right. Note that both components appeared earlier when the base (*a*) rather than the tip (*b*) of the tail was stimulated.

induced two components of inhibition with different latencies. Such phenomena were observed systematically for all the convergent neurones studied ($n = 19$). Fig. 2 also shows that the two components were earlier when triggered from the base (*a*) as opposed to the tip (*b*) of the tail.

The cumulated results show that, for the onset of the earlier component of inhibition, the mean difference between the latencies obtained from the two sites of stimulation was $d_1 = 13{\cdot}6 \pm 1{\cdot}9$ ms which, taking account of the 100 mm between the sets of electrodes, corresponds to a peripheral conduction velocity of $7{\cdot}3 \pm 0{\cdot}3$ m/s. A mean difference of $21{\cdot}4 \pm 6{\cdot}4$ ms, which corresponds to $4{\cdot}7 \pm 1{\cdot}4$ m/s, was observed for the end of this inhibitory period.

For the onset of the late component of inhibition the mean difference obtained by

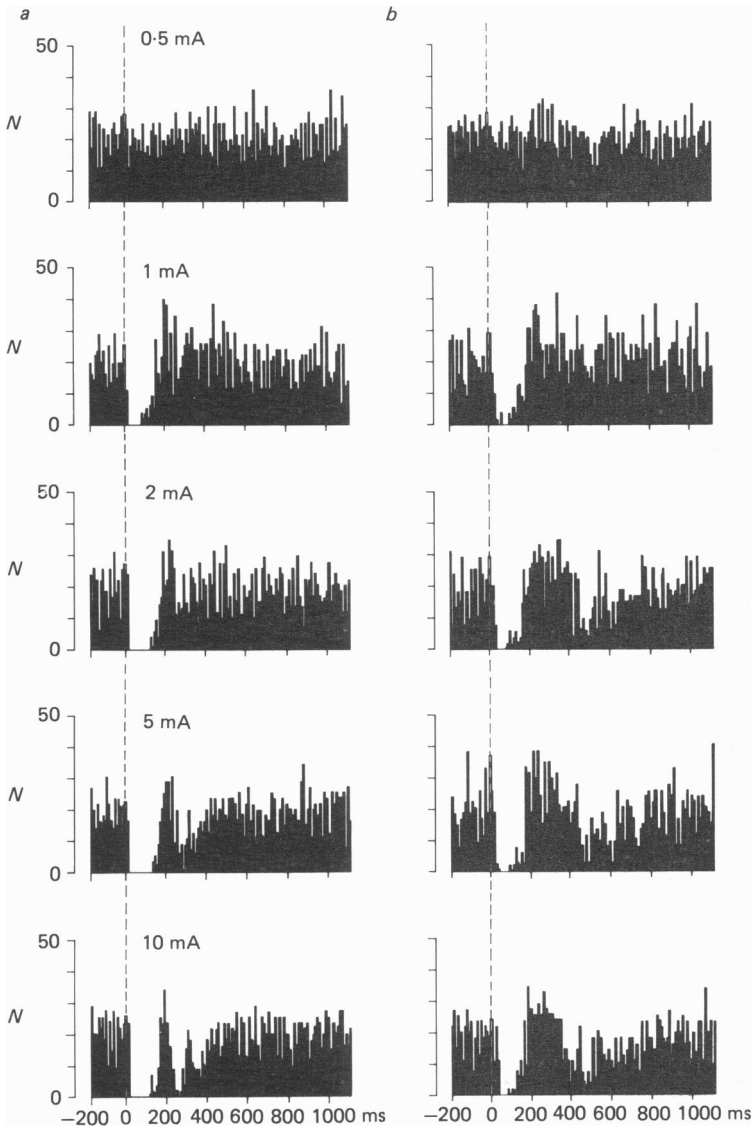

Fig. 3. Individual example of the effects of different intensities (0·5, 1, 2, 5 and 10 mA) of percutaneous electrical stimuli (0·66 Hz; 2 ms duration; 200 ms delay) applied to the base (*a*) or the tip (*b*) of the tail, on the DLH-evoked activity (30 nA) of a trigeminal convergent neurone (p.s.h.s, bin width 5 ms, 100 trials in both cases). Note that the Aδ component appeared with currents of 1 mA and presented essentially the same characteristics in terms of magnitude and duration when the highest intensities were employed. By contrast, the C component became perceptible at a higher stimulus intensity (2 mA) and was more pronounced when 5 or 10 mA was applied.

stimulating the base and the tip of the tail was $d_2 = 147{\cdot}7 \pm 14{\cdot}9$ ms, which corresponds to a peripheral conduction velocity of $0{\cdot}68 \pm 0{\cdot}07$ m/s. Measurements of the end of the late component gave a mean difference of $234{\cdot}5 \pm 16{\cdot}5$ ms, corresponding to a peripheral conduction velocity of $0{\cdot}43 \pm 0{\cdot}03$ m/s.

Thus, with the 10 mA stimuli, the inhibitory processes were triggered by peripheral

fibres with conduction velocities in the ranges of $4{\cdot}7 \pm 1{\cdot}4$–$7{\cdot}3 \pm 0{\cdot}3$ and $0{\cdot}43 \pm 0{\cdot}03$–$0{\cdot}68 \pm 0{\cdot}07$ m/s, respectively. According to Gasser & Erlanger (1927) and Burgess & Perl (1973) these values correspond to peripheral conduction velocities in the Aδ- and C-fibre range respectively. We will therefore subsequently refer to these as the Aδ and C components.

TABLE 1. Proportions of cells exhibiting an Aδ and/or C fibre component of inhibition induced by various intensities of electrical stimuli applied to the tail (+: presence of the component; 0: absence of inhibition)

Current values (mA)	Number of cells	Base of the tail		Tip of the tail	
		Aδ	C	Aδ	C
10	19/19	+	+	+	+
5	11/11	+	+	+	+
2	6/11	+	+	+	+
	5/11	+	0	+	0
1	1/11	+	+	+	+
	9/11	+	0	+	0
	1/11	0	0	0	0
0·5	4/11	+	0	+	0
	7/11	0	0	0	0
0·25	1/11	+	0	+	0
	10/11	0	0	0	0

Effects of various intensities of percutaneous electrical stimuli, applied to the tail, on DLH-evoked activities of trigeminal convergent neurones

Currents of different intensities were applied percutaneously to the base and the tip of the tail to determine the threshold for obtaining each component of inhibition. Fig. 3 illustrates the results of an individual example of this type of study which was fully carried out on eleven neurones. Note that in this example the inhibitory effects could not be elicited by applying 0·5 mA stimuli to the tail; at 1 mA and above, an Aδ component was always present, with the inhibition being almost identical for all intensities tested. With respect to the C component, the inhibition appeared clearly at 2 mA when the tip was stimulated (*b* in Fig. 3) and to a lesser extent when the base was stimulated (*a*); increasing the current resulted in increased inhibitory effects regardless of whether the stimuli were applied to the tip or the base of the tail.

Table 1 summarizes the results qualitatively. In most cases inhibitory phenomena were not observed with the lowest intensities employed (0·25 mA). However, although the Aδ component was only observed in some cases (four out of eleven) with 0·5 mA, it was present in most cases (nine out of eleven) with 1 mA; in contrast a C component was rarely seen with these intensities (one out of eleven at 1 mA). At 2 mA, all the cells presented an Aδ component and six out of eleven showed a C component. Finally, all the convergent units exhibited both an Aδ and a C component when 5 or 10 mA were applied to the base or the tip of the tail. Note that the occurrence of each component was identical for a given cell, regardless of whether the tip or the base was stimulated.

Fig. 4 summarizes the results quantitatively in terms of percentages of inhibition

which, for each intensity, was calculated during the total period between the onset and the end of the inhibitory components observed with 10 mA stimuli (see Methods). Note that on this semilogarithmic plot there is a clear linear relationship between current intensity and percentage inhibition in the 0·25–2 mA range for the Aδ component, and in the 1–5 mA range for the C component. Further inhibitory

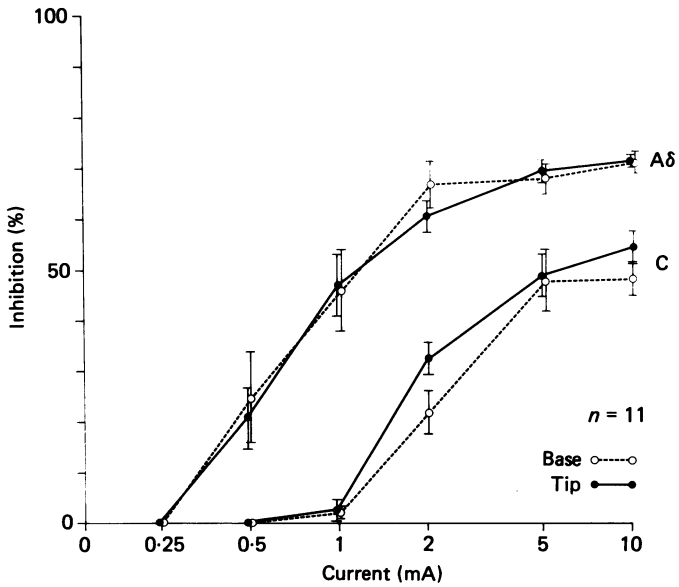

Fig. 4. Mean percentages of inhibition of the DLH-evoked activities of trigeminal convergent neurones (ordinate) induced by different current intensities (abcissa) applied to the base (dashed line) or the tip (continuous line) of the tail. Note that a clear current–inhibition relationship was observed in the 0·25–2 mA range for the Aδ component and in the 1–5 mA range for the C component. Although the C-fibre components of the inhibitions were of longer duration when the tip was stimulated, there was no clear difference between the curves obtained by stimulating the tip or the base of the tail.

processes could not be triggered in the 2–10 mA range for the Aδ component. Note again that stimulation of the tip or of the base of the tail produced nearly identical curves.

Effects of various frequencies of percutaneous electrical stimuli, applied to the tail, on DLH-evoked activities of trigeminal convergent neurones

In the light of the possible involvement of the activation of Aδ fibres in analgesic procedures based on peripheral electrical stimulation (see Discussion), currents of 1 mA were applied percutaneously to the base of the tail with different frequencies. Such intensity was chosen because it was found to induce clear Aδ but no C components of inhibition (Fig. 4). Fig. 5 illustrates the results of an individual example of this type of study which was fully carried out on four neurones. Note that the inhibitory processes were able to follow increasing frequencies of stimulation, although they were slightly less effective with the highest frequency employed (8 Hz).

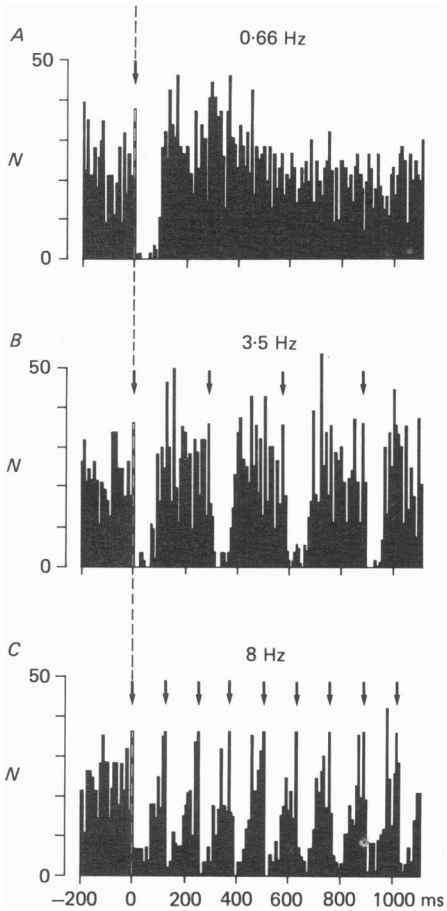

Fig. 5. Individual example of the inhibitory effects induced by percutaneous electrical stimuli (1 mA; 2 ms duration; 200 ms delay) applied at different frequencies on the base of the tail, on the DLH-evoked activity (15 nA) of a trigeminal convergent neurone (p.s.h.s, bin width 5 ms, 100 trials). Note that the Aδ component presents the same magnitude when frequencies of 0·66 and 3·5 Hz were applied, whereas it decreased slightly with the highest frequency employed (8 Hz).

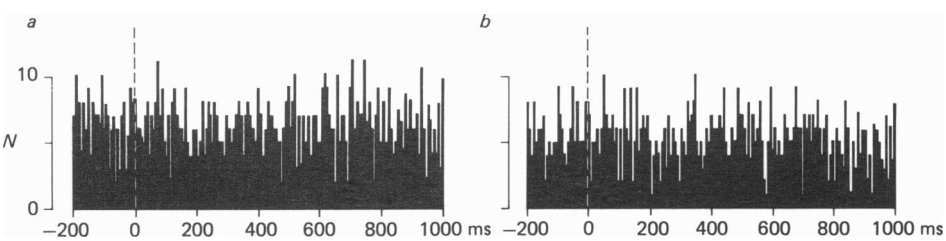

Fig. 6. Individual example of the lack of effect induced by percutaneous electrical stimuli (10 mA; 2 ms duration; 0·66 Hz; 200 ms delay) applied to the base (*a*) or the tip (*b*) of the tail, on the DLH-evoked activity (29 nA) of a trigeminal non-noxious only neurone (p.s.h.s, bin width 5 ms, 100 trials).

Effects of percutaneous electrical stimuli, applied to the tail, on DLH-evoked activities of trigeminal non-noxious only neurones

Fig. 6 shows a typical example of the results obtained for all the non-noxious only neurones tested. The repeated application (100 trials; 0·66 Hz) of percutaneous electrical stimuli (10 mA; 2 ms) to the base (Fig. 6*a*) or the tip (Fig. 6*b*) of the tail did not induce any noticeable change in the steady discharges induced by the

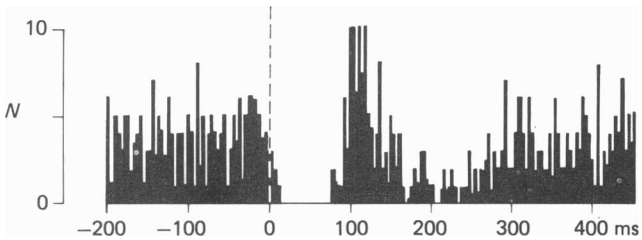

Fig. 7. Individual example of the biphasic inhibitory effect induced by repetitive single electrical stimuli (10 mA; 0·66 Hz; 2 ms duration; 200 ms delay) applied to the muzzle, on the DLH-evoked (39 nA) discharge of a lumbar dorsal horn convergent neurone (p.s.h., bin width 2 ms, 100 trials).

electrophoretic ejection of DLH. The failure to induce any inhibitory effects on the the DLH-evoked activities of non-noxious only neurones was observed both with the highest (10 mA) and the lowest (1 mA) currents employed in the fourteen neurones tested.

General properties of convergent neurones recorded in the dorsal horn of the lumbar spinal cord

A total of eleven convergent neurones were studied; they were located in the deeper layers of the dorsal horn (laminae IV–VI). All the units were under the influence of d.n.i.c. triggered by noxious stimulation (pinch) of the muzzle or the forepaws, and gave statistically steady discharges (mean $42{\cdot}3 \pm 5{\cdot}4$ spikes/s) during the electrophoretic ejection of DLH (mean current, $26{\cdot}4 \pm 3{\cdot}6$ nA).

Effects of percutaneous electrical stimuli, applied to the muzzle, on DLH-evoked activities of dorsal horn convergent neurones

Fig. 7 illustrates the effects of the repetitive application (100 trials; 0·66 Hz) of percutaneous electrical stimuli (10 mA; 2 ms duration) to the muzzle. As observed during recordings of trigeminal convergent neurones this procedure induced the appearance of the two components of inhibition. This was a constant finding obtained in the whole population ($n = 11$) of lumbar dorsal horn convergent units tested. Interestingly, the application of lower-intensity currents (1 mA) always induced the appearance of only the earlier component.

DISCUSSION

In the present study we have demonstrated that single-shock, percutaneous electrical stimuli can induce extrasegmental post-synaptic inhibitory processes which are triggered exclusively by activation of peripheral Aδ, or Aδ and C fibres. These processes acted on convergent neurones recorded in trigeminal nucleus caudalis and most probably were the same as were responsible for the inhibitions we observed on convergent neurones in the dorsal horn of the spinal cord.

The use of micro-electrophoresis of DLH to elicit neuronal activity allowed us to obtain statistically regular and continuous levels of firing from the neurones studied. The analysis of p.s.h.s obtained under such conditions provided an adequate method for studying post-synaptic inhibitory processes which under other conditions might have been subliminal and impossible to detect.

The results of the experiments in which recordings were made from convergent neurones in trigeminal nucleus caudalis, led to the conclusion that the inhibitory processes were triggered exclusively by the activation of peripheral fibres with conduction velocities in the 4·7–7·5 and 0·43–0·68 m/s range. According to Gasser & Erlanger (1927) and Burgess & Perl (1973) there are three types of cutaneous peripheral fibres, namely Aα, Aδ and C fibres, and these have peripheral conduction velocities of 30–100, 4–30 and 0·4–2 m/s respectively. Thus we can deduce that in the present study the inhibitory components were triggered exclusively by slowly conducting Aδ and C fibres. It may be noted that in previous studies in the rat it has been shown that the responses of dorsal horn neurones to percutaneous electrical stimulation reflect a similar pattern of activation by peripheral fibres as do the responses of these cells to direct nerve stimulation (Menétrey *et al.* 1977).

For obvious reasons it was impossible to estimate the conduction velocities of the peripheral fibres sustaining the early and late inhibitory components when recordings were made at the lumbar level and stimuli were applied in the trigeminal region. However, similarly to trigeminal convergent neurones, lumbar convergent neurones exhibited two inhibitory components when 10 mA were applied extrasegmentally (to the muzzle) and only the earlier component when 1 mA was applied. It is therefore most probable that identical mechanisms were brought into play in the two different situations.

The distance between the coccygeal segments and trigeminal nucleus caudalis on the one hand and those between the trigeminal system and lumbar segments on the other hand precludes the possibility that segmental systems were involved in either series of experiments, and reduces to a minimum the possible involvement of propriospinal (or more correctly spino-trigeminal or trigemino-spinal) mechanisms. The extrasegmental feature of the inhibitory processes described herein suggests that they belong to the d.n.i.c. system already described by our group (Le Bars *et al.* 1979*a*,*b*; Dickenson *et al.* 1980). Indeed, further to the fact that all the convergent neurones recorded were under the influence of d.n.i.c., several other characteristics were shared by the inhibitory processes induced by extrasegmental percutaneous electrical stimulation and d.n.i.c., as follows.

(a) Neuronal specificity: only convergent neurones, whether spinal or trigeminal, are affected by d.n.i.c. (Le Bars *et al.* 1979*a*,*b*; Dickenson *et al.* 1980), and in the

present study, we showed that non-noxious only neurones were unaffected by extra-segmental percutaneous electrical stimulation, whereas all the spinal and trigeminal convergent neurones were affected. It is important to note that non-noxious only neurones presented a similar level of firing (35·0 ± 3·3 spikes/s) to the convergent neurones (31·8 ± 1·3 spikes/s), thus excluding a possible bias in such a comparison.

(b) The triggering by nociceptive events: d.n.i.c. are specifically triggered by noxious stimuli (Le Bars, Chitour & Clot, 1981; Villanueva & Le Bars, 1985). In the present study, only Aδ and C fibres gave rise to the inhibitory processes. Although peripheral unmyelinated and thin myelinated fibres can respond to stimuli below the pain threshold (Van Hees & Gybels, 1972; Gybels, Handwerker & Van Hees, 1979; Adriansen, Gybels, Handwerker & Van Hees, 1983; Torebjörk, Lamotte & Robinson, 1984), the relationship between the activation of such fibres and nociceptive reactions or pain is a classical one (Van Hees & Gybels, 1972; Dubner & Beitel, 1976; Lamotte & Campbell, 1978, Gybels *et al.* 1979; Adriansen *et al.* 1983, Torebjörk *et al.* 1984). However, the thresholds for triggering the Aδ- and the C-fibre components were found to be in the 0·25–0·5 and 1–2 mA range respectively, which could be interpreted as suggesting a possible contribution by non-nociceptive afferents.

(c) The encoding of nociceptive information: a close relationship exists between the strength of the heterotopic nociceptive stimuli and the d.n.i.c.-related inhibitions acting on convergent neurones; such a relationship was found to be particularly clear when temperatures in the 44–52 °C range were used as conditioning stimuli (Le Bars *et al.* 1981; Villanueva & Le Bars, 1985). In the present study such a relationship was found in the 0·25–2 mA range for the Aδ component of inhibition and the 1–5 mA range for the C component. However, a contribution by non-nociceptive afferents is again possible, especially at the lower intensities of stimulation. Certainly the stimulus parameters employed would have been expected to activate some low-threshold afferents.

(d) Involvement of a post-synaptic inhibitory mechanism: d.n.i.c. affect all the activities of convergent neurones, whether those elicited from the periphery or as in the present study, by micro-electrophoresis of excitatory amino acids (Villanueva *et al.* 1984*a*,*b*). Glutamate and its analogues such as DLH excite neurones by acting directly on their membranes (see Puil, 1981), and since in the present study the tip of the electrophoresis pipette was in contact with the recording electrode 10 μm from its tip an indirect action of DLH via surrounding interneurones would appear to be very unlikely. We can therefore conclude that the two inhibitory components corresponded to inhibitory post-synaptic potentials.

There exist numerous studies concerning analgesia produced by transcutaneous electrical nerve stimulation in humans (for a review see Andersson, 1979). Although they can often be explained by the inhibitory processes triggered by Aα inputs at the segmental level (Noordenbos, 1959; Melzack & Wall, 1965; Wall & Sweet, 1967), the conclusions from human experiments are often contradictory and the mechanisms underlying transcutaneous electrical nerve stimulation and electro-acupuncture applied to sites with or without segmental relation to the pain locus are still controversial (Satran & Goldstein, 1973; Nathan & Rudge, 1974; Andersson & Holmgren, 1975; Andersson, Holmgren & Roos, 1977; Jeans, 1979; Melzack, 1984) and difficult to explain with a single hypothesis. In this respect it is important to

point out that the proposed gate-control theory (Melzack & Wall, 1965) is mainly focused on segmental spinal inhibitory mechanisms and therefore the pain-alleviating effects of remote stimulation require a different functional basis.

Although transcutaneous electrical nerve stimulation can be effective when applied at high frequencies and intensities below the pain threshold, the resulting pain relief is more localized and often limited to the stimulated segment (see Andersson, 1979). Furthermore, it has also been shown that stronger analgesic effects can be obtained with transcutaneous electrical nerve stimulation by using a critical level of stimulation which produces an unpleasant, but not quite painful sensation (see Andersson, 1979; Melzack, 1984). Analgesic effects produced by electro-acupuncture always employ intensities of stimulation strong enough to induce the feeling of 'teh chi', an unpleasant sensation reminiscent of pain (Shanghai Acupuncture Anesthesia Co-ordinating Group, 1977); this procedure results in a widespread distribution of pain relief.

The importance of Aδ-fibre activation in the production of analgesia or anti-nociceptive effects by somatic electrical stimulation has been suggested by several authors (Woolf, Mitchell & Barrett, 1980; Kawakita & Funakoshi, 1982; Chung, Lee, Hori, Endo & Willis, 1984; Lee, Chung & Willis, 1985; Sjölund, 1985). In this respect, our results showed that, as compared to the C component, the Aδ component of inhibition was easier to obtain and more constant in terms of magnitude and duration: it was observed with lower intensities of percutaneous electrical stimulation and rapidly reached its maximum effect when the current was increased; applying stronger intensities of peripheral stimuli gave rise to inhibitory responses of similar magnitude (see Fig. 3). This difference between the Aδ and C components was probably due to the fact that, in addition to having lower thresholds, the Aδ fibres responsible for the earlier inhibitions produce a more synchronized input to the spinal cord than do the slower C fibres. The 'safety' of the Aδ component of inhibition is also illustrated in Fig. 5, where it was elicited by various frequencies of extra-segmental percutaneous stimuli. This observation reinforces the proposal that Aδ fibres play an important role in the induction of analgesia or hypoalgesia by procedures using transcutaneous stimulation, since such procedures often involve these frequencies of stimulation.

In conclusion, there is now a substantial amount of data which implicates the activation of Aδ fibres in analgesic procedures based on peripheral electrical stimulation. Such activation also triggers post-synaptic inhibitory mechanisms which act on convergent neurones through the d.n.i.c. circuitry and induce widespread inhibitory effects. Although we have shown that C fibres participate in these effects on convergent neurones, it remains unknown whether they also contribute to the analgesic procedures. However, the fact that in humans both Aδ and C polymodal nociceptors respond to a wide range of stimuli, including non-nociceptive ones (Van Hees & Gybels, 1972; Torebjörk & Hallin, 1974; Gybels *et al.* 1979; Adriansen *et al.* 1983, Torebjörk *et al.* 1984), seems to support such a possibility.

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