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STUDIES OF BRAIN STRUCTURES INVOLVED IN DIFFUSE NOXIOUS INHIBITORY CONTROLS IN THE RAT: THE ROSTRAL VENTROMEDIAL MEDULLA

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SUMMARY

1. Previous electrophysiological, pharmacological and anatomical evidence had suggested a possible participation of rostral ventromedial medulla (RVM) in the supraspinal part of the loop underlying diffuse noxious inhibitory controls (DNICs). In order to test this hypothesis, two experimental series were performed during which DNICs were compared in control sham-operated rats and rats with lesions of the RVM and of an adjacent candidate for such a role, the nucleus gigantocellularis reticularis (Gi).

2. In the first experimental series, lesions were induced, in anaesthetized animals, by injections of quinolinic acid (0·3–0·8 μl of a 360 nmol/μl solution) into the RVM or Gi. In the control animals ($n = 10$), the vehicle alone (artificial cerebrospinal fluid) was injected. Histological lesion reconstructions were performed after each electrophysiological experiment. Three groups of animals were considered: in the first group ($n = 5$), the lesion was centred on the RVM, including the two caudal thirds of nucleus raphe magnus (NRM) and adjacent reticular areas; in the second group ($n = 5$), the lesions extended more rostrally and involved the rostral pole of NRM; in the third group ($n = 5$), the lesion extended more laterally and dorsally and included nucleus reticularis gigantocellularis pars alpha (GiA), nucleus reticularis paragigantocellularis lateralis (LPGi) and the Gi. In each case, all the neuronal cell bodies within the lesioned area were destroyed.

3. In the second experimental series, electrolytic lesions of the total rostrocaudal extent of the NRM ($n = 5$) were induced, in anaesthetized animals, by passing cathodal current (5 mA, 8 s). In the control animals ($n = 5$), the electrode was lowered but current was not applied.

4. One week after lesioning, the animals were anaesthetized, paralysed, artificially ventilated and recordings were made from convergent neurones in trigeminal nucleus caudalis. These neurones gave responses due to activation of A and C fibres when percutaneous electrical stimuli were applied to their receptive fields. DNICs were triggered by immersion of each paw in a 50 °C water-bath. Both the general properties of the convergent neurones and the inhibitions of the C fibre-evoked responses produced by these heterotopic noxious stimuli were compared in the different groups of animals.

5. The sizes of receptive field, spontaneous activities, thresholds for C fibre-evoked

responses and responses to C fibre activation were not different in the control and lesioned animals. The percentage inhibitions of the C fibre-evoked responses both during and in the 44 s following the conditioning periods were also very similar in the different groups of animals.

6. These results indicate that neither the RVM nor Gi are involved, at least directly, in DNIC. Taken together with the results of previous studies using midbrain lesions it is concluded that the midbrain and medullary structures which are known to be involved in the modulation of pain, do not play a direct role in DNIC. The possible modulation of DNIC by these structures and the theoretical implications of these results are discussed.

INTRODUCTION

In the rat, the rostral ventromedial medulla (RVM) includes the nucleus raphe magnus (NRM) and the adjacent reticular area, namely the nucleus reticularis gigantocellularis pars alpha (GiA) and the nucleus paragigantocellularis lateralis (LPGi). The RVM has been extensively studied in the rat and in other species and is thought to play a key role in the modulation of pain. Electrical stimulation of these structures produces both antinociception and inhibition of the responses of spinal and trigeminal neurones involved in the transmission of nociceptive information. The RVM sends massive projections to the spinal cord via the dorsolateral funiculus (DLF); this region contains the somata of 5-HT neurones which terminate in spinal dorsal horn and trigeminal nucleus caudalis, and the participation of serotoninergic mechanisms in modulating pain is well established (for reviews see Besson & Chaouch, 1987; Le Bars, 1987; Willis, 1988; Fields & Basbaum, 1989; Fields, Heinricher & Mason, 1991).

On the basis of these behavioural, neurophysiological, pharmacological and anatomical data, the RVM is considered to be a major relay in the postulated endogenous analgesic system (see references in Fields & Basbaum, 1989). Since the natural means of triggering this system were unclear, it has been proposed that such structures might be involved in diffuse noxious inhibitory controls (DNICs) (see references in Le Bars & Villanueva, 1988). DNICs are very powerful descending inhibitory controls, which act specifically on convergent neurones in the spinal dorsal horn and trigeminal caudalis nucleus, and which are triggered specifically by heterotopic noxious stimuli. Several lines of evidence suggested the involvement of the RVM in these controls (see references in Le Bars & Villanueva, 1988): (1) DNICs are subserved by a loop which involves supraspinal structures, the descending part of which is confined to the DLF, (2) several analogies exist between the effects of DNIC and of electrical stimulations of NRM, (3) the participation of serotoninergic mechanisms in DNIC is very likely since these controls are reduced in *p*-chlorophenylalanine-pretreated animals or after the administration of 5-HT receptor antagonists, (4) preliminary results indicated that DNICs were reduced immediately following acute electrolytic lesioning of NRM and adjacent reticular areas (Dickenson, Le Bars & Besson, 1980). In addition to these lines of evidence which suggest a role for the RVM in the DNIC circuitry, it is worth pointing out that the properties of some neurones recorded in this area also suggested such a role. Three classes of neurones whose activity is correlated with noxious stimuli have been

described in this region (see references in Fields *et al.* 1991). The activity of one group of these cells (called 'on-cells') is enhanced during noxious heating and just prior to flicking of the tail; this response can be blocked by low doses of morphine given systemically or microinjected within the periaqueductal grey (PAG). These data suggested a possible neuronal link which would fit with the requirements for DNIC itself and for its blockade by low systemic doses of morphine or by morphine microinjected within the PAG or the third ventricle (see references in Bouhassira, Villanueva & Le Bars, 1988).

On the basis of these data, the participation of the RVM in the circuitry underlying DNIC appeared reasonable. In order to test this hypothesis directly, we aimed to study the effects of lesions of the RVM induced by quinolinic acid, on DNIC acting on trigeminal convergent neurones. We also studied the effects of lesions of the more rostral portion of NRM and of the more dorsally situated nucleus reticularis gigantocellularis (Gi), since this structure has also been implicated in systems which modulate pain (see Discussion). We also investigated in the present study the effects of chronic electrolytically induced lesions of the RVM.

Unexpectedly, as described herein, we found that DNICs were not modified by any chronic lesions of these regions in the brainstem.

METHODS

The experiments were carried out on male Sprague–Dawley rats weighing 200–250 g and involved three stages: first, the lesions were induced either by quinolinic acid injections or electrolytically; then 1 week later, electrophysiological experiments were performed; finally, the brain lesions were reconstructed after examination of serial histological sections.

Quinolinic acid-induced lesions (first series of experiments)

The animals were anaesthetized with chloral hydrate (450 mg/kg I.P.), mounted in a stereotaxic head holder and a hole made in the skull with a drill in order to stereotaxically place a cannula into the NRM or adjacent reticular area, using the co-ordinates defined by Paxinos & Watson (1986). Quinolinic acid was injected slowly over a 3 min period via a 1 μl Hamilton syringe, and the needle was left *in situ* for 5 min before withdrawal, to allow time for diffusion. During the surgical procedure, the level of anaesthesia was assessed by the absence of corneal reflexes and of significant modifications of the heart rate.

Lesions of the RVM were made by injecting 0·3–0·8 μl of a 360 nmol/μl quinolinic acid solution (co-ordinates: 10 mm caudal to bregma; 0 mm lateral to the midline; 0·2 mm below the interaural axis). Figure 1 shows an example of a lesion produced by such a procedure. Identical concentrations and volumes were injected more rostrally (co-ordinates: 11·5 mm caudal to bregma; 0 mm lateral to the midline; 0·2 mm below the interaural axis) or more laterally and dorsally (co-ordinates: 10·5 mm caudal to bregma; 1·2 mm lateral to the midline; 0·5 mm above the interaural axis) to produce lesions of the rostral pole of NRM and of Gi areas respectively.

In 60% of cases, the animals exhibited twitching over a 1–2 h period, but convulsions were not observed during the recovery from anaesthesia. After recovery, no obvious change in gross behaviour was observed; a slight loss of body weight was seen in the following 2 or 3 days, but this was corrected within a week.

Control animals underwent identical microinjection procedures except that the vehicle alone (artificial cerebrospinal fluid) was microinjected; we will refer to these animals as '(first) control group'. One week after the injection, the animals were prepared for electrophysiological experiments.

Electrolytically induced lesions (second series of experiments)

The surgical procedure and anaesthesia were identical to those used for microinjections of neurotoxins except that a stainless-steel electrode, insulated except for 0·5 mm at the tip, was

lowered into the NRM (co-ordinates: 11 mm caudal to bregma; 0 mm lateral to the midline; 0·2 mm below the interaural axis). Lesions were induced by passing cathodal current (5 mA; 8 s). In control sham-operated animals, the electrode was lowered but current was not passed; we will refer to these animals as '(second) control group'.

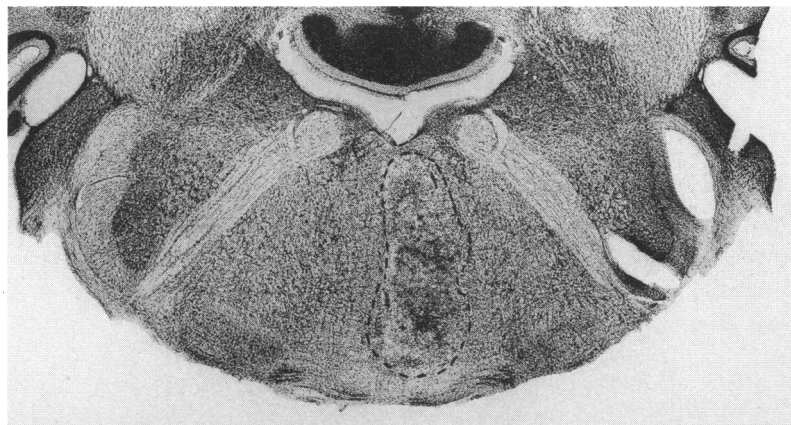

Fig. 1. Photomicrograph showing an example of an RVM lesion elicited by an injection of quinolinic acid (0·4 μl of a 360 nmol/μl solution) 1 week earlier. Within the lesion (delineated by the dashed line), only small glial cells and degenerated or degenerating neurones can be observed.

One week after the lesioning procedure, these animals were prepared for electrophysiology as with the animals with quinolinic acid-induced lesions (see below). However, in order to study the effects of NRM lesions on the arterial blood pressure, a catheter was placed in the carotid artery to perform arterial blood pressure measurements during the electrophysiological experiments.

Animal preparation for electrophysiology

After an intraperitoneal injection of 100 μg atropine sulphate, the animals were deeply anaesthetized with 2·5% halothane in a nitrous oxide–oxygen mixture (2:1). A tracheal cannula was inserted, a jugular vein cannulated, and the animals were paralysed by intravenous injection of gallamine triethiodide (Flaxedil) and artificially ventilated. The rate (50–55 strokes/min) and volume of ventilation were adjusted to maintain a normal acid–base equilibrium as assessed using a capnometer (Capnomac II, Datex instruments, Helsinki, Finland) which continuously measured end-tidal CO_2, N_2O and halothane levels throughout the experiments. The measurements of CO_2, N_2O and halothane are performed by infrared absorption and O_2 levels with a fast paramagnetic analyser. Such parameters are digitally displayed, and each of them is under control of alarms. Heart rate was monitored continuously and core temperature maintained at $37 \pm 0{\cdot}5$ °C (mean ± S.E.M.) by means of a homeothermic blanket system.

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. After surgery, the level of halothane was reduced to 0·5%.

Under this anaesthetic regime, in each animal tested in the present study, heart rate (mean: 396 ± 5 beats/min) was not significantly modified (less than 5 beats/min) during the application of either electrical or thermal stimuli (see below).

Recordings

Unitary extracellular recordings were made from convergent neurones in trigeminal nucleus caudalis with glass micropipettes (10–15 MΩ) filled with a mixture of 5% NaCl and Pontamine Sky Blue dye. Penetrations were made in each animal on the left and right side, successively,

1·5–2·0 mm caudal to the obex and 1·5–2·5 mm lateral to the midline. Stability was achieved by placing a glass frame held in position with a micromanipulator and 2% Ringer–agar gel, over the surface of the medulla. Neurones were classified as convergent on the basis of their responses to both mechanical and transcutaneous electrical stimulation of their receptive fields. They all

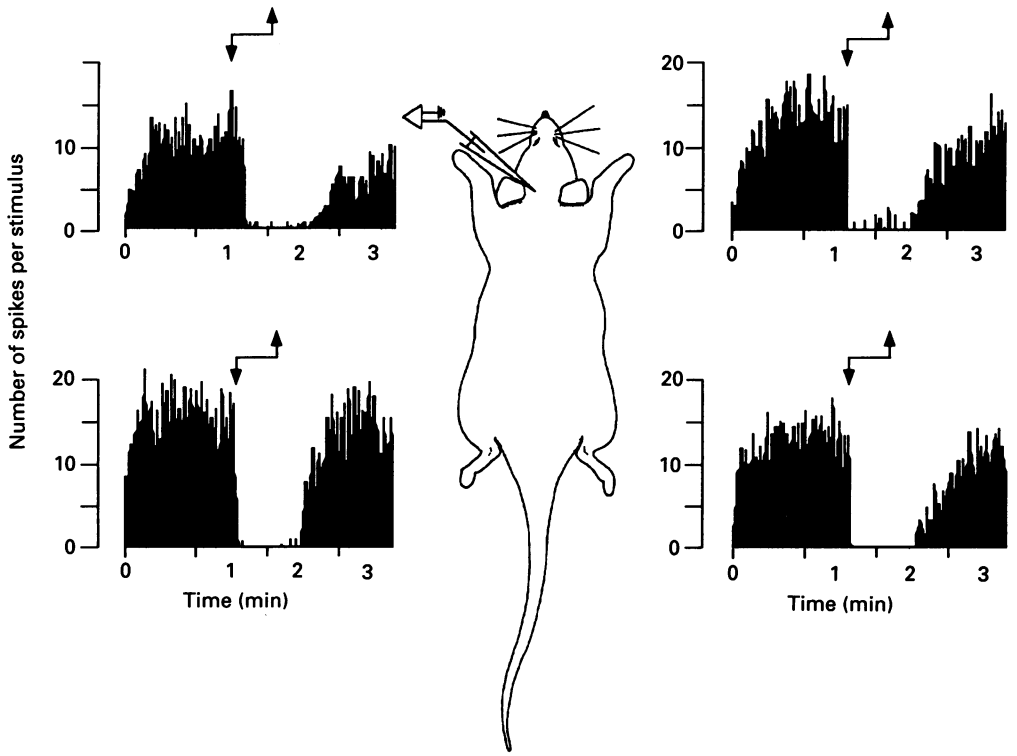

Fig. 2. Example of inhibitory effects observed in a control animal. A convergent neurone with a receptive field located ipsilaterally on the muzzle was recorded in the left trigeminal nucleus caudalis. C fibre responses evoked by percutaneous electrical stimulation of the centre of the receptive field were conditioned by the immersion of each paw successively in a 50 °C water-bath. For each sequence, the histograms represent the temporal evolution of the C fibre responses that were conditioned by immersion of a paw between the 65th and the 90th stimulus (arrows). Large inhibitions involving almost complete blockade of the responses and long-lasting post-effects were observed regardless of whether the left or right forepaw (top left and right respectively) or the left or right hindpaw (bottom left and right respectively) was stimulated.

responded to innocuous and noxious mechanical stimuli and gave responses with latencies corresponding to A and C fibre inputs. Once a cell had been identified, the extent of its receptive field was determined. Only cells presenting no serious alteration in spike amplitude or waveform during the complete experimental procedure were considered.

Experimental design (*see Fig. 2*)

The experimental procedure consisted of sequences of 120 suprathreshold electrical stimuli (single square-wave pulses of 2 ms duration) applied repetitively (0·67 Hz) to the excitatory

receptive field; under our experimental conditions, these stimuli give rise to stable and reproducible responses. During such sequence, a paw was immersed in a 50 °C water-bath from the 65th to the 90th stimulus presentation (i.e., for 37 s). For each cell, sequences were performed at 10 min intervals for each of the four paws, in random order.

A multichannel analyser (Tracor TN 1710) was used on-line to build post-stimulus histograms (PSHs). For each sequence, two types of analysis were performed: the first aimed at the evaluation of dynamic phenomena such as habituation or more usually 'wind up'; the second was devoted to the study of DNIC. In both cases the PSH built from the 50th to the 65th stimulations was used as a control for the sequence.

In order to appreciate dynamic phenomena, the mean responses observed for each successive period of five stimulations were calculated during the first fifty responses and expressed in terms of the percentage of the control response for the sequence. Regarding the inhibitory phenomena, the PSH built from the 75th to 90th responses was used to assess the effects of the conditioning stimulus, the PSHs built from the 91st to the 105th responses and 106th to the 120th responses allowed after-effects to be observed during two successive 22 s periods after the cessation of the conditioning stimulus. The PSHs were analysed to distinguish responses due to A and C fibre inputs by their latencies.

Inhibitions were expressed as percentage decreases in the number of spikes for both the A and C fibre-evoked responses with reference to the control PSH. The temporal evolution of individual responses was also visualized on a two-channel chart recorder on which the bins were set to give the cumulative individual responses due to either A or C fibre inputs. Only the C fibre component will be considered in the present study because it was not always easy to differentiate between $A\alpha\beta$ and $A\delta$ responses; in addition, as previously reported, DNICs have been found to be less potent against these latter responses because of the supramaximal nature of the electrical stimuli applied to the peripheral field.

Analysis of variance (ANOVA) was used for statistical purposes. *Post hoc* tests were not employed because no statistically significant differences were found in these studies.

Histological controls

At the conclusion of the experiments, the recording sites were marked by electrophoretic deposition of Pontamine Sky Blue. The animal was deeply anaesthetized with 3% halothane, and the brain was perfused through the heart with 0·9% NaCl followed by 10% formaldehyde. The brain was removed, frozen, cut in serial 100 μm-thick sections, and Nissl stained with Cresyl Violet or Carmin. Brainstem lesions were reconstructed from camera lucida drawings of serial sections.

RESULTS

In the first experimental series, the electrophysiological results in the animals with quinolinic acid-induced lesions ($n = 13$) were very similar to those observed in the (first) controls ($n = 10$). On the basis of the lesion reconstructions three groups of animals have been considered. In the first ($n = 5$), the lesions were centred on the RVM, including the caudal pole of the NRM and adjacent reticular areas; in the second group ($n = 5$), the lesions extended more rostrally and involved the most rostral part of the NRM; in the third group ($n = 5$), the lesions were more laterally and dorsally situated to include the GiA–LPGi–Gi area and in some cases, the NRM. In the second experimental series, the NRM of the group of animals with electrolytic lesions ($n = 5$) was destroyed in all its rostrocaudal extent and the electrophysiological results were not significantly different to those observed in the (second) control sham-operated animals ($n = 5$). The effects of these lesions on the general properties of the recorded units and on DNIC acting on these neurones, are detailed in the following sections.

General properties of the neurones

In the present study, the convergent neurones were located within the magnocellular layer of nucleus caudalis as depicted by the electrophoresing dye at the end of the experiments. Their excitatory receptive fields were found on the

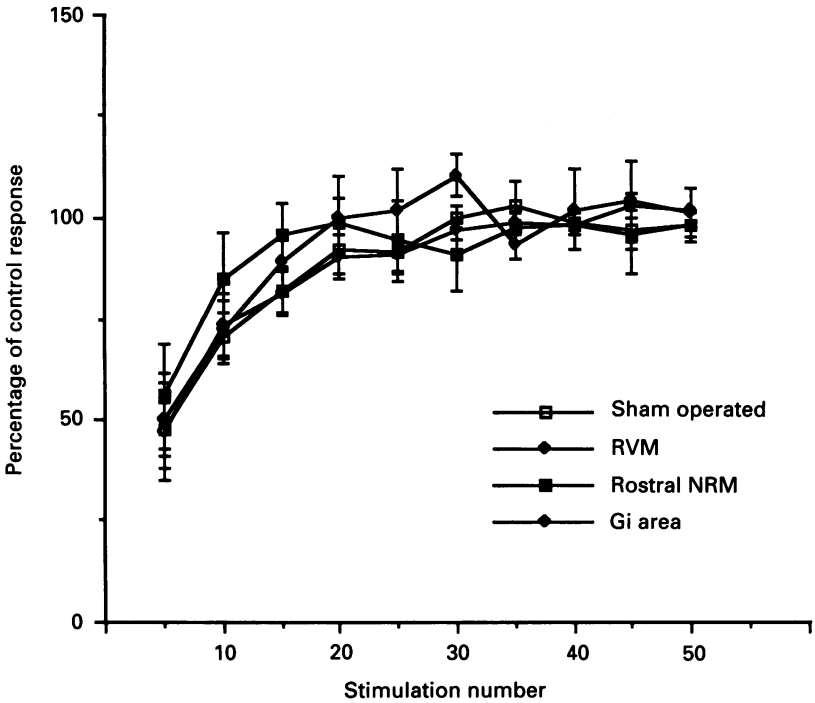

Fig. 3. Diagram illustrating the dynamic properties of the successive responses to the fifty first stimulations. The mean C fibre-evoked responses observed for each successive period of five stimulations are expressed in terms of the percentages of the control response (responses to the 50th and 65th stimulations of each experimental sequence (see Methods)). Note that a 'wind-up' phenomenon is present during the twenty-five first responses in the different groups of animals: sham-operated animals, animals with lesions of the rostral ventromedial medulla (RVM), lesions of the rostral pole of nucleus raphe magnus (rostral NRM) or lesions of the giganto-paragigantocellular reticular nuclei (Gi area). No intergroup differences were revealed by variance analysis.

ipsilateral part of the muzzle. The cells could be activated by both innocuous (hair movements, stroking, light pressure) and noxious (pinch) mechanical stimuli applied to their receptive fields. The excitatory receptive fields and spontaneous activities of the neurones recorded in the different groups of lesioned animals were qualitatively similar to those observed in the control animals.

Effects of quinolinic acid-induced lesions

By applying 2 ms percutaneous electrical stimuli to the centres of their excitatory receptive fields, responses due to activation of peripheral A and C fibres were

observed. In the (first) control animals, the mean threshold for obtaining C fibre-evoked responses was $5{\cdot}8 \pm 0{\cdot}6$ mA. This threshold was not significantly different in the lesioned animals: $5{\cdot}3 \pm 0{\cdot}9$, $4{\cdot}7 \pm 0{\cdot}6$ and $4{\cdot}8 \pm 0{\cdot}9$ mA after RVM, rostral NRM and giganto-paragigantocellular reticular nuclei (GiA–LPGi–Gi) lesions, respectively (F_{3-78} (3–78 degrees of freedom) = 0·44; n.s.).

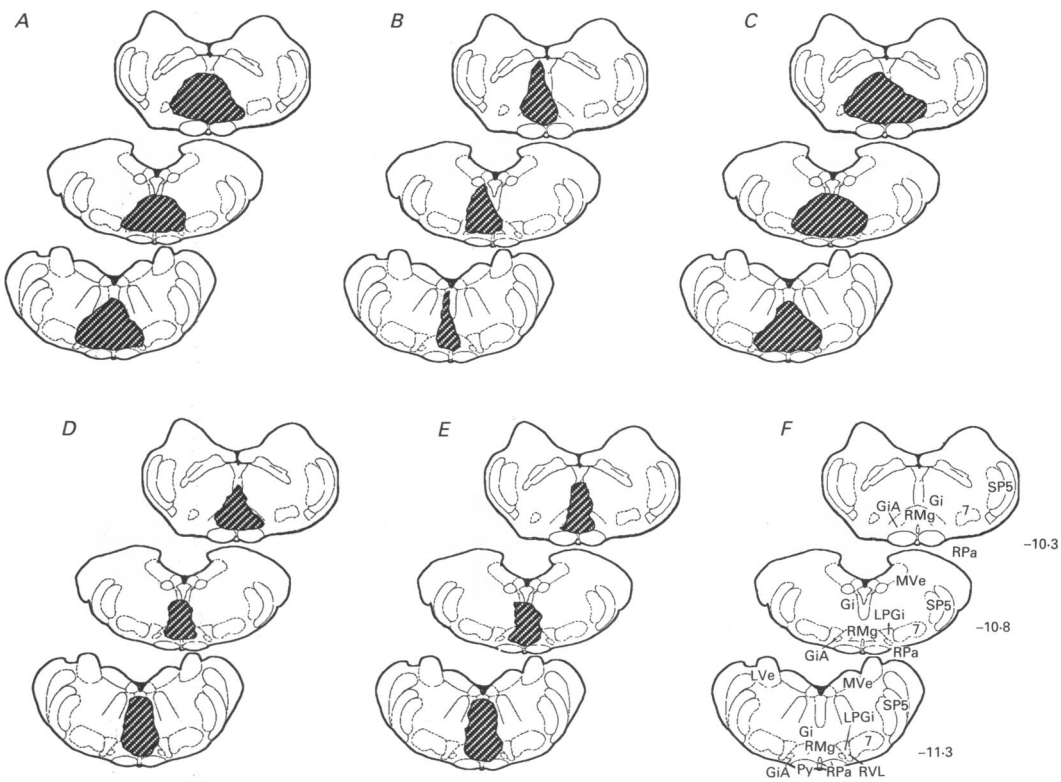

Fig. 4. Schematic representation (*A–E*), after camera lucida reconstruction, of the lesions (hatched area) of the RVM. The drawings are simplified from Paxinos & Watson (1986). Key for medullary structures indicated by abbreviations in *F*: 7, facial nucleus; Gi, nucleus reticularis gigantocellularis; GiA, nucleus reticularis gigantocellularis pars alpha; LPGi, nucleus reticularis paragigantocellularis lateralis; LVe, lateral vestibular nucleus; MVe, medial vestibular nucleus; Py, pyramidal tract; RMg, nucleus raphe magnus; RPa, nucleus raphe palidus; RVL, rostroventral lateral reticular nucleus; SP5, spinal trigeminal nucleus. Levels indicated are caudal to bregma.

After suprathreshold stimulation (2 times threshold) in the (first) control animals, a mean of $11{\cdot}2 \pm 1{\cdot}3$ C fibre latency spikes were evoked per stimulus; not significantly different responses ($F_{3-78} = 1{\cdot}53$; n.s.) were recorded in the lesioned animals ($13{\cdot}8 \pm 2{\cdot}0$; $15{\cdot}0 \pm 2{\cdot}5$; $9{\cdot}9 \pm 1{\cdot}3$ C fibre-evoked spikes after RVM, rostral NRM and GiA–LPGi–Gi lesions, respectively).

The dynamic properties of the responses to the successive stimulations are shown in Fig. 3. On average, a 'wind-up' phenomenon was observed during the twenty-five

first responses in all groups of animals. No inter-group differences were revealed by variance analysis.

Effects of electrolytically induced lesions

The mean threshold for obtaining C fibre-evoked responses was not significantly different ($F_{1-23} = 0{\cdot}05$; n.s.) between lesioned and (second) control animals ($5{\cdot}9 \pm 0{\cdot}9$

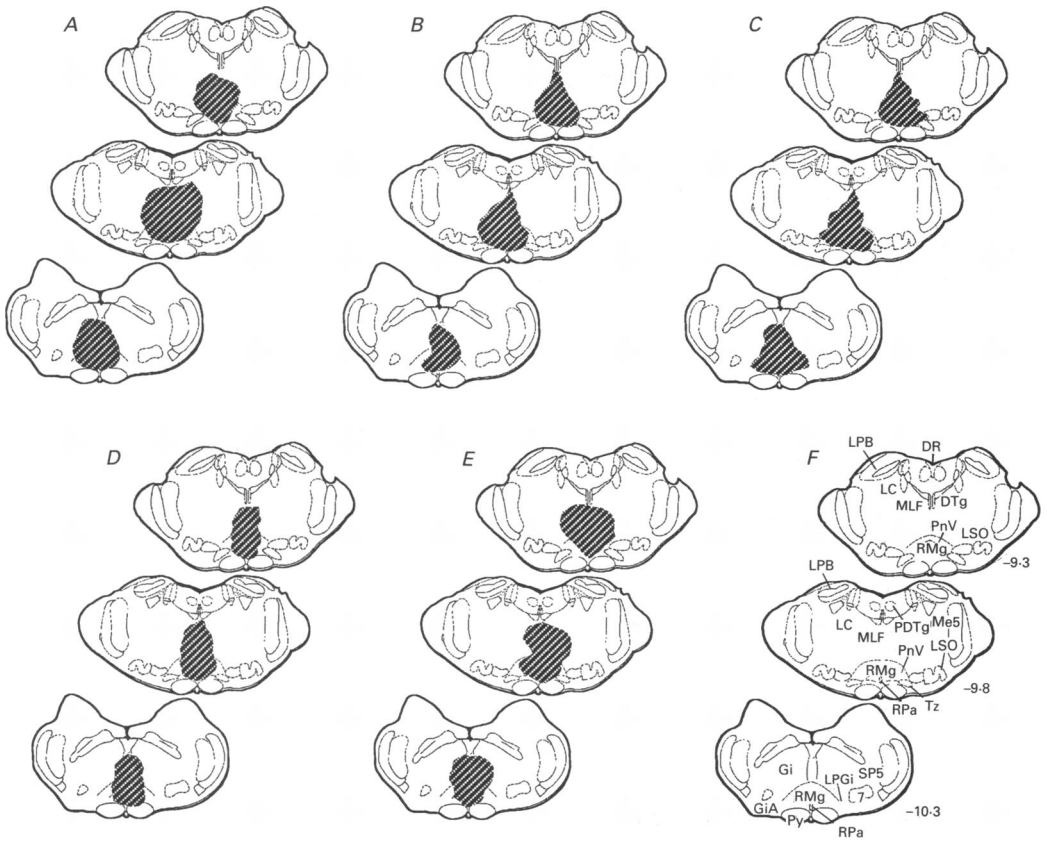

Fig. 5. Schematic representation (*A–E*) after camera lucida reconstructions, of the lesions (hatched area) of rostral NRM area. Drawings are simplified from Paxinos & Watson (1986). Key for medullary structures indicated by abbreviations in *F* as in Fig. 4 plus DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus; LC, locus coeruleus; LPB, lateral parabrachial nucleus; LSO, lateral superior olive; Me5, trigeminal mesencephalic nucleus; MLF, medial longitudinal fasciculus; PDTg, posterodorsal tegmental nucleus; PnV, ventral pontine reticular nucleus; Tz, nucleus of the trapezoid body.

and $6{\cdot}1 \pm 0{\cdot}3$ mA, respectively). After suprathreshold stimulation, a mean of $9{\cdot}8 \pm 1{\cdot}5$ C fibre latency spikes were evoked in control animals; not significantly different responses ($F_{1-23} = 0{\cdot}3$; n.s.) were recorded in the lesioned animals ($10{\cdot}7 \pm 0{\cdot}9$).

Effects of quinolinic acid-induced lesions of the RVM and rostral NRM on DNIC

All the convergent neurones could be inhibited by heterotopic noxious stimuli, i.e. immersion of any of the paws in a 50 °C water-bath. Figure 2 shows a typical individual example recorded in a control rat, i.e. an animal that received a

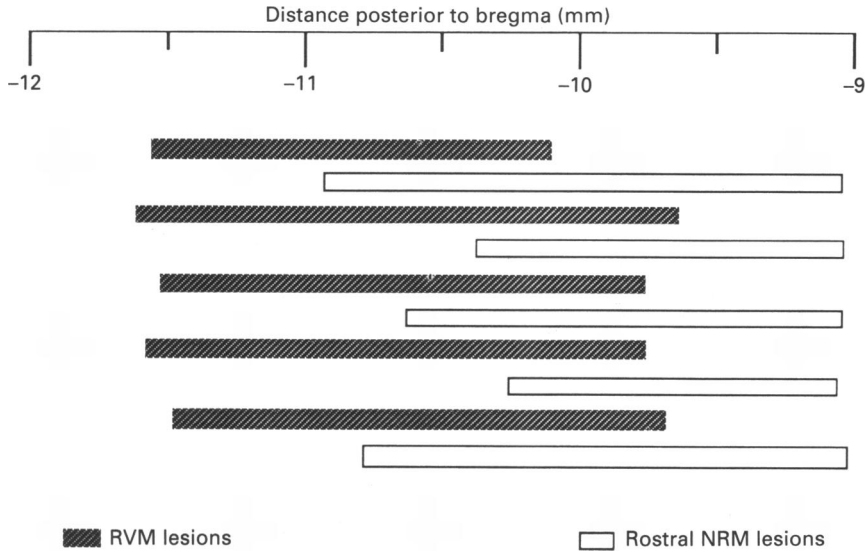

Fig. 6. Schematic representation of the total rostrocaudal extent of each selected lesion of the NRM area. Anteroposterior co-ordinates are adapted from Paxinos & Watson, (1986) and expressed in mm posterior to bregma. After camera lucida reconstructions, animals were divided into two groups: in the first (hatched bars) the lesion included, within the RVM, approximately the caudal two thirds of the NRM (total extension of NRM: −9·16 to −11·6 mm from bregma); in the second group (open bars) the rostral two-thirds of NRM were destroyed. Note the overlap between the two groups.

microinjection of vehicle within the brain and did not have any obvious brain damage. Note that all four noxious thermal stimuli elicited strong inhibitions of the C fibre-evoked responses, which were followed by marked after-effects. The mean inhibitions in the control animals when the left and right forepaws were stimulated, were 85·0±3·7 and 92·8±1·8%, respectively, while left and right hindpaws stimulation produced inhibitions of 94·6±1·1 and 96·7±0·7%, respectively. After-effects of around 50 and 30%, respectively, were observed during the 0–22 and 22–44 s periods after the end of the conditioning periods.

Figures 4 and 5 show reconstructions taken from camera lucida drawings of all the lesions included in the group designated as RVM (Fig. 4) and of all the lesions included in the group designated as the rostral pole of the NRM (Fig. 5). In the first group the lesions were centred on the NRM (caudal two-thirds) and in all cases, the lesions involved the major part, and in some cases (Fig. 4*A* and *C*) all, of the reticular formation adjacent to the NRM, namely the GiA and LPGi. In the second group, the lesions involved the rostral two-thirds of the NRM and the ventral pontine reticular

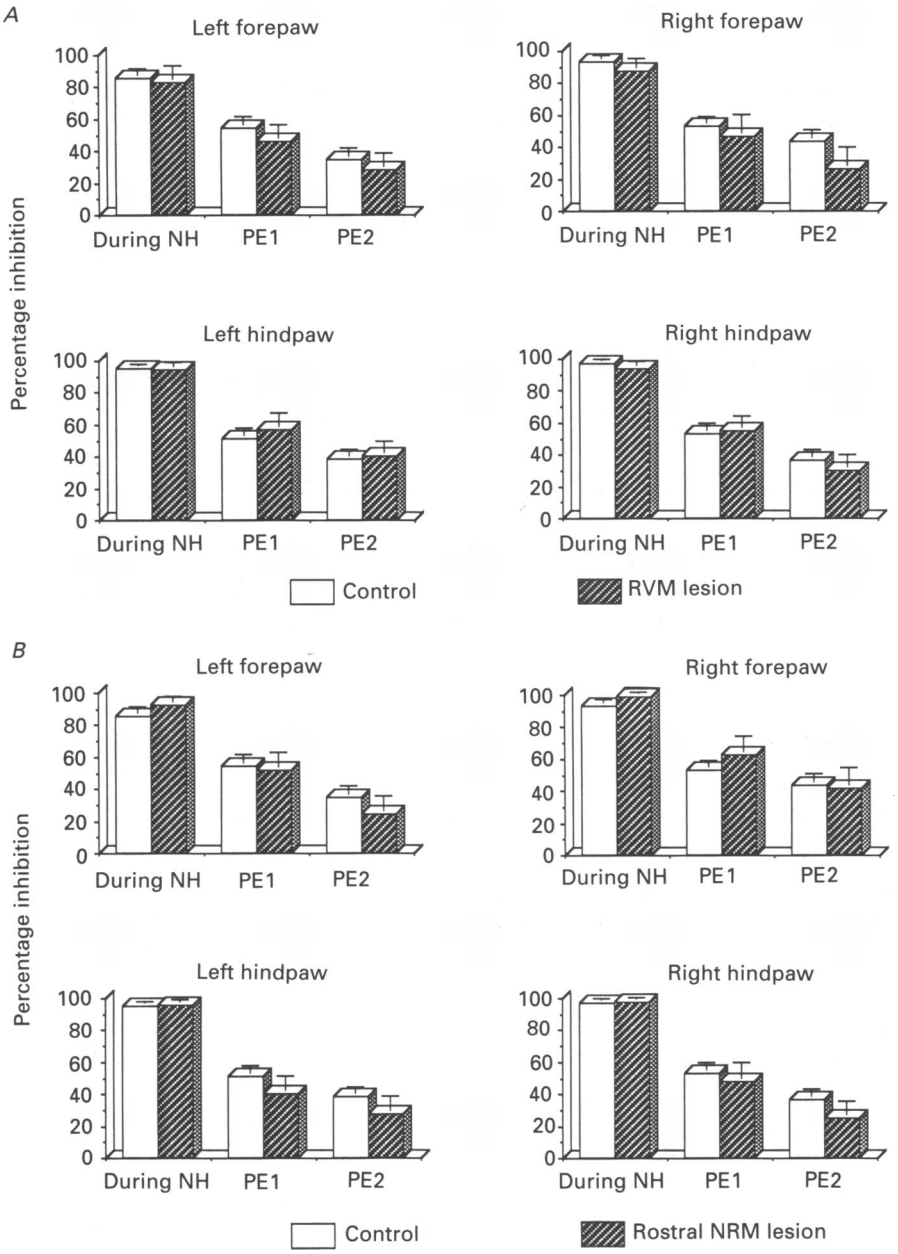

Fig. 7. *A*, effects of RVM lesion (shown in Fig. 4) on DNIC: histograms represent the percentage inhibitions (ordinates) observed during ('during NH') the immersion of a paw in a 50 °C water-bath and the post-effects observed during the 0–22 s (PE1) and 22–44 s (PE2) periods after the end of such conditioning. Inhibitions observed in the controls (□) and lesioned animals (▨) were not significantly different. *B*, effects of rostral NRM lesion (shown in Fig. 5) on DNIC (presentation as in Fig. 7*A*). The percentage inhibitions of the C fibre-evoked responses (ordinate) observed during and after the immersion of each paw in a 50 °C water-bath were not significantly different in controls (□) than in animals with lesions of the rostral NRM area (▨).

nucleus in the adjacent reticular area (Fig. 5). In both groups, the lesioned area contained only small glial cells and degenerating or degenerated neurones. Figure 6 shows a schematic representation of the rostrocaudal extents of the lesions and the overlap between the two groups.

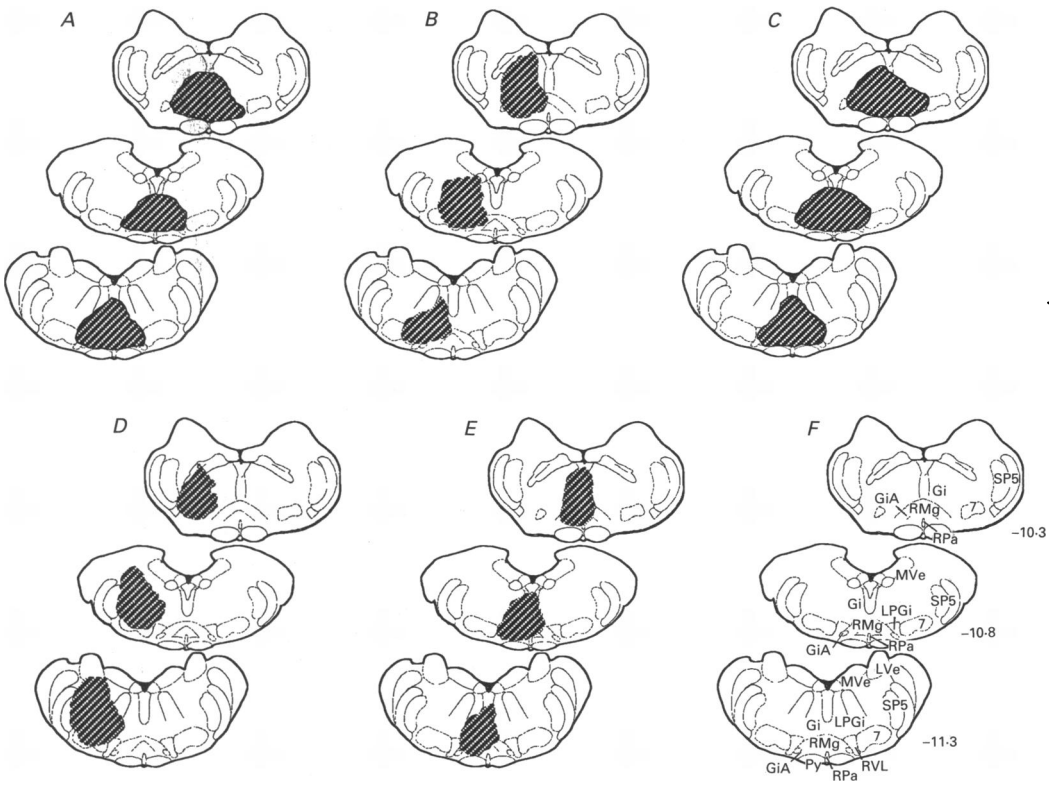

Fig. 8. Schematic representation (*A–E*) after camera lucida reconstructions of the lesion of giganto-paragigantocellular reticular nuclei. Presentation and abbreviations in *F* as in Figs 4 and 5. Note that lesions *A* and *C* are also included in the caudal NRM lesions group in Fig. 4*A* and *C*.

Fourteen and eleven neurones were recorded in the RVM and rostral NRM lesioned groups respectively (at least two in each animal); they were all subject to DNIC and, as shown by the cumulative results in Fig. 7, there were no significant differences in the DNIC-mediated inhibitions between the (first) control (41 neurones recorded) and lesioned animals ($F_{2-65} = 0{\cdot}11$; n.s.). Whether in a control or a lesioned animal, the percentage inhibitions of the C fibre-evoked responses elicited by immersion of a paw in a 50 °C water-bath was always $> 85\%$. The after-effects during the 44 s following the conditioning period were also similar in the different groups.

Effects of quinolinic acid-induced lesions of the giganto-paragigantocellular reticular nuclei on DNIC

The extent of all the lesions of these reticular nuclei are shown in Fig. 8; the lesioned areas contained only small glial cells without neuronal cell bodies. The

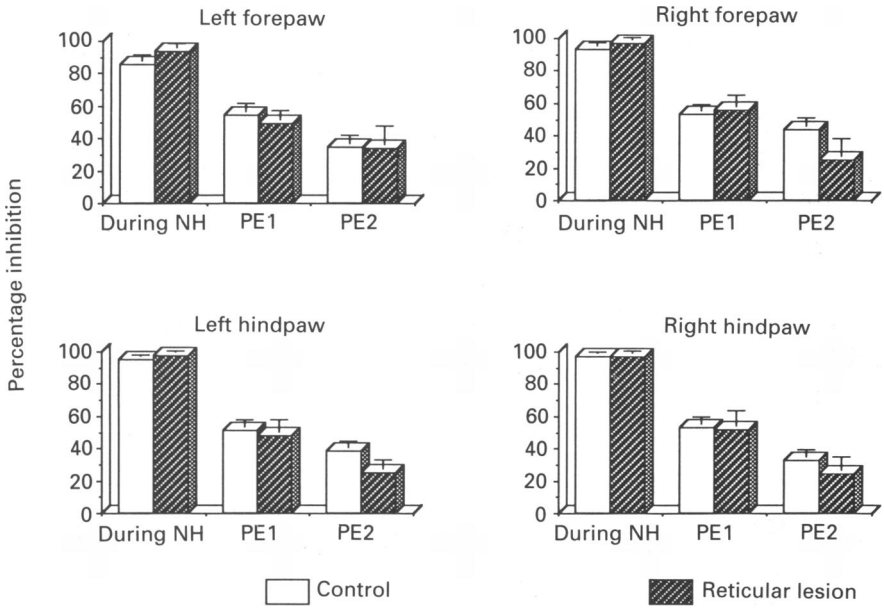

Fig. 9. Inhibitory effects observed in an animal with lesions (shown in Fig. 8) of the giganto-paragigantocellular reticular nuclei (presentation as in Fig. 7*A*). The percentage inhibitions of the C fibre-evoked responses of convergent neurones observed during and within the 44 s following the immersion of each paw in a 50 °C water-bath are presented on the ordinates. There were no significant differences between control (□) and lesioned animals (■).

lesions involved the GiA and LPGi either unilaterally (Fig. 8*B*, *D* and *E*) or bilaterally (Fig. 8*A* and *C*); in each individual case, a large part of the Gi was also destroyed.

Thirteen convergent neurones were recorded in these animals and the results are presented in Fig. 9. As there were no significant differences between neurones recorded ipsilaterally or contralaterally to the lesion in the unilaterally lesioned animals (Fig. 8*B*, *D* and *E*), the results obtained for each side in these animals were pooled. Figure 9 shows that there were no significant differences between the two groups either during or after the application of the noxious conditioning stimuli ($F_{1-53} = 1{\cdot}70$; n.s.). Moreover, in the two cases in which the lesion was bilateral including the GiA–LPGi–Gi area bilaterally and the caudal NRM (Fig. 8*A* and *C*), the electrophysiological results were not significantly different; indeed, in these two cases, the percentage inhibition of the C fibre-evoked responses was always over 90% during the immersion of any of the paws.

In a remaining group of animals which were not categorized because the lesions were smaller (15 rats; not shown), the electrophysiological results (general properties of neurones ($n = 35$) and DNIC; not shown) were not different from those presented above.

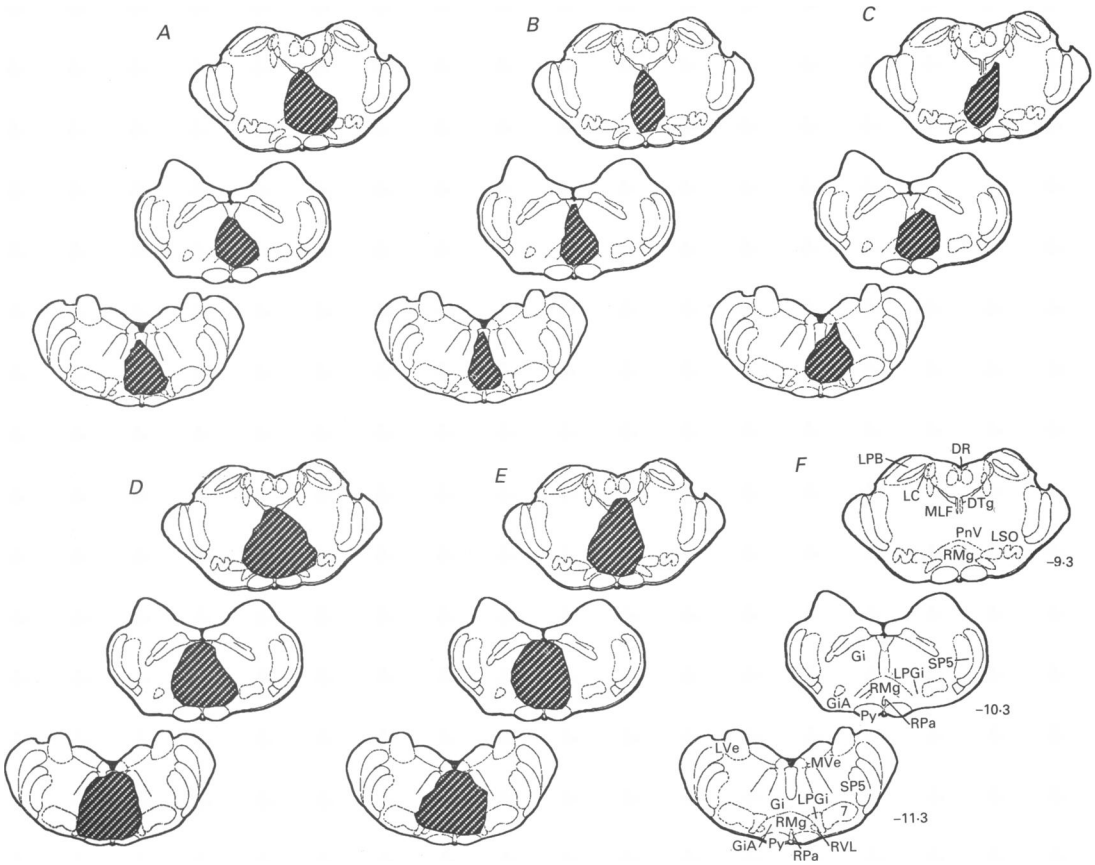

Fig. 10. Schematic representation (*A–E*) after camera lucida reconstructions of the electrolytic lesions of the NRM. Presentation and abbreviations in *F* as in Figs 4 and 5.

Effects of electrolytic lesions of the NRM on DNIC

Figure 10 shows a reconstruction taken from camera lucida drawings of all the lesions in this experimental group. In each case the lesion included the NRM in all its rostrocaudal extent and in some cases (Fig. 10*D* and *E*) the reticular formation adjacent to the NRM.

Thirteen convergent neurones were recorded in the NRM-lesioned animals; they were all subject to DNIC and, as shown by the cumulative results in Fig. 11, there were no significant differences in the DNIC-mediated inhibitions between the (second) control animals (11 neurones recorded) and lesioned animals ($F_{1-23} = 0{\cdot}3$; n.s.).

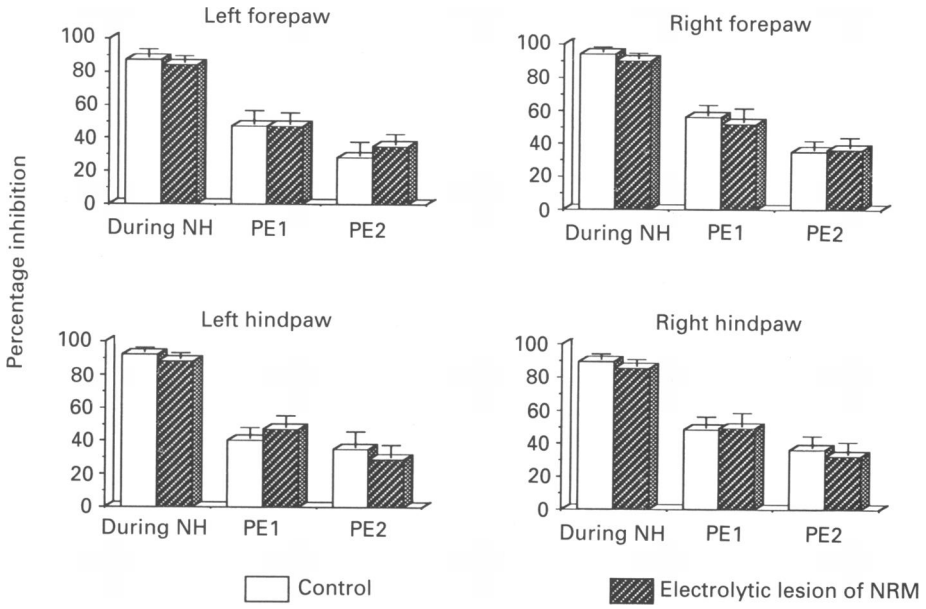

Fig. 11. Inhibitory effects observed in an animal with electrolytic lesions (shown in Fig. 10) of the NRM (presentation as in Fig. 7*A*). The percentage inhibitions of the C fibre-evoked responses of convergent neurones observed during and within the 44 s following the immersion of each paw in a 50 °C water-bath are presented on the ordinates. There were no significant differences between control (□) and lesioned animals (■).

The arterial blood pressure measurements performed in these animals indicated that the mean arterial blood pressure was not significantly different between lesioned (98·4 ± 2·6 mmHg) and (second) control animals (102·6 ± 3·7 mmHg).

DISCUSSION

The present study constitutes the third in a series in which we are trying to determine which supraspinal structures are involved in the circuitry which subserves DNIC in the rat. In a very similar way to our previous works in which midbrain structures such as periaqueductal grey, cuneiformis nucleus, parabrachial area, locus coeruleus/subcoeruleus were lesioned, (Bouhassira, Bing & Le Bars, 1990, 1992), the results presented here are negative in that no significant differences were observed between the controls and the animals with lesions of RVM or Gi, either in respect of the general properties of trigeminal convergent neurones or of DNIC acting on these neurones.

The methodology used in those studies was identical except for the lesioning procedure. Like the lesions of the locus coeruleus/subcoeruleus area (Bouhassira *et al.* 1992), the RVM lesions were induced by quinolinic acid, while the lesions of the PAG area were induced by ibotenic acid. The decision to use quinolinic acid in the present study was made because of the very high level of fatalities (> 75%), which occurred within 2–3 h of injecting ibotenic acid into the medulla. As with kainate injections

in this area (Chan, Chong, Ong, Chong & Lim, 1985), the death was almost always due to respiratory failure. Although the fatality rate after quinolinic acid injections was still high (around 25%), it was judged to be more acceptable. The selective neuronal loss induced by quinolinic acid is very similar to that induced by ibotenic acid (Schwarcz, Whetsell & Mangano, 1983); in both cases, focal injections do not result in lesions distant from the injection site (Kohler, Schwarcz & Fuxe, 1979; Guldin & Markowitsch, 1981). Following quinolinic acid injections, the lesions were very similar to, although slightly less clearly delimited than, those induced by ibotenic acid injections; no lesion distant from the injection site, notably within the mesencephalon and medulla, were seen.

Although the RVM contains a large population of 5-HT-containing cells, we did not use specific neurotoxins against these neurones since a complete loss of neuronal cell bodies was obtained with the quinolinic acid-induced lesions.

The majority of reports concerning the RVM and the modulation of pain (see references in Introduction) dealt with the area from the caudal pole of the facial nucleus to the level of the trapezoid body, a region of the RVM which was included in our lesions. In addition, we tested, in the present study, the effects of lesions of the most rostral portion of the NRM. However, the electrophysiological results observed in animals with lesions in these areas were not significantly different from those observed in control animals. When considering the different subgroups of our first experimental series, it appears that the NRM was not destroyed in all its rostro-caudal extent in any individual animals and, therefore, its direct participation in DNIC could not be definitively ruled out. Attempts to produce such a lesion, by using two injections 1 mm apart, always resulted in the death of the animal. Although the theoretical possibility that approximately one third of the NRM, either the caudal or rostral portion, is sufficient to sustain DNIC remained difficult to assume, we tested in a second experimental series the effects of electrolytically induced complete lesions of the NRM. The electrophysiological results observed in these animals confirm that this structure is not directly involved in DNIC.

As regards our electrophysiological findings, it appears that the general properties of trigeminal convergent neurones, namely type of receptive field, spontaneous activity and excitability (i.e. threshold for, and magnitude of, C fibre-evoked responses) were not statistically different in the lesioned animals as compared with the controls. This suggests that the RVM and Gi are not involved in the tonic descending inhibition of trigeminal convergent neurones in the rat. This is in accord with Hall, Duggan, Johnson & Morton (1981) who showed that electrolytic lesions of the medullary raphe do not reduce tonic descending inhibition of dorsal horn neurones in the cat. Some behavioural studies have produced results consistent with these data, notably the lack of change in a nociceptive reflex which has been reported in association with lesions of NRM (Yaksh, Plant & Rudy, 1977; Chance, Krynock & Rosecrans, 1978; Abbot & Melzack, 1982; Abbot, Melzack & Samuel, 1982) or of gigantocellularis and paragigantocellularis areas (Mohrland, McManus & Gebhart, 1982). However, an increase in nociceptive reactions has also been reported after either lesions of (Halpern & Halverson, 1974; Proudfit & Anderson, 1975; Casey & Morrow, 1989), or microinjections of local anaesthetics into (Proudfit, 1980), this region.

The second electrophysiological finding in this study, namely that DNIC acting on convergent trigeminal neurones was unaltered after the RVM or Gi lesions, indicates that the neurones and synapses of the RVM (NRM + GiA + LPGi) and Gi are not directly involved in the loop subserving DNIC (the participation of more caudal portions of LPGi or Gi is not excluded by the present study). This is in contradiction with the abstract published by Dickenson *et al.* (1980). However, it is noteworthy that these results were of a very preliminary nature and needed confirmation; indeed the procedure used, viz. acutely inducing electrolytic lesions during neuronal recordings, is very much open to criticism. Such a procedure does not spare the axons which pass through the lesioned area and therefore does not permit definitive conclusions to be reached when positive results are obtained. However, the results obtained in animals with complete lesions of the NRM, electrolytically induced one week before recordings, suggest that the effects observed by Dickenson *et al.* (1980) were not due to lesions of passing fibres but probably to either the small size of the sample of animals and/or to short-term effects – within the half-hour range – of the DC current used for electrolysis.

The electrophysiological, anatomical and behavioural arguments supporting the participation of the RVM in the modulation of pain and possibly in DNIC were presented in the Introduction. The role of Gi in nociception has been emphasized by the findings that a significant proportion of neurones in this structure respond to noxious peripheral stimuli and that electrical stimulation within it usually elicits escape/avoidance behaviour (Casey, 1971; Goldman, Collins, Taub & Fitzmartin, 1972). However, antinociceptive effects have also been reported following both stimulation and microinjection of morphine in the ventral part of this nucleus (Satoh, Akaike, Nakasawa & Takagi, 1980; Zorman, Hentall, Adams & Fields, 1981). In keeping with such results, electrical stimulation in Gi can also inhibit nociceptive activity in spinal and trigeminal neurones (Zorman *et al.* 1981; Dostrovsky, Shah & Gray, 1983; Gray & Dostrovsky, 1983; Pretel, Guinan & Cartens, 1988; Yezierski, 1990). Finally, anatomical data are compatible with the participation of this structure in spino–bulbo-spinal loops since it receives afferents from, and projects to, the spinal cord (Basbaum, Clanton & Fields, 1978; Zemlan, Behbehani & Beckstead, 1984; Matsuyama, Ohta & Mori, 1988). In view of these data, the possibility that Gi plays a direct role in DNIC had to be tested; however, the present data clearly demonstrate that it does not.

Taken together, the present results and those of our previous studies (Bouhassira *et al.* 1990, 1992) indicate that medullary and midbrain structures known to be involved in the descending inhibitory control of the spinal transmission of nociceptive information, do not play a direct role in DNIC.

Although unexpected, these results are not contradictory with the fact that DNIC was found to be reduced by serotonergic antagonists (see references in Le Bars & Villanueva, 1988). In the latter studies the drugs were administered systemically and therefore their site(s) of action could not be determined. The RVM is not the sole source of serotonergic projection to the dorsal horn of the spinal cord. Thus, in the light of the present data, the serotonergic structures involved in DNIC remain to be determined. The present results do not rule out either the hypothesis which implicates DNIC in the detection of nociceptive signals, or the proposition that these

controls could constitute the neurophysiological basis for counter-irritation phenomena in animals and humans. According to the 'contrast' hypothesis, DNIC could facilitate the recognition of nociceptive signals by higher centres through an enhancement of the signal-to-noise ratio between a population of activated convergent neurones and the remaining population of spinal and trigeminal convergent neurones. However, such a hypothesis suggested a new interpretation of the physiological meaning of some descending inhibitory controls (see Le Bars & Villanueva, 1988).

On the basis of our studies, we would now suggest that DNIC may contribute to pain detection independently of the postulated 'endogenous pain inhibitory system' which includes notably the periaqueductal grey (PAG) and the RVM, since it appears quite clear that the two systems are sustained by pathways which are different, at least in part. In view of this conclusion, there are no longer incompatibilities between the contrast hypothesis and the postulated antinociceptive negative feedback loop proposed by some authors (see Fields & Basbaum, 1989). Since the PAG, which is not directly involved in the DNIC circuitry, can indirectly modulate these controls (Bouhassira, Villanueva & Le Bars, 1992), one can suggest interactions between the systems of modulation of pain.

The problem of determining which brain structures are involved in DNIC remains unresolved. In our previous papers we addressed this problem and suggested some candidates. Thus, if the participation of the lateral thalamus has already been excluded (Villanueva, Peschanski, Calvino & Le Bars, 1986), the involvement of other diencephalic structures remains possible. Indeed, structures such as the medial thalamus which receives nociceptive afferents from the spinal cord (Craig & Burton, 1981; Giesler, Yezierski, Gerhart & Willis, 1981) or the lateral hypothalamus, stimulation of which can produce both analgesia and inhibition of spinal dorsal horn neurones (Carstens, 1982; Mokha, Goldsmith, Hellon & Puri, 1987; Lumb, 1990), could be involved in the DNIC circuitry. We also suggested that other structures which have not yet been shown to be involved in the modulation of pain could play a role in DNIC. Most notably, the participation of subnucleus reticularis dorsalis (SRD) in the caudal medulla was envisaged because of the electrophysiological properties of the neurones recorded within it. These neurones are exclusively or preferentially activated by noxious stimuli applied to any part of the body (Villanueva, Bouhassira, Bing & Le Bars, 1988), can encode nociceptive information and respond to the activation of peripheral Aδ and C fibres (Villanueva *et al.* 1988; Villanueva, Bing, Bouhassira & Le Bars, 1989). Since DNIC can be triggered by Aδ and C fibre volleys evoked by single percutaneous electrical stimuli and can encode nociceptive stimuli (see Le Bars & Villanueva, 1988), subnucleus reticularis dorsalis is an obvious candidate. In addition, it has been shown recently that the ascending pathways in the spinal cord involved in the activation of subnucleus reticularis dorsalis neurones are located in the anterolateral quadrant (Bing, Villanueva & Le Bars, 1990) as is the ascending part of the loop subserving DNIC (Villanueva *et al.* 1986); furthermore, this structure projects to the spinal dorsal horn via the DLF (Bernard, Villanueva, Carroué & Le Bars, 1990). The study of DNIC in rats with neurotoxin-induced lesions of SRD therefore seems to be a priority for further investigations.

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