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DIFFUSE NOXIOUS INHIBITORY CONTROLS (DNIC). I. EFFECTS ON DORSAL HORN CONVERGENT NEURONES IN THE RAT

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SUMMARY

(1) Sixty-eight convergent dorsal horn neurones have been recorded at the lumbar level in anaesthetized intact rats. All cells received prominent Aα and C fibre afferents and correspondingly could be activated by high and low threshold stimuli applied to the peripheral excitatory receptive field.

(2) The activity of 67/68 of these neurones was powerfully inhibited by noxious stimuli applied to various parts of the body. Since non-noxious stimuli were ineffective in this respect, the term "diffuse noxious inhibitory controls" (DNIC) is proposed.

(3) DNIC could be evoked by noxious pinch applied to the tail, the contralateral hind paw, the forepaws, the ears and the muzzle; the most effective areas were the tail and muzzle. Noxious heat applied to and transcutaneous electrical stimulation of the tail were extremely effective in eliciting DNIC as was the intraperitoneal injection of bradykinin.

(4) DNIC strongly depressed by 60—100% both the C fibre response following suprathreshold transcutaneous electrical stimulation and the responses to noxious radiant heat.

(5) The spontaneous activity and the responses to low threshold afferents induced either by Aα threshold electrical or natural stimulation were also powerfully inhibited.

(6) In the majority of cases, long lasting post-effects directly related to the duration of conditioning painful stimulus were observed.

INTRODUCTION

It is well known that the transmission of painful messages is strongly modulated at the spinal level. As postulated by Melzack and Wall [28,40],

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this transmission is controlled both by segmental mechanisms and by systems involving supraspinal structures [see refs. in 2,3,7,25,28,34,40,42]. Segmental inhibitory effects induced by the activation of large diameter fibres on responses of dorsal horn neurones to noxious stimuli have been demonstrated by various authors and would seem to be the neural basis for certain clinical analgesic effects such as those induced by transcutaneous nerve stimulation and some forms of acupuncture. In the same way the stimulation of the dorsal columns is able both to depress the activity of dorsal horn neurones involved in nociception and in some cases to successfully relieve pain in man. The stimulation of some brain stem structures is also efficacious in inhibiting the responses of dorsal horn neurones to painful stimuli. Correspondingly the stimulation of some well delimited structures of the brain such as the periaqueductal grey matter and raphe nuclei induces very powerful analgesia in animals; this kind of stimulation has been used in man for pain relief with varying success. One interesting point regarding both types of inhibitory effects — segmental or supraspinal — is their common final effects on dorsal horn nociceptive neurones especially on convergent units, i.e. cells receiving both low and high threshold afferent inputs.

However, there exist a number of pain relieving stimuli that do not fit into the neural schema described above. For instance, under the terms of counterirritation or "hyperstimulation analgesia" [26] are designated various phenomena the common feature of which is that painful stimuli applied to one area of the body are at the origin of analgesic effects observed on other areas. This has been observed on the basis of both experimentally induced or clinical pain [10,15,26,35,41]. In addition, it has been recently postulated [8,21,27] that some analgesic effects induced by acupuncture may be based on mechanisms similar to those underlying counterirritant procedures. Furthermore there is some evidence suggesting that the experimentally induced pain threshold is raised in suffering patients [16,32]. In animals, such phenomena have also been described [1].

Thus, these data prompted us to postulate that the responses of certain neurones in the central nervous system to noxious stimulation may be inhibited by another noxious stimulus. The aim of the present study was to investigate the possible existence of such inhibition on dorsal horn cells in the anaesthetized intact rat. The present paper is concerned with such an investigation on convergent neurones receiving both noxious and non-noxious input; the following paper [20] will describe results obtained with non-convergent neurones, i.e. noxious only, non-noxious only and proprioceptive cells.

METHODS

Male rats weighing between 200 and 275 g were used in these experiments. Following an intraperitoneal injection of 100 μg atropine sulphate, the animals were anaesthetized with 1.5—2% halothane in a nitrous oxide/oxygen mixture. A tracheal cannula was inserted, the jugular vein cannulated

and an intraperitoneal cannula sewn in place. A laminectomy was then made between segments T11 and L2 over the spinal cord and the vertebrae mounted onto a rigid frame. The dura was then opened over the area of the cord giving maximal dorsum potentials to electrical stimulation of the hind paw. Finally the wound borders were infiltrated with xylocaine, the skin overlying the cord retracted to form a pool and the animal was immobilized with gallamine triethiodide (Flaxedil). The level of halothane was lowered to 0.5% and the rat artificially ventilated. The rate (70—80 counts/min) and the volume were adjusted to maintain a normal acid-base equilibrium [9]. With this level of anaesthesia clear neuronal effects could be produced with no corresponding change in the EEG during the noxious stimulation. The EEG was monomorphic with regular slow waves (3—5 Hz) and was stable throughout the experimental period. Heart rate was continuously monitored and the central temperature kept constant by means of an homeothermic blanket system.

An NaCl pontamine blue filled glass micropipette (resistance 8—12 MΩ) was lowered into the dorsal horn and the pool filled with agar to minimize respiratory movements. Electrode descents were made throughout the dorsal horn at the T11—L2 levels and those cells responding to natural stimulation of the ipsilateral hind paw were considered. Once a cell had been identified according to the effect of touch, pressure, pinch, noxious heat and other stimuli applied to the periphery, the extent of the receptive field was determined. Cells responding to non-noxious temperatures were not studied. The cells were classified into 4 groups according to the criteria of Iggo [18] and Menétrey et al. [30], i.e., noxious only units, convergent units, innocuous units and proprioceptive cells. In this paper only the convergent neurones are considered. These units have also been described as wide dynamic range [29], lamina V type [17,39], class II [18,30] or polymodal [43] neurones. The sizes (0.57 ± 0.12 cm^2) of their peripheral excitatory receptive fields were variable but generally extended to include several toes and could include the whole paw. Ipsilateral cutaneous inhibitory fields which could be activated by both noxious pinch and more often, repetitive light touch, were found for several neurones.

When possible, 5 types of activity were studied. When encountered, spontaneous activity was recorded; if this was not present for the convergent and innocuous units sustained pinch applied to the peripheral receptive field by means of an artery clip produced a high level of activity. This stimulation was noxious when applied to the investigator. For the convergent and noxious units the response to radiant heat applied by a focussed bulb was studied. The temperature of the skin was measured by means of a thermistor, applied in the centre of the 6 mm^2 irradiated spot, which also controlled the bulb intensity hence providing reproducible heating steps. For the convergent and innocuous neurones light tactile stimulation regularly applied to the peripheral field by air-jet, paint-brush or blunt probe was used as an activating stimulus. Finally for all neurones transcutaneous electrical stimulation by two needles inserted in the centre of the excitatory receptive field

was used to characterize the fibre input of the neurones. In the rat, this stimulation produces similar responses to those induced by direct nerve stimulation [30]. Single square pulses of 0.2 or 2 msec duration were applied at 1000–3000 msec intervals and the current for threshold and supramaximal Aα and C fibre responses was measured. For each sequence of electrical stimulation, a poststimulus histogram was built and analysed to distinguish the responses due to Aα, Aδ and C fibre inputs according to their latency and using the classification of Gasser and Erlanger [11] and Burgess and Perl [4]. However, the responses to Aδ input were not considered in the present study since they were rarely encountered and when present were not easily reproducible and often difficult to differentiate from the total A response. The responses were recorded on magnetic tape and conventional display techniques and analysis were used.

The effects of various noxious peripheral stimuli were tested against the 5 types of activity described above. These stimuli consisted of:

— Pinch, from serrated forceps, adjudged to be clearly noxious when applied to the investigator, was applied to various parts of the body. The areas of the body tested were the tail (base, medial portion or tip), contralateral hind paw, muzzle, forepaws and ears. The ipsilateral hind paw was not tested because of possible confusion resulting from the presence of ipsilateral inhibitory fields and interference with the often large excitatory receptive fields.

— Noxious heat applied by hot water at a constant temperature to the 3 cm final portion of the tail by means of an insulated beaker.

— Transcutaneous electrical stimulation of the tail as this stimulus is known to be noxious from behavioural studies [5]. We used the same parameters as those we have utilized in the chronic animal to produce vocalisation [6] (1 msec square pulses in a 500 msec train at 50 Hz) and the threshold for inhibition of the conditioned response was searched in a similar manner for the threshold for vocalisation in the chronic rat. 0.2 mA increments in current were applied until the threshold for inhibition was reached.

— Bradykinin (8 μg in 1 ml saline) injected via an intraperitoneal cannula. Injection of 1 ml saline was used as control and at the end of the experiments, the spread of injection within the peritoneum was verified by injection of a dye. This stimulus has been reported to be both painful in man [23] and to induce pseudo-affective reactions in animals [14,22].

At the end of the testing of a neurone, the descent was marked by iontophoresis of pontamine blue. Conventional histological techniques were used to localize the recording site. Statistical analyses were made using the unpaired Student's *t*-test.

RESULTS

(I) General findings

We found prominent inhibitory effects on convergent neurones induced by noxious stimuli applied to widespread areas of the body. For convenience

we have designated these controls as diffuse noxious inhibitory controls (DNIC). DNIC was found to exert a powerful effect on 67/68 convergent units studied, cells responding to noxious radiant heat, strong pinch, pressure and often light touch — neurones with a pronounced Aα and C fibre input. Fig. 1 shows the localization of 49 of these convergent units, the majority being in the medial part of the dorsal horn.

The DNIC could be induced by noxious stimulation applied to various areas of the body with the most powerful inhibitory effects often completely abolishing the response of the neurones. DNIC could be evoked by noxious stimulation applied to the tail, the contralateral hind limb, both forelimbs, the muzzle, the ears and the viscerae. These inhibitions could be induced by noxious transcutaneous electrical stimulation, noxious heat, strong pinch and intraperitoneal injection of bradykinin. Innocuous stimulation was completely ineffective. The DNIC reduced the response of the convergent neurones to C fibre stimulation, noxious radiant heat, pinch and also the spontaneous firing rate. Responses to light tactile stimulation and to A fibre stimulation were also affected but to a lesser extent. Long post-effects were frequently observed.

Noxious only neurones, innocuous cells and proprioceptive units were not affected. In the spinal preparation we were unable to demonstrate the existence of DNIC. The results will be considered in detail for convergent

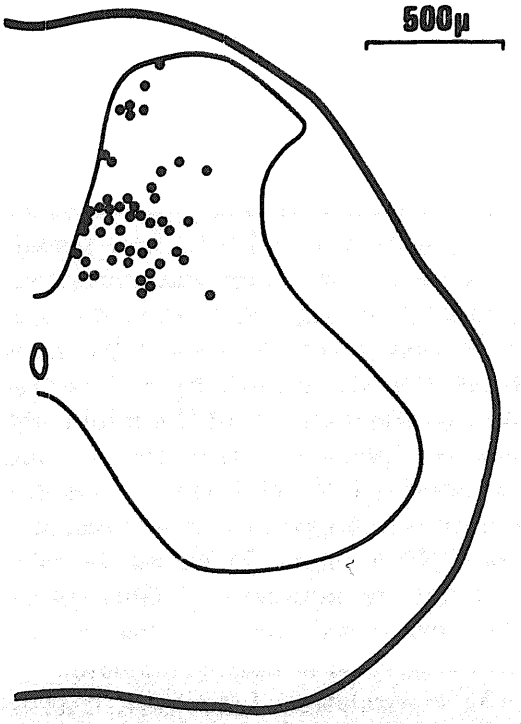

Fig. 1. Histological localization of the 49 convergent neurones succesfully marked by pontamine blue illustrated on a transverse section of the lumbar spinal cord.

neurones below; results from other types of neurones and those obtained in the spinal animal will be presented in the following paper [20].

(II) DNIC versus responses to transcutaneous electrical stimulation

(1) C fibre responses

All 68 convergent cells were tested for their response to transcutaneous electrical stimulation of the excitatory receptive field. This stimulation produced clear Aα and C fibre responses, which in most cases correlated well with the responsiveness of the neurones to natural non-noxious and noxious stimulation of the peripheral field. The A fibre response will be considered in a following section.

The C fibre response of these convergent neurones had a mean threshold of 3.46 ± 0.32 mA for a 2 msec duration pulse. Increasing the current to a suprathreshold level (threshold × 1.5—3) produced a maximal C fibre response against which DNIC was tested. In the majority of neurones this response consisted of several bands, each containing a train of spikes. The mean latency of the maximal firing due to C fibres was 260 msec, indicating a mean peripheral conduction velocity of about 0.6 m/sec.

The full range of plurisegmental noxious stimuli were tested for their efficacy in inhibiting the C fibre response of these convergent neurones. Whereas every cell tested except one was clearly inhibited, the degree of inhibition was quantified in only those cells showing an extremely stable response.

(a) Noxious pinch versus C fibre response. Intense pinch applied to the tail resulted in an almost complete block (mean 87.9 ± 2.7% inhibition; n = 26) of the C fibre response induced by supramaximal stimulation. The weakest effect seen was a 12% inhibition in one neurone; by contrast, 12 neurones had the C fibre response completely inhibited by noxious pinch applied to the tail. In no case did innocuous stimulation such as stroking or jets of air applied to the tail produce any inhibitory effect in all neurones tested. During the stimulation of the tail the degree of inhibition was unaltered, remaining constant throughout the period of pinching and following cessation of the pinch post-effects were clearly seen (mean duration 1.55 times the period of pinch). Fig. 2 illustrates these effects. In most cases the duration of the post-effect was directly related to the duration of the inhibitory stimulation and gradual recovery of response followed this period. During the inhibitory period, in those cases of incomplete inhibition, the spike amplitude was unchanged and the poststimulus histogram did not reveal any obvious change in the peak latency of the C fibre bands. There was no relationship between the percentage inhibition and the number of C fibre spikes present. Furthermore there was no relationship between the size of the peripheral excitatory receptive field and the degree of inhibitory control.

Noxious mechanical stimulation of the contralateral hind paw, muzzle and ears, also produced clear inhibition of the C fibre response (see Fig. 3). However, there were significant differences between the degree of inhibitory

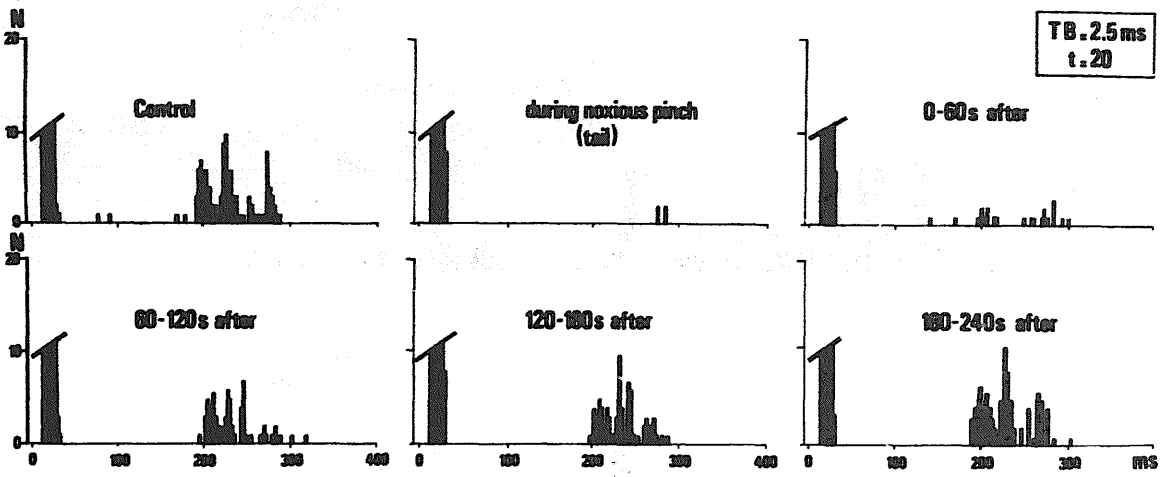

Fig. 2. Poststimulus histograms (t = 20 trials; TB = time base; N = number of spikes) showing the complete inhibition of the long latency C fibre response of a convergent neurone to supramaximal transcutaneous electrical stimulation by noxious pinch applied to the tail. Note the long post-effect following the period of pinch (1 min). The short latency A fibre response was unaffected and has been truncated for clarity of presentation.

control activated by the different areas of the body. There was no difference between the tail (87.9 ± 2.9%, n = 26) and the muzzle (85.0 ± 5.9%, n = 9) in producing these inhibitions but the stimulation of these areas induced significantly greater inhibitions ($P < 0.01$ for the tail, $P < 0.05$ for the muzzle) than those produced by pinch applied to the contralateral hind paw (70.1 ± 7.3%, n = 15) or the ears (62.3 ± 7.9%, n = 13); pinching the forepaws produced inhibitions of 70.1 ± 8.3% (n = 5).

(b) Noxious heat applied to the tail versus C fibre response. Ten of the above convergent units were tested for the effect of noxious heat on the final 3 cm portion of the tail on the C fibre response. This was produced by the application of hot water at various constant temperatures. Noxious heat (mean temperature of 48.2° C) produced inhibitions in all cells tested with a mean percentage inhibition of 74.0 ± 4.7 (n = 10). There was often an increase in the degree of inhibition over the first 2 or 3 sec following application of the stimulus presumably due to the time necessary to heat the tail skin to a noxious level. However within the 10—30 sec heating periods, the maximal inhibitory effect remained constant. Water at innocuous temperatures (less than 42° C) produced no effect on the C fibre response (n = 6). Because of difficulties such as the tail skin temperature after the end of the stimulation, durations of the post-effects have not been calculated; however, clear inhibitory effects were seen outlasting the period of heating by up to 100 sec. Fig. 4 provides a typical example of these effects.

It should be pointed out that, in contrast to the effects described above, restricted noxious radiant heat stimuli (6 mm^2) applied to the tail produced no clear inhibitory effect.

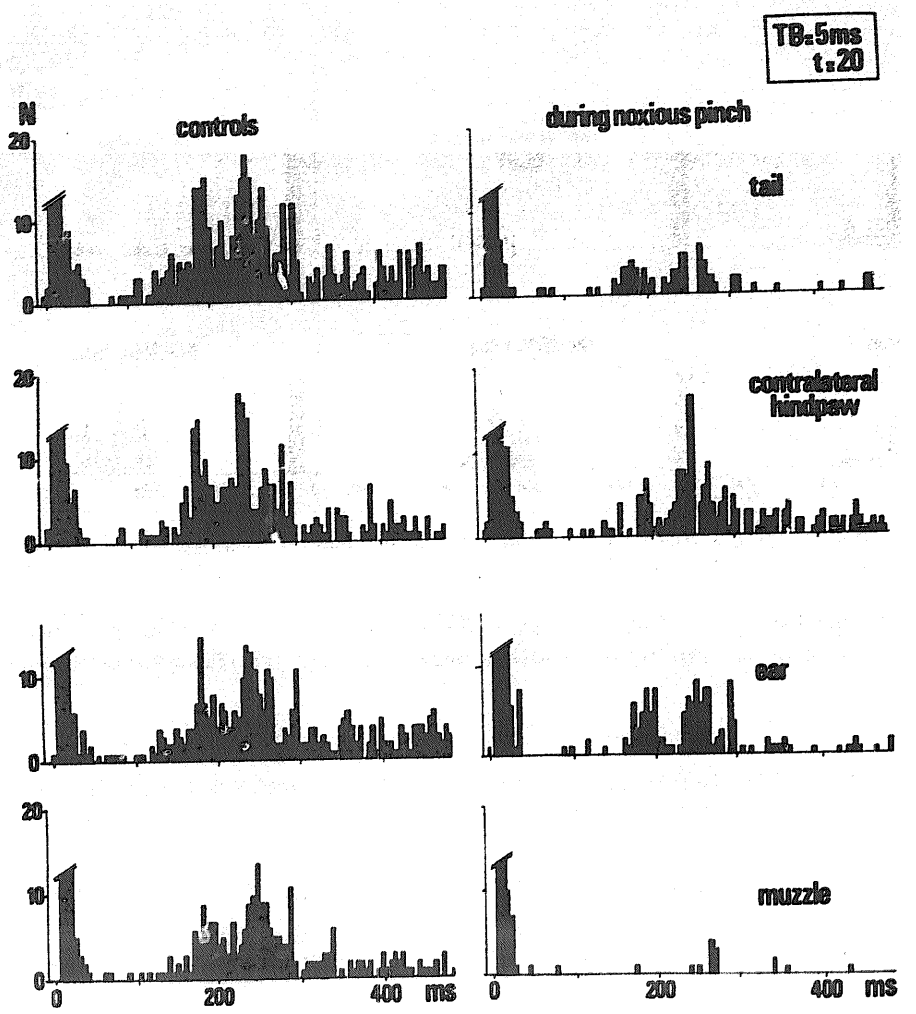

Fig. 3. Poststimulus histograms comparing the inhibitory effect of noxious pinch applied successively to various parts of the body on the C fibre response (A fibre response truncated) of a convergent neurone. The inhibitory effect from the tail and muzzle are most effective. Note that because of the post-effects the control responses are progressively diminished despite well spaced test periods.

(c) Intraperitoneal bradykinin versus C fibre response (Fig. 5). Bradykinin is well known to be a potent stimulant of visceral nociceptors. As the previous noxious stimuli were all externally applied, we have tested the effect of 8 μg bradykinin i.p. on the C fibre response to gauge whether noxious visceral stimulation could induce similar inhibitory effects. Seven cells, all with a prominent C fibre input, were tested with bradykinin. Powerful inhibitory effects were seen with a latency of 5—20 sec after the injection. The mean inhibitory effect was 84.3 ± 6.0% (n = 7), remarkably similar to that produced by noxious pinching of the tail and muzzle. Post-effects of long duration were seen in all cases with a mean inhibitory period of 170 sec before full re-establishment of the response. During this period the C fibre

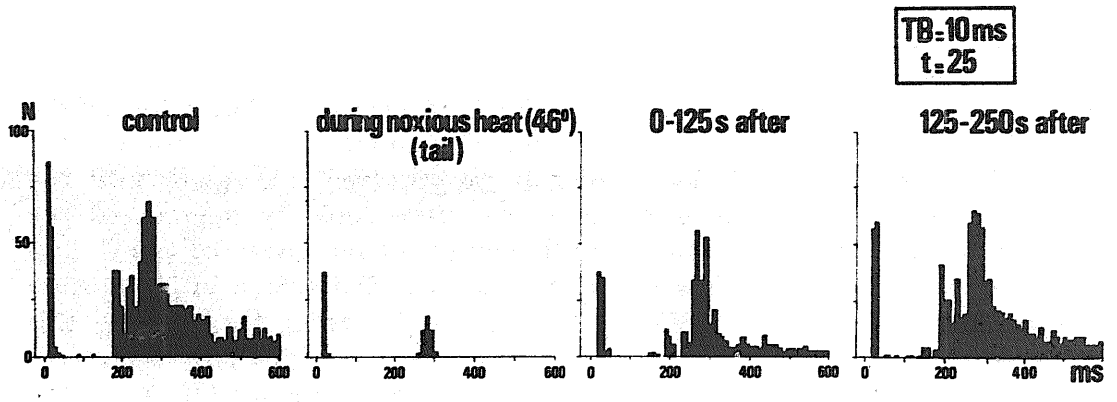

Fig. 4. A set of poststimulus histograms illustrating the inhibitory effect of noxious heat applied to the tail on, in this case, both the A and C fibre responses of a convergent neurone.

response slowly recovered, again with no change in spike amplitude or response latency. Intraperitoneal injection of saline produced no change in the activity of these neurones.

(d) Electrical stimulation of the tail versus C fibre response. In behavioural experiments [6] we have used electrical stimulation of the tail as a

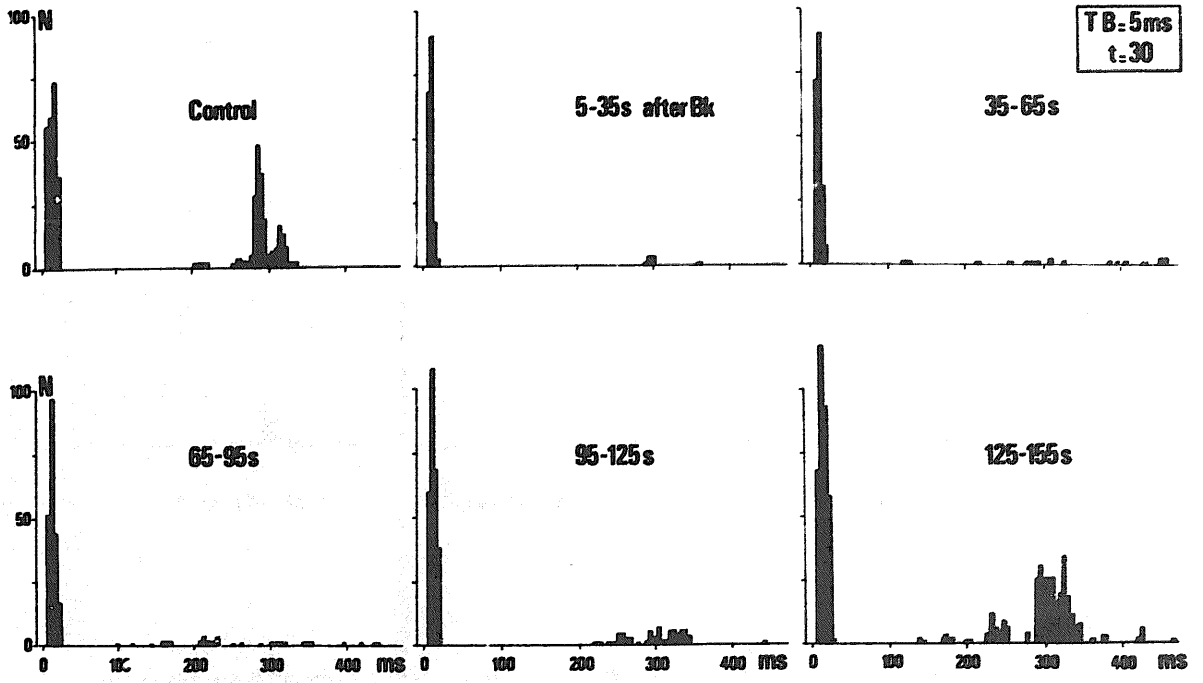

Fig. 5. Poststimulus histograms illustrating the inhibitory effect of intraperitoneal bradykinin (Bk; 8 μg) on the response of a convergent neurone to electrical stimulation of the peripheral field. Both the A and C fibre responses were inhibited; the latter was completely inhibited for up to 2 min after the injection; only the later components of the A fibre response were inhibited with a lesser post-effect.

nociceptive stimulus in chronic rats, using the threshold current for vocalisation as the criterium for nociception. Because this stimulus is obviously nociceptive we have investigated the effect of electrical stimulation of the tail on the C fibre response, using the same parameters as in the chronic rat.

In the chronic animal the current is progressively increased until the threshold for vocalisation is reached. We have similarly augmented the current in the acute animal until the threshold for inhibition of the C fibre response was reached. This was taken as a clear diminution in the C fibre response of the order 30—50%. The threshold current for eliciting this response was 2.94 ± 0.43 mA (n = 9). In the chronic animal [6], the threshold for vocalisation was 2.14 ± 0.59 mA (n = 84). Augmentation of the current progressively increased the degree of inhibition until a 100% inhibition was reached (Fig. 6).

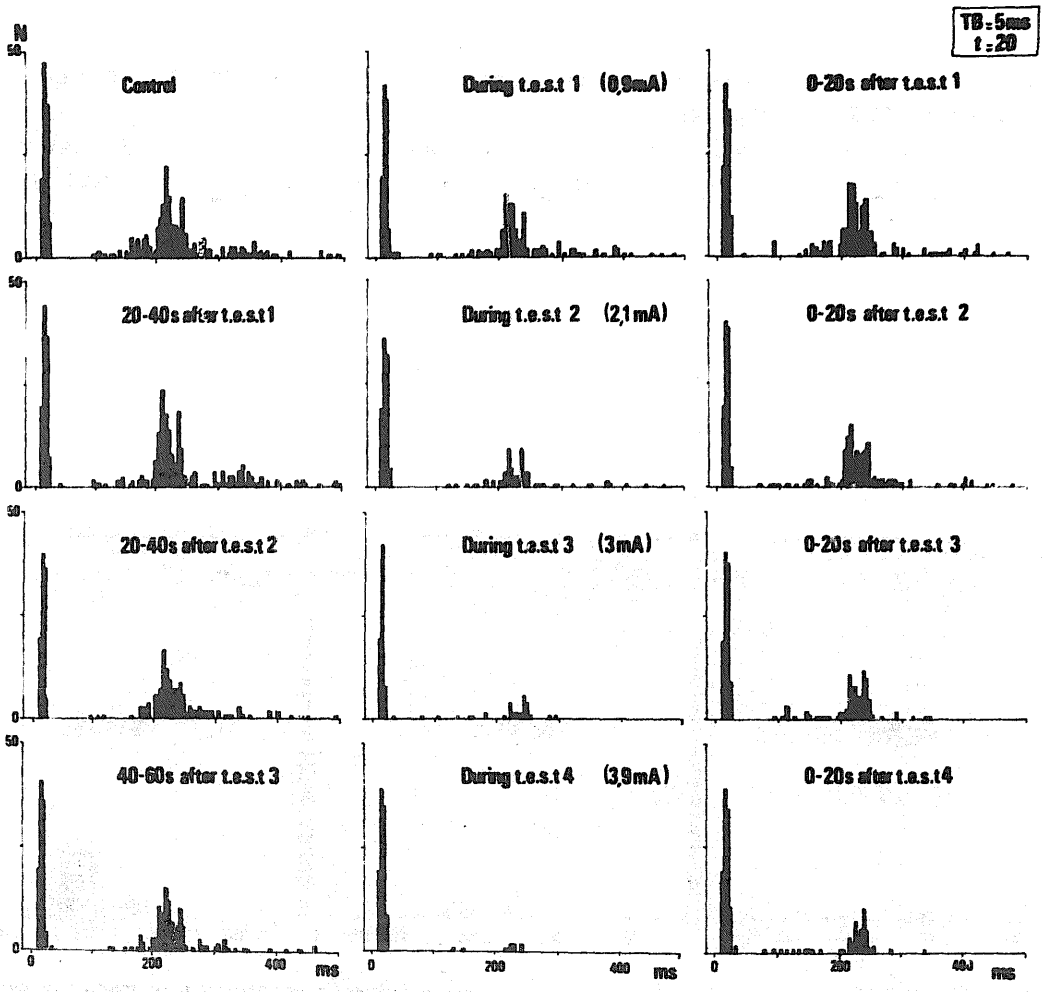

Fig. 6. The increased inhibitory effect of and increased post-effects after transcutaneous electrical stimulation of the tail (t.e.s.t.) on the Aα and C fibre response of a convergent neurone as the current is increased. The histograms are continuous; due to the increasing post-effects the control after test 3 was delayed.

(2) Aα fibre responses

All these convergent units received Aα fibre input (latency 5—7 msec) in addition to the C fibre input. The effect of the plurisegmental noxious stimulation was tested aginst the Aα fibre responses in two ways. The first was to qualitatively examine the effect of the noxious stimulation with the supramaximal Aα fibre response concurrent with the C fibre response. With this extreme suprathreshold stimulation of Aα fibre, inhibitory effects were seen in 45% of the neurones (see Figs. 4, 5 and 6). Innocuous stimulation was completely ineffective in this respect. Because of the supramaximal nature of this stimulation, 10 neurones with a stable Aα fibre response were tested at a threshold current producing 1—4 spikes per stimulus (mean threshold current: 0.64 ± 0.07 mA for a 0.2 msec duration pulse; whole population). No effects were seen in two of these neurones but, as illustrated in Fig. 7, in the other 8 pinching the tail produced a 81.8 ± 8.8% (n = 8) inhibition, the contralateral hind paw 79.2 ± 13.6% (n = 7) and the ear a 60% inhibition. Pinch of the forepaw was also effective. In 4 cases tested augmentation of the voltage to 3 or 4 times suprathreshold reduced the effect of noxious mechanical stimulation of the tail to a mere 18.7 ± 11.9% inhibition. This suggests that by increasing stimulation parameters above threshold for the A fibre response, the effect of DNIC may be overriden. As for the C fibre responses, innocuous peripheral stimulation was completely without effect (see Fig. 7).

With the A fibre stimulation the inhibitory post-effects were slight, being a mean 89% of the period of pinch for the tail and 54% for the contralateral hind paw.

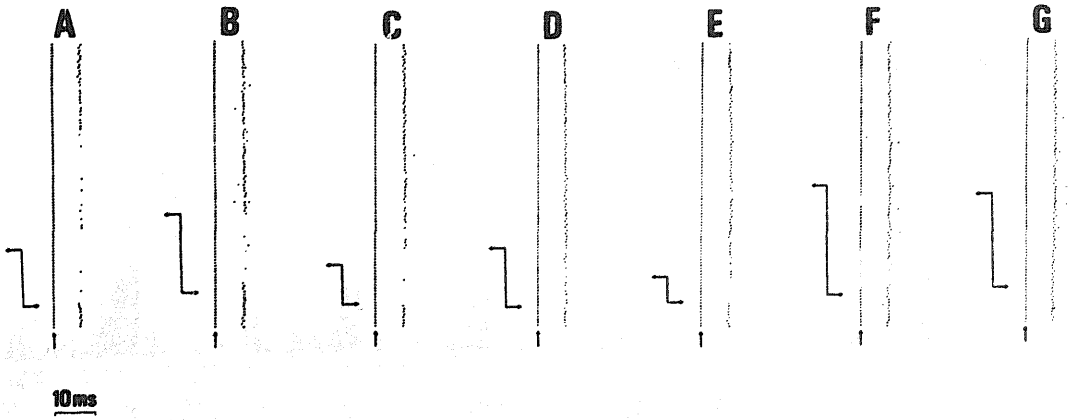

Fig. 7. Dot display (each dot represents one spike) of the Aα response of a convergent neurone following electrical stimulation of the receptive field (shock artefact marked by the vertical arrow). The period of plurisegmental stimulation is shown by the horizontal double arrows. A, noxious pinch (tail); B, noxious heat 49°C (tail); C, noxious pinch (hind paw); D, noxious pinch (forepaw); E, noxious pinch (muzzle); F, air jet (tail); G, air jet (muzzle).

(III) DNIC versus responses to natural stimulation

(1) Responses to radiant heat (Fig. 8.)

All 68 units exhibited a clear excitatory response following application of noxious radiant heat to the peripheral field from a focussed bulb. However, due to habituation, sensitization or densensitization phenomena, the responses to ramp heating steps were not always stable with repetitive testing. A stable response was tested in 9 cells, i.e., at least 4 identical control responses followed by a clear recovery after the test inhibition. In all these cells mechanical noxious stimulation of the tail produced a complete block of the response to noxious heat (above 42° C). The post-effect was extremely pronounced and lasted on average 4.27 times the period of

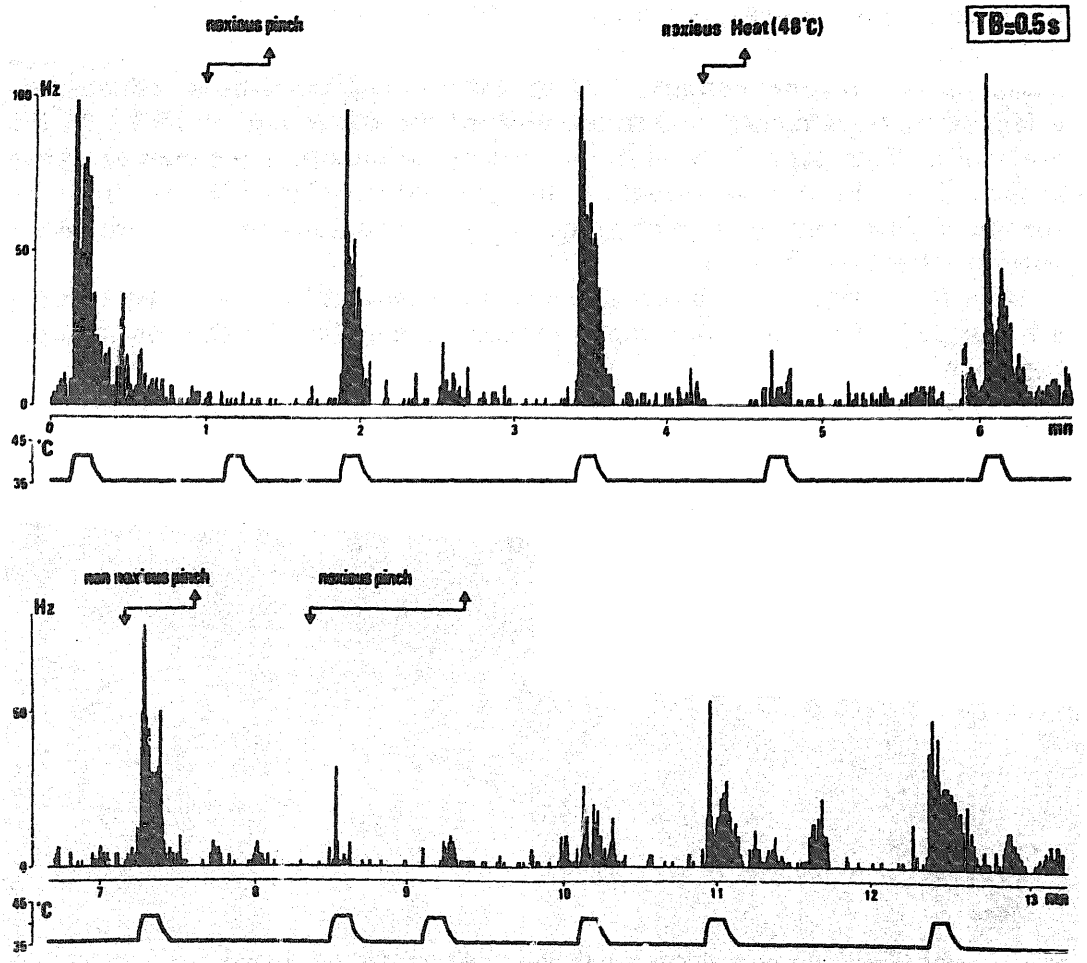

Fig. 8. The effect of DNIC on the response of a convergent neurone to radiant heat applied to the excitatory peripheral receptive field. Whereas noxious pinch and noxious heat (both applied to the tail) produce complete inhibition of the response, non-noxious light pinch is without effect. Note the pronounced post-effect following the sustained final period of pinch. The heating steps are shown below the ratemeter record.

noxious stimulation. It is interesting to note the effect of noxious heat applied to the tail against the response of the neurone to radiant heat. In all 3 cases tested water at 48° C completely blocked the response of the neurone to noxious heat. Although these were the only cells tested systematically, another 9 cells responding to the application of radiant heat showed inhibitions of this activity during pinch of the tail, contralateral paw and muzzle.

(2) Responses to sustained noxious pinch

In 10 convergent cells with no spontaneous activity, a high level of regular firing was induced by sustained pinch applied to the peripheral field by means of a small artery clip. Often a phasic outburst of activity was seen following onset of the pinch which rapidly habituated to produce a stable level of activity. During this latter period the effect of noxious stimulation applied to various parts of the body was tested.

Noxious pinching of the tail produced a powerful inhibition of this activity (84.5 ± 4.0%, n = 10), identical to that resulting when tested against the spontaneous activity (see section IV). The post-effects following the period of stimulation were again long, on average 2.53 times the duration of the inhibitory pinch. In 3 cases a complete cessation of activity was seen. There was no habituation of the inhibitory effects, for example, pinch applied to the tail over 2 min caused a complete inhibition of this tonic activity and recovery of the response only commenced after the end of the noxious stimulation (Fig. 9). In addition, noxious heating of the tail to 48° C also produced clear inhibitions.

The activity induced by this sustained pinch could also be powerfully reduced by pinch applied to the muzzle (82.5 ± 7.5%, n = 4). The contralateral hind paw was less efficacious in reducing the response to the constant

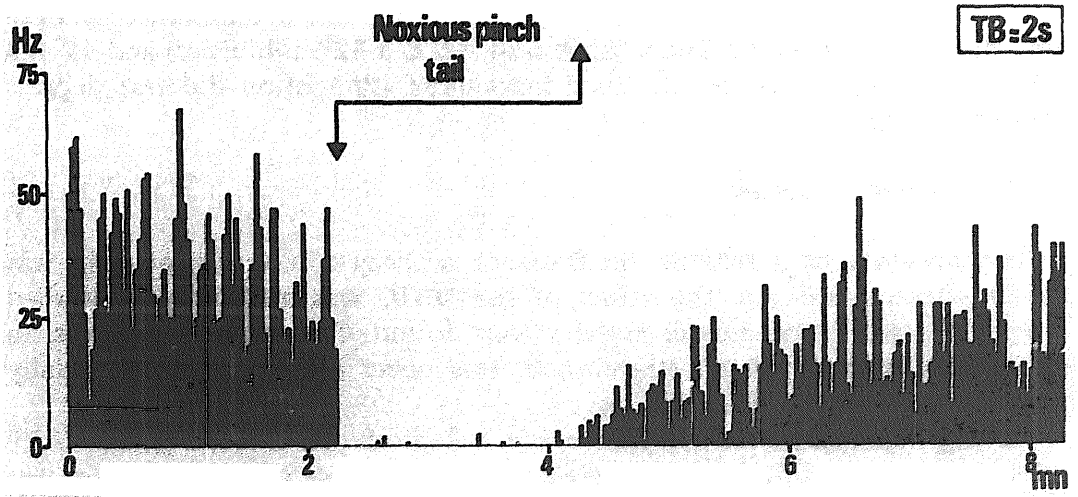

Fig. 9. The complete inhibition and long post-effect seen after noxious pinch of the tail on the activity of a convergent neurone induced by sustained pinch applied to the hind paw peripheral receptive field (see text).

pinch (57.5 ± 4.7, n = 4), being significantly less than that produced by the tail and muzzle ($P < 0.05$). In the case of the tail and muzzle, noxious stimulation produced an identical level of inhibition to that seen with the spontaneous rate (see section IV) even though the activity produced by the tonic pinch was up to 60 spikes/sec, whereas the spontaneous activity rarely exceeded 10 spikes/sec.

(3) Responses to light natural stimuli

As a corollary to the Aα fibre response and as result of the tactile sensitivity of the peripheral fields of most of these convergent neurones, we tested the effect of noxious stimulation on the response of the cells to natural tactile stimulation such as stroking, hair movement and light pressure.

Thirteen cells with a marked response to such regularly applied stimuli were tested. One cell was uninfluenced by the noxious stimulation but the remaining were all inhibited though to a lesser degree than that seen using C fibre stimulation as the activating input. The mean percentage inhibition seen was 68.7 ± 7.8% (n = 13) after pinching the tail which when compared to the 87.9% inhibition seen with C fibre responses is significantly less ($P < 0.01$). Similarly the effect of pinching the muzzle (60.0 ± 11.0%, n = 6) and injection of bradykinin (56.6 ± 6.6%; n = 5) are less important on responses to tactile stimulation than to C fibre responses ($P < 0.05$ in both cases). Pinching the hind paw or the ear also produced clear inhibitions (51.8 ± 6.3%; n = 10, and 40.1 ± 12.0%; n = 6 respectively). Fig. 10 shows the comparative effect of pinch applied on various areas of the body against this type of response. In 6 cases, extremely long post-effects were seen lasting between 2 and 6 times the period of stimulation; on the other hand, in 7 neurones no post-effects were seen at all. The inhibitory effects produced were efficient whatever the tactile stimulation or the level of the induced neuronal activity. Heating the tail also inhibited the activity produced by tactile stimulation. In one unit (Fig. 11) 3 temperatures were tested; 51°C produced a 100% inhibition, 45°C a 62% inhibition and 42°C a 50% inhibition. Again in all cases innocuous stimulation did not depress these responses.

(IV) Spontaneous activity

The presence of a reliable spontaneous activity of convergent units was rarely encountered but the effect of the DNIC was methodically studied when cells with spontaneous activity were found. The spontaneous rate, in absence of any peripheral stimulation, was never of a high order, usually being between 5 and 10 spikes/sec.

Strong noxious pinch applied to the tail produced a mean 82.5 ± 6.2% inhibition (n = 8) of the spontaneous activity of these cells. The effect was almost instantaneous, did not alter the spike amplitude and produced long lasting post-effect inhibitions following the end of the period of pinch. These lasted a mean 3.07 times the period of stimulation and in every case

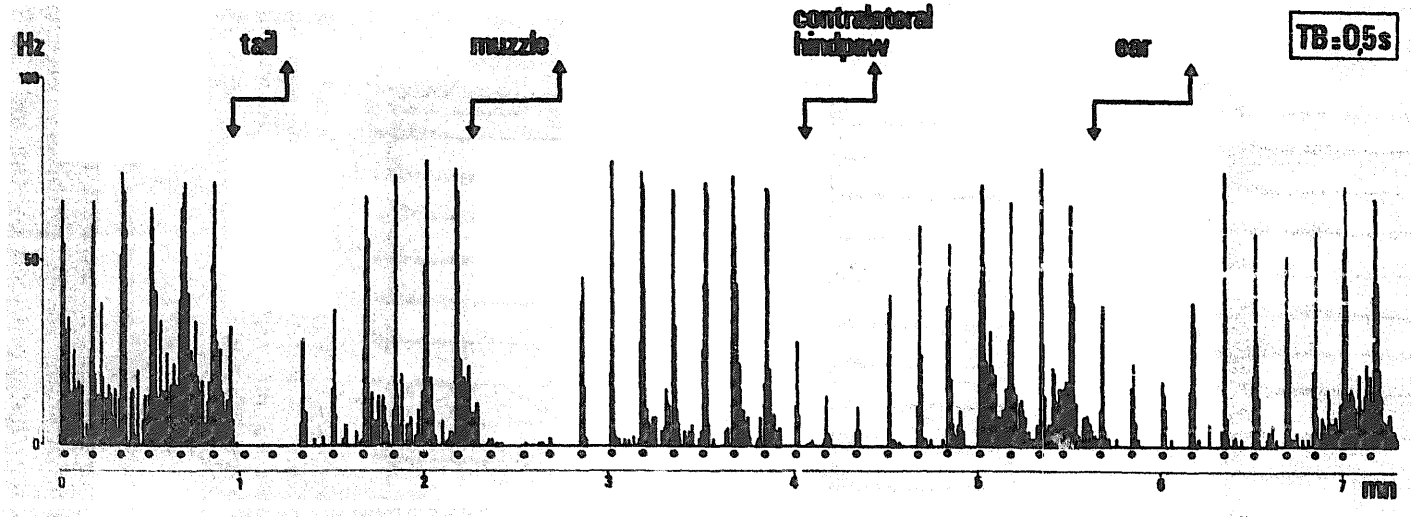

Fig. 10. The inhibitory effect of noxious pinch applied to various parts of the body (period of pinch arrowed) on the response of a convergent neurone to regular light stroking (black circles) applied each 10 sec. Note the greater effect of pinch on both the spontaneous and this evoked activity when applied to the tail and muzzle compared to the paw and ear.

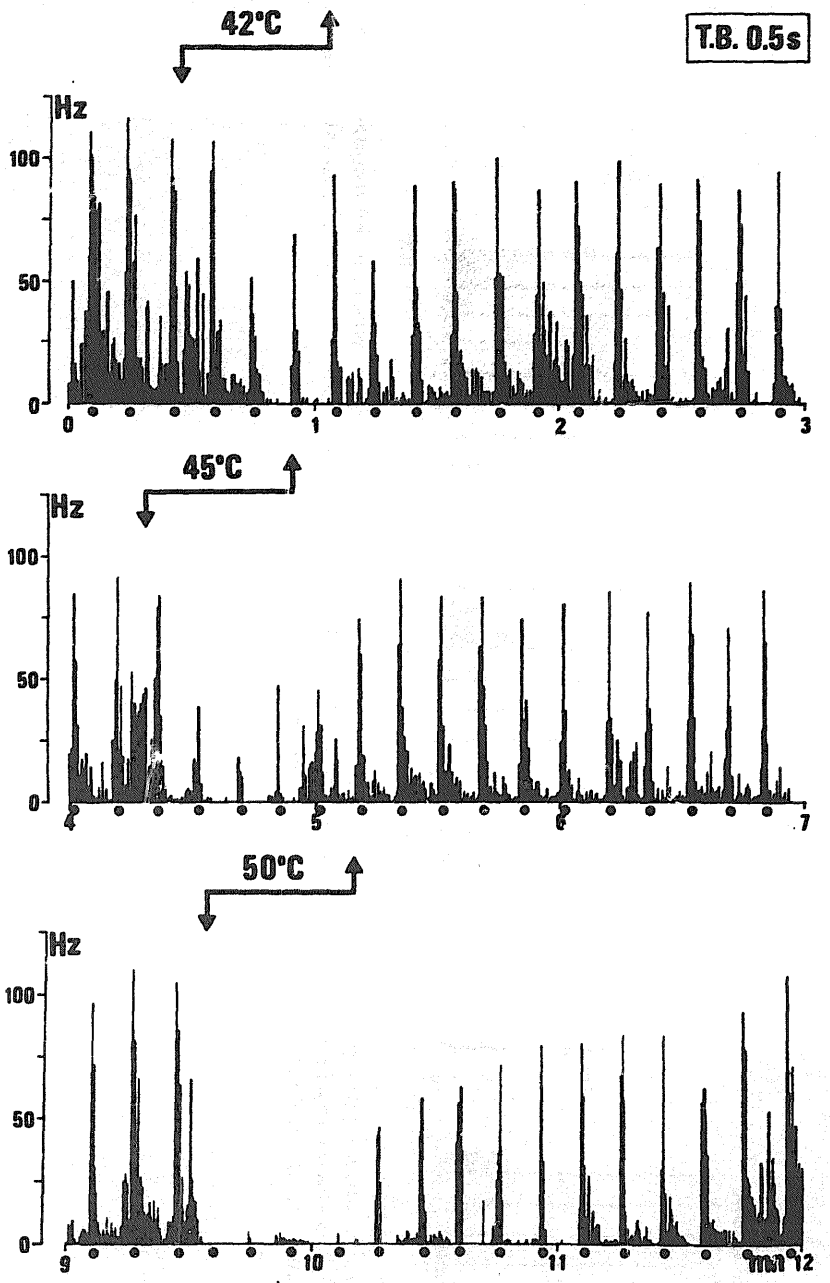

Fig. 11. The inhibitory effect of noxious heat applied to the tail on the response of a convergent neurone to regular tactile stimulation (in this case, stroking the peripheral field represented by the black circles). Increasing the level of noxious heat increases the degree of inhibition of both the evoked and spontaneous activity of the neurone and prolongs the period of the post-effect.

such a post-effect was seen manifested as a gradual increase in activity until the pretest baseline was regained. Pinch applied to the contralateral hind paw was also efficient in producing these inhibitory effects, though the mean inhibition was less than that produced by the tail (70.7 ± 6.4, n = 8)

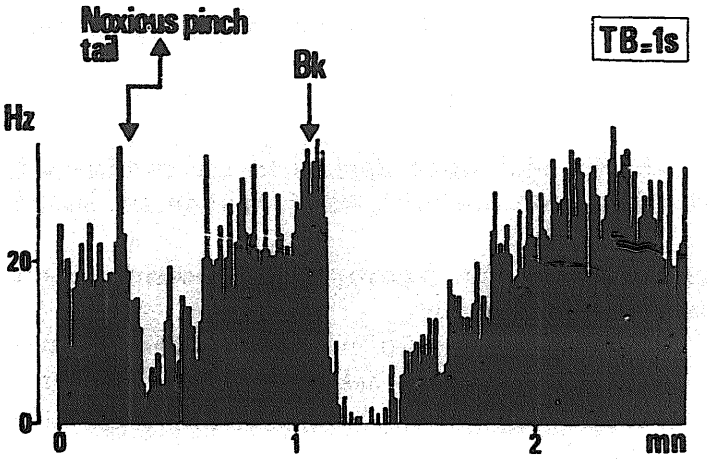

Fig. 12. The inhibitory effect of noxious pinch of the tail and bradykinin i.p. on one of the few convergent neurones exhibiting a high level of spontaneous activity.

and correspondingly shorter post-effects were observed (2.32 times the period of stimulation). Though tested less frequently pinching the muzzle (n = 2) and ears (n = 3), noxious heating of the tip of the tail to 48°C (n = 3) and intraperitoneal injection of bradykinin (n = 3) also produced long lasting inhibitions (Fig. 12). In all cases, innocuous stimulation did not alter the spontaneous firing rate.

(V) Ipsilateral inhibitory receptive field

As expected inhibitory receptive fields, ipsilateral to the excitatory receptive fields, were encountered [17,30,39]. However, these controls and the widespread inhibitory effects we have described are different phenomena. The ipsilateral fields could be activated by non-noxious stimuli as well as pinch whereas DNIC was only induced by purely noxious stimuli. Furthermore in cells without ipsilateral inhibitory fields (51/67), the widespread DNIC system was still present.

DISCUSSION

These results demonstrate that in the intact animal, dorsal horn convergent neurones responding to both noxious and innocuous stimuli and correspondingly receiving both C and Aα fibre inputs can be strongly inhibited by peripheral noxious stimuli applied to areas of the body remote from the peripheral excitatory receptive field. As all except one of these neurones were influenced by DNIC, one can presume that DNIC can act on the messages transmitted towards higher centres as many convergent neurones have been shown to be at the origin of the spinothalamic and spinoreticular tracts in the rat [12,30]. These inhibitions, observed at the lumbar level, can be induced from widespread areas: the tail, contralateral hind paw, fore-

paws, ears, muzzle and from the viscerae. By contrast, innocuous stimuli were ineffective when applied to these same areas.

The responses of convergent neurones can therefore be inhibited by a variety of noxious stimuli:

— Pinch applied by forceps (painful when applied to the investigator) produced strong inhibitory effects; on the other hand, light tactile stimuli and moderate pressure were ineffective.

— Intraperitoneal injection of bradykinin, known to be a potent noxious stimulation in man [23], was equally effective.

— Noxious heat applied to the tail by means of hot water at temperatures above noxious threshold in man also produced these inhibitions. The degree of inhibition increased with the level of noxious heat; this form of stimulation is nociceptive in chronic rats [33].

— Electrical stimulation of the tail was equally effective using the same parameters of stimulation as those which produce vocalisation in the chronic rat. The threshold for the inhibition of these convergent neurones was 2.94 mA; the threshold for vocalisation observed in a previous study [6] was 2.14 mA. Thus one can presume that the activation of nociceptors is at the origin of the inhibitory effects we have observed. Furthermore, when applied to the excitatory receptive field, this stimulation evokes C fibre responses. Augmentation of the current above the threshold produces an increased degree of inhibition of these convergent neurones.

In all these cases, it seems that clear inhibitory effects require a certain degree of recruitment of peripheral nociceptors. For example, noxious radiant heat applied to a restricted area of the tail (a few mm^2) is almost ineffective whereas noxious heat applied to several cm^2 by means of hot water produces the powerful inhibitory effects we have described. The strength of the inhibitory effects of bradykinin could well be due to the degree of spatial summation induced by the stimuli as the intraperitoneal injection will presumably act on a large pool of visceral nociceptors. The efficacy of pinch, applied to a relatively large area of the periphery in these experiments, in inducing the inhibitions would seem again to relate to an effect of spatial summation. Furthermore the greater degree of inhibition produced by pinch applied to the tail or muzzle than that produced from the paws or ears may result from the higher degree of central representation of the former areas as demonstrated by their importance in the behaviour of rats. In this respect it is interesting to note that the receptive fields of dorsal horn neurones responding to noxious heat applied to the tail are large and bilateral [33].

In addition to the influence of spatial summation there seems to be a major role of temporal summation in these inhibitory effects, as demonstrated by the electrical stimulation of the tail. We have used trains of 500 msec as the conditioning stimulus: single shock or short trains do not produce clear inhibitory effects. In the chronic animal, whereas a 500 msec train produces stable vocalisation, shorter stimuli result in either no vocalisation or extremely variable effects. Thus there seems to be a correlation between

the stimulus duration and both the DNIC and the degree of nociception as represented by the vocalisation of the chronic rat.

The effective stimuli suggest that nociceptors, whether superficial, deep or visceral, are at the origin of the inhibitions we have described. Noxious heat induces an activation of cutaneous nociceptors [see refs. in 19], whereas intraperitoneal injection of bradykinin activates visceral nociceptors [22]. In the case of strong pinch or repetitive electrical stimulation of the tail, both cutaneous and deep nociceptors may be involved. In any case, these different kinds of stimulation are known to activate unmyelinated and fine myelinated fibres and also to induce a strong firing of the dorsal horn cells involved in pain transmission [see refs. in 2,13,18,36,43].

The type of effective stimuli might suggest that vasomotor reactions play a role in the inhibitions we observed. This is certainly not the case for the 3 following reasons: our experiments were carried out in anaesthetized animals in which such reactions are minimized; secondly, it is difficult to imagine an involvement of nociceptive induced vasomotor reactions in DNIC as the same stimuli produce reliable activations when applied to the excitatory receptive field of neurones; thirdly, more importantly, as will be presented in the following paper, the DNIC only affects convergent units; other dorsal horn neurones are not influenced; such a specificity indicates that a central neuronal mechanism subserves DNIC.

The types of activity inhibited include both the spontaneous and induced activities, these latter being evoked by either noxious or non-noxious stimuli. The spontaneous activity in these neurones was always inhibited as were the responses produced by light tactile stimulation. Bearing out this latter point is the inhibition of the Aα fibre response. But the responses to noxious stimulation (noxious heat, tonic pinch and C fibre response) were more powerfully inhibited by DNIC. In fact, differential effects were revealed by considering the responses to electrical stimulation of the peripheral excitatory receptive field of the cells. The inhibitions produced lesser effects against Aα fibre than C fibre responses evoked by electrical stimulation in terms of the percentage of cells inhibited; the degree of inhibition depended on the stimulation parameters. Whereas at supramaximal stimulation 98% of C fibre responses were inhibited by pinch applied to the tail (88% inhibition), only 45% of the A fibre responses were inhibited and to a much lesser extent than the C response. However, at threshold stimulation parameters for the Aα fibres, the proportion of responses inhibited was increased to 80% and the extent of the inhibition was at 80%. In the case of the responses to light mechanical stimulation, although the DNIC produced lesser inhibitions (69%) against this activity than against C fibre responses (88%), the effect was still considerable.

The order of efficacy in producing DNIC from the various parts of the body was similar in all these cases notwithstanding the evoked response they were tested against. For instance the tail and muzzle were always more effective than the hind paw and ears.

One interesting point we wish to emphasize is the constant observation

of inhibitory post-effects which outlasted the period of stimulation by up to 4 times. However, the duration of the post-effects differed depending on the activating stimulus applied to the receptive field of the neurone. For example, with the C fibre response the post-effect after tail pinch was 1.55 times the period of stimulation whereas the post-effect with the Aα fibre response was only 0.89 times the period of stimulation. Similarly although there was a variability in the presence or otherwise of post-effects with light tactile stimulation there was a tendency for these to be less than those obtained for similar levels of activity induced by radiant noxious heat. Thus after a period of inhibition the Aα fibre response recovers before the C fibre response.

This differential recovery is compatible both with a post- and a presynaptic inhibitory effect. In the former case, if the level of polarisation of the cell is increased, the grouped Aα EPSP may achieve the threshold level of depolarisation before the flatter C fibre EPSP [37]. On the other hand, since the C fibre responses were always inhibited whereas the Aα fibre response was at times not affected, a presynaptic mechanism may be implicated as well. Present results do not allow us to conclude as to the mechanisms of these inhibitions. However, the presence of post-effects lasting up to several minutes is compatible with the involvement of a neuromodulator rather than a classical neurotransmitter.

Although the results we have found seem to have been not previously reported in the literature, there are some electrophysiological observations which may be relevant to the system we have described. Thus, spontaneous and evoked activity in noxious units of the monkey dorsal horn can be reduced by pinch applied to the contralateral paw [38] and stimulation of the infraorbital nerve can inhibit some mechanical responses of spinothalamic cells [24].

In conclusion, the present results illustrate that the activity of convergent dorsal horn neurones is powerfully inhibited by the application of nociceptive stimuli to widespread segments of the body. Therefore, these controls are totally different from the segmental inhibitory effects induced on these neurones by the activation of large diameter fibres. This assertion is furthermore supported by the following paper which demonstrates the involvement of supraspinal structures in DNIC.

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