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MODIFICATIONS OF DORSAL HORN CELL ACTIVITIES IN THE SPINAL CORD, AFTER INTRA-ARTERIAL INJECTION OF BRADYKININ

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SUMMARY

1. A method is described to study the modification in the activity of the lamina IV and V cells of the dorsal horn under the intra-arterial administration of bradykinin into the hind limb of the spinal cat.

2. The modifications induced by intra-arterial injection of bradykinin preferentially affected the lamina V cells (77 % of the units) and produced few changes in the lamina IV cells (16 % of the units showed variations).

3. 52 % of the lamina V cells were activated; the mean increase in the firing rate was 700 %. The mean latency of the effect was 20 sec and the mean duration was 47 sec. However, there was large variance in the excitatory effect across cells. On the other hand, for the same cell, the observed effects were perfectly reproducible and injections of physiological fluid induced no response.

4. 25 % of the lamina V cells were inhibited. Generally, the activity of the cell was reduced to 13 % of its initial value; in some cases, a total suppression of activity was observed. The mean latency of the inhibitory effect was 12 sec and its duration 28 sec. All these units had a very wide inhibitory field (activated by stimulations of low intensity) which asymmetrically surrounds the excitatory field.

5. This study confirms the role played by the lamina V cells in the transmission of nociceptive messages. The existence of inhibitory phenomena is in favour of the gate control theory described by Melzack & Wall (1965) without deciding the pre- or post-synaptic nature of these mechanisms.

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INTRODUCTION

Mendell & Wall (1964) described a hyperpolarization of primary afferents produced by stimulating C fibres. From these results, Melzack & Wall (1965) proposed a new theory of pain mechanisms called 'gate control theory'; this theory is based on the existence at the spinal level of inhibitory and facilitatory interactions between cutaneous afferents of low and high threshold. Today the existence of the gate control is sharply debated since some authors (Zimmermann, 1968; Franz & Iggo, 1968; Vyklicky, Rudomin, Zajac & Burke, 1969; Burke, Rudomin, Vyklicky & Zajac, 1971) did not observe positive dorsal-root potentials after stimulation of C fibres. However, Mendell (1970) confirmed his first observation (Mendell & Wall, 1964) by showing a hyperpolarization of the primary afferent fibres following stimulation of muscle fibres of group III. Recent microphysiological studies (Hillman & Wall, 1969) in the lamina V of the dorsal horn have clearly shown the existence of an interaction between inputs driven by high and low threshold fibres. Latter results confirmed the existence of a gate control mechanism, the nature of which does not appear to be an exclusively presynaptic one, since Hongo, Jankowska & Lundberg (1968) have also seen post-synaptic inhibitory effects on cells which are at the origin of the spinocervical tract.

In the dorsal horn, the cells of Rexed's lamina V are of special interest because they show specific and graduated responses under intense stimulations (Wall, 1967; Hillman & Wall, 1969; Wagman & Price, 1969; Price & Wagman, 1970) and moreover they are activated by splanchnic afferents (Pomeranz, Wall & Weber, 1968; Selzer & Spencer, 1969*a*, *b*). These results have been obtained either after electrical stimulation of the nerves or after mechanical stimulations.

Moreover it is known that some chemical agents, when injected by intra-arterial means, can induce nociceptive reactions in man or in animals. Among these substances, bradykinin has been particularly studied. Armstrong, Dry, Keele & Markham (1953), Armstrong, Jepson, Keele & Stewart (1957) showed that, in man, this polypeptide triggered a 'cutaneous pain' when put on the base of a bare phlycten. Furthermore, some authors found the presence of this substance during the course of inflammatory processes in animals (Rocha e Silva & Antonio, 1960) and in man (Chapman, Ramos, Goodell & Wolff, 1963). During inflammation, this substance is released from the $\alpha2$ globulin (Van Arman, 1952) and is inactivated by an enzymic system in the plasma (Schachter, 1964). These effects of bradykinin have been especially studied by Lim and co-workers (see Lim, 1968); in the chronic dog, the intra-arterial injection of bradykinin into the splenic artery leads to nociceptive reactions manifested by drastic changes in

cardiovascular and respiratory functions, accompanied by vocalization and by the appearance of aggressive behaviour (Guzman, Braun, Lim, Potter & Rodgers, 1964; Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Enge, 1964). From the electrophysiological point of view, these manifestations are associated with the appearance of action potentials in the splanchnic nerve.

In man, the intra-arterial (Burch & De Pasquale, 1962; Guzman, Braun & Lim, 1962; Sicuteri, Franchi & Fanciullacci, 1964) and intraperitoneal (Lim, Miller, Guzman, Rodgers, Rodgers, Wang, Chao & Shih, 1967) injection of bradykinin is followed by a painful sensation.

According to these results, bradykinin is one of the most potent pain producing agents known. It is therefore interesting to use intra-arterial injections of this drug to determine which structures of C.N.S. are involved in the transmission of nociceptive messages. In the following study, by reference to earlier electrophysiological results, we intend to analyse the effects of intra-arterial injection of bradykinin on the activity of the cells in Rexed's lamina IV and V of the cat's dorsal horn.

METHODS

Fourteen cats, each weighing 2–3 kg, were used. After halothane anaesthesia they were immobilized by gallamine triethiodide (Flaxedil), artificially ventilated and placed in a stereotaxic apparatus. A spinal cord section was performed at C1. Arterial blood pressure, central temperature and end tidal CO_2 were monitored continuously: blood pressure always being above 80 mm Hg, central temperature being maintained around 37·5° C and end tidal CO_2 being adjusted between 4 and 4·5 %.

After laminectomy, the dura mater was opened and the cord exposed from L4 to S1. Then the dura mater was folded back and maintained thus with threads making a tank so that the cord could be lightly supported and consequently the effects of respiratory movements reduced. By using very thin forceps, the pia-mater was subsequently opened with the aid of a dissecting microscope so that the micro-electrode could pass through. The exposed cord was then covered with paraffin oil which was maintained at 37° C by a thermostatic heater.

The recordings were made in S1, L7 and L6 with micropipettes filled with 3 M-KCl, the resistance of which remained between 4 and 20 MΩ.

The cells were localized by Howland's technique (Howland, Lettvin, McCulloch, Pitts & Wall, 1955; Wall, 1960). They were also characterized by their electrophysiological criteria as described by Wall (1967). According to their electrophysiological properties, the lamina IV cells are easy to characterize. As regards the lamina V, the most important problem consists of the extreme difficulty in the differentiation between the ventral part of this layer and the dorsal part of the lamina VI by means of the anatomical technique. The electrophysiological criteria are not entirely satisfactory since the lamina VI cells, as they receive monosynaptically muscular afferents, are also polysynaptically activated from the lamina V by cutaneous afferents (Wall, 1967). So that, in this study, as we use both the anatomical and the electrophysiological techniques, the denomination of lamina V must be

taken in a relatively large meaning, for a certain number of cells were likely situated in the dorsal part of the lamina VI.

A polyethylene tube of 0·9 mm diameter was introduced upstream in the popliteal artery. The continuity between the tube and the artery was maintained by a continuous and very slow perfusion of physiological fluid (2–4 ml./hr). This perfusion was performed by means of a pressure infusor (Fenwall) and a Vygon microtube which reduced the flow (Fig. 1). Synthetic bradykinin (Sandoz) was injected through a three-way tap. 5 μg bradykinin was injected in 1 ml. solution. Further tests were systematically performed by injection of 1 ml. physiological fluid.

Unitary recordings were effected 3 or 4 hr after the elimination of volatile anaesthetics.

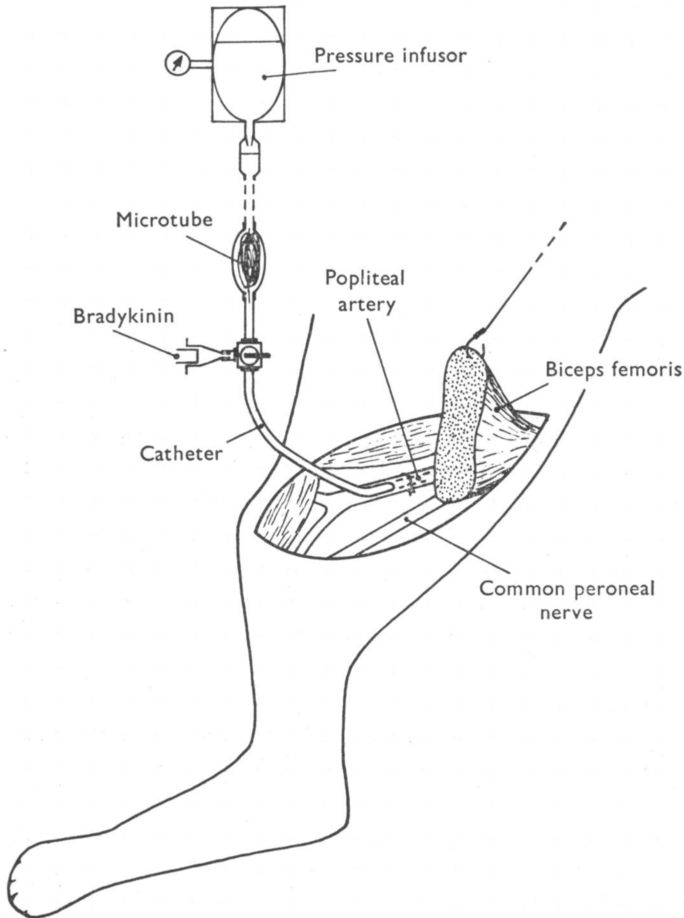

Fig. 1. Experimental schema representing the system of slow perfusion into the popliteal artery (2–4 ml./hr) and the intra-arterial injection of bradykinin.

RESULTS

A. General findings (Table 1)

Ninety cells were included in this study, twenty-nine in the lamina IV and sixty-one in the lamina V. The importance of the effects following the intra-arterial injection of bradykinin was different, depending on the lamina since 17% of cells had modifications in lamina IV, whereas, in lamina V, 77% of them were affected.

Among those cells, whose activity was modified, two different kinds of effects were observed: an excitatory effect and an inhibitory effect, the former being twice as frequent.

TABLE 1. Summary of the effects of bradykinin injection

	Lamina IV	Lamina V
No effect	24 (83%)	14 (23%)
Excitation	4 (13·7%)	32 (52%)
Inhibition	1 (3·4%)	15 (25%)
Total	29	61

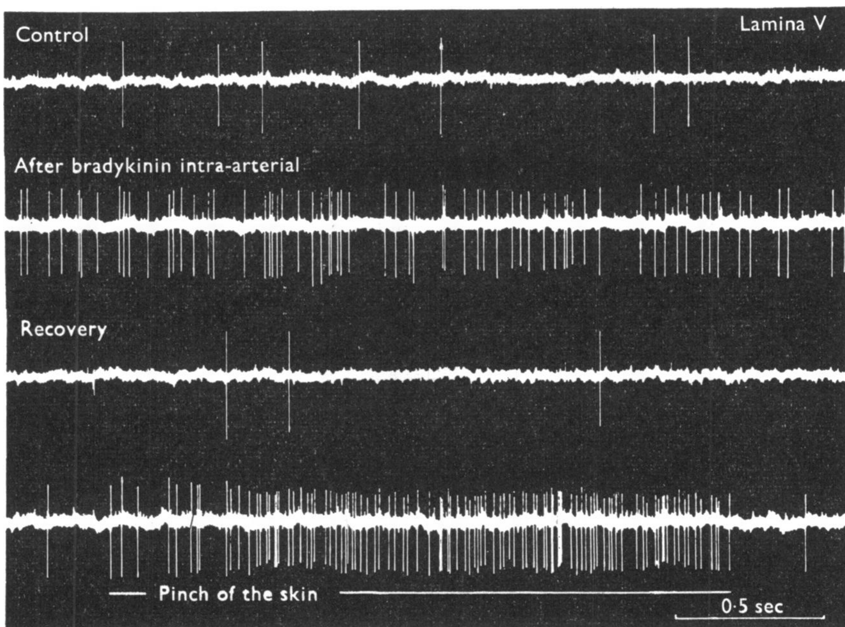

Fig. 2. Comparison between the excitatory response of a lamina V cell after intra-arterial injection of bradykinin and the natural response after a strong stimulation (pinch of the skin).

B. *Effects on lamina V cells*

Excitatory effects

Fifty-two per cent of the lamina V cells (32/61) were excited. These cells presented analogous characteristics to those described by Wall (1967): wide peripheral field, important increase of firing according to the stimulus intensity, and weak accommodation.

Consequently it appears that the cells which were activated by nociceptive stimuli (pressure or pinch of the skin) were preferentially activated by bradykinin intra-arterial injection. One example is given in Fig. 2 in

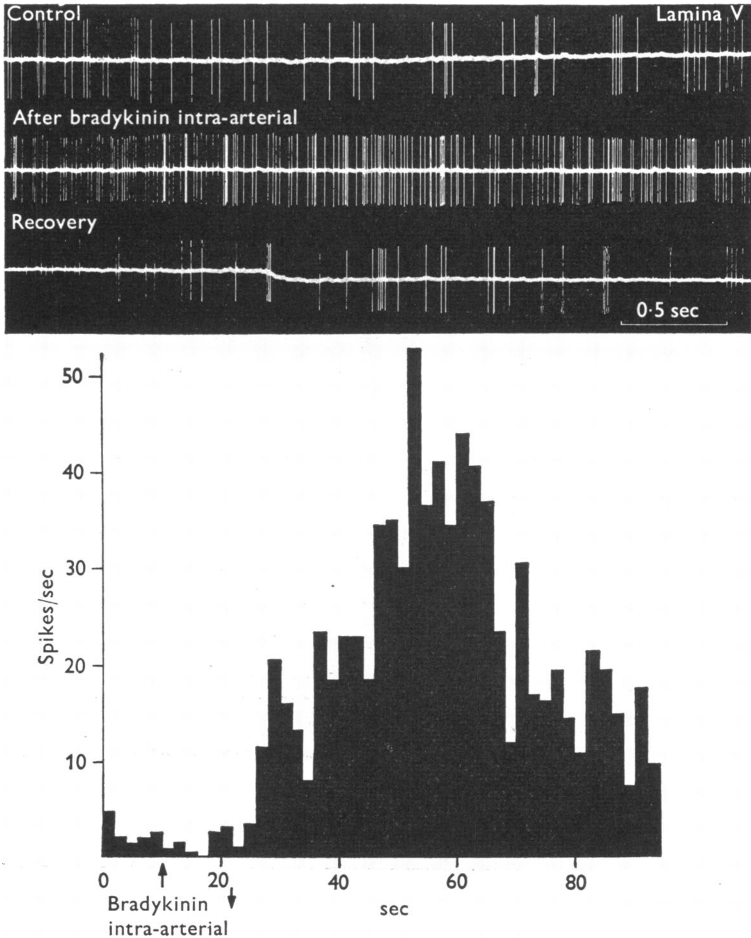

Fig. 3. Typical excitatory response of a lamina V cell after intra-arterial injection of bradykinin.

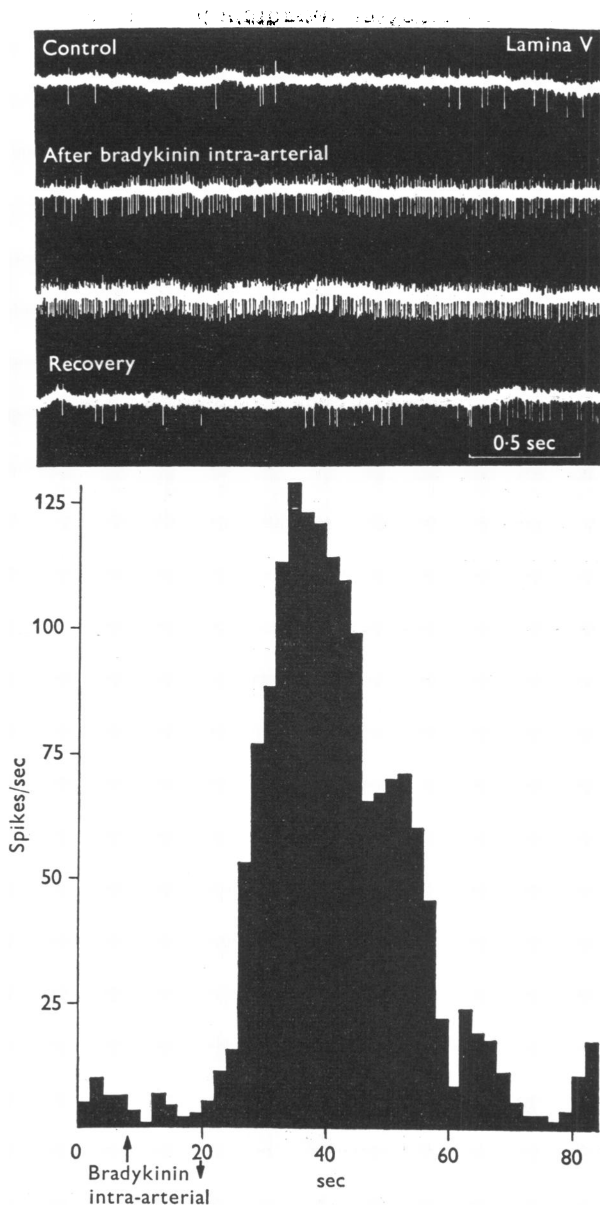

Fig. 4. Excitatory response of a lamina V cell. The spontaneous activity of this cell was relatively low, but was greatly increased by bradykinin injection.

which the effects produced by bradykinin injection and by natural stimulation (strong pressure) are respectively shown.

These excitatory effects do not appear to depend on the spontaneous

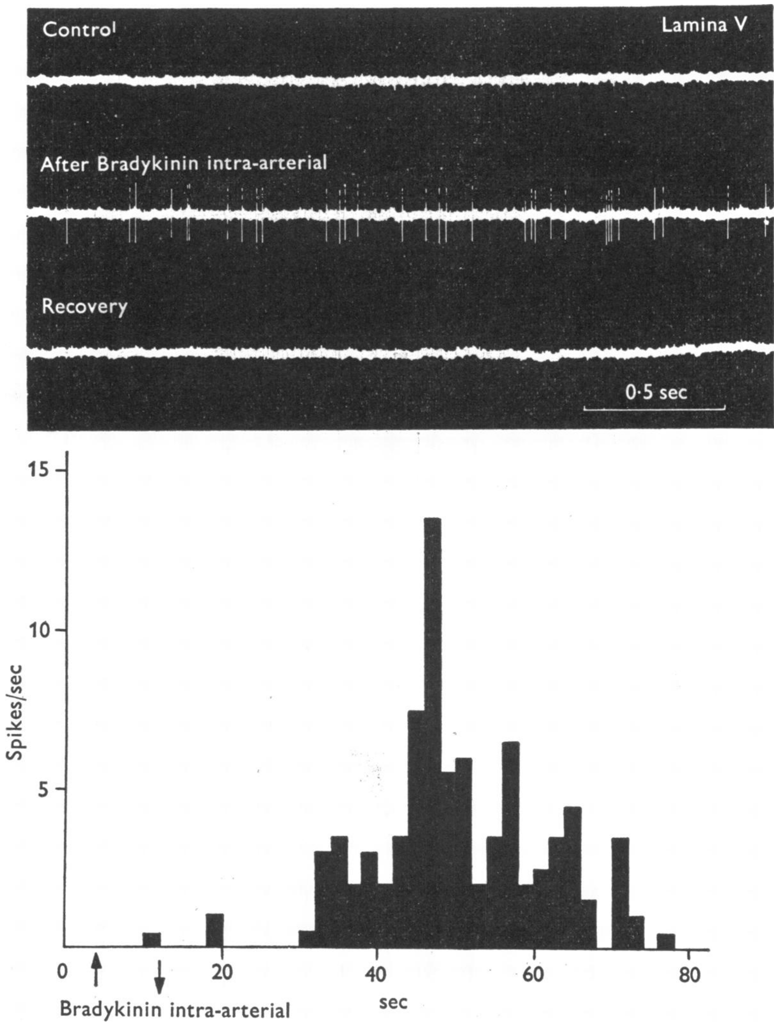

Fig. 5. A very weak excitatory response of lamina V cell whose spontaneous activity was absent prior to stimulation.

activity of the cells, which was highly variable (from 0 to 45/sec). Very important differences in the degree of activation between cells were observed: after bradykinin injection, the increase of firing rate was between 145 and 4200 %. However, these modifications are relatively

homogeneous since, in 75% of the cases, the firing increase was in the range of 200–800%. In Fig. 3, the most frequently observed effect is represented. In some cases (Fig. 4), bradykinin administration induced a very intense cellular activation, in other cases, the activation was not very striking (Fig. 5). Concerning the latency and the duration of the phenomenon, notable variations were apparent between cells, as shown in Figs. 3, 4 and

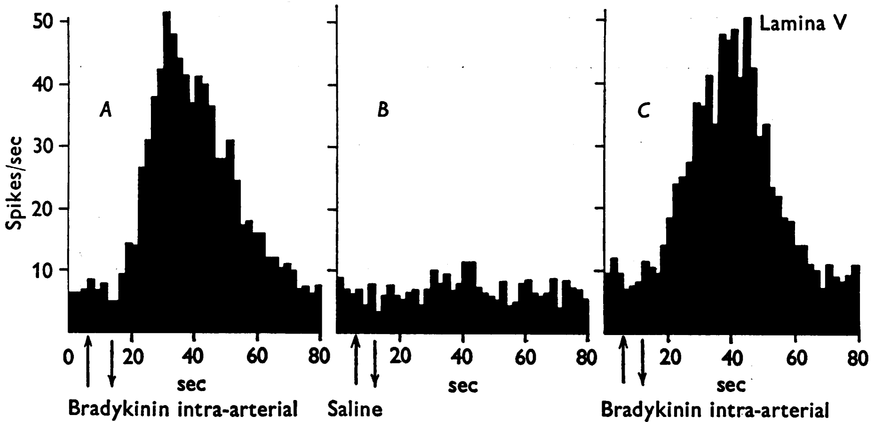

Fig. 6. Effects of repeated administrations of bradykinin. Repeated injections of bradykinin (*A* and *C*) produced identical excitatory effects, whereas injection of physiological fluid (*B*) did not induce significant variations of the firing rate.

5. These differences may have been due to circulatory problems induced by the injection technique (see Discussion). The mean latency of the excitatory effect following injection was 20 ± 13.

In the same cell, the effects of bradykinin were easily reproducible (Fig. 6) and we could regularly verify that the injection of physiological solution produces no modification of the firing rate.

Inhibitory effects

Twenty-five per cent of the cells in lamina V (15/61) were inhibited after bradykinin injection. We could see that these units had an excitatory field similar to that of the cells which were excited by bradykinin. In contrast to these neurones, the inhibited cells had a wide inhibitory field which was activated by natural stimulations of low intensity.

As shown in Fig. 7, the time course of the inhibitory effect was very similar to that of the excitatory effect. However, the latency of the inhibitory effect is significantly shorter (12 ± 11 sec of its initial value).

In certain cases, for the same cell, we observed both inhibitory and excitatory effects, the former always preceding the latter (Fig. 8).

Unaffected cells

Twenty-three (14/61) cells in lamina V showed no change in their activity after bradykinin injection. Generally, these cells showed, either a low level of spontaneous activity or none at all (eight cells) and a small increase in firing after intense peripheral stimulation.

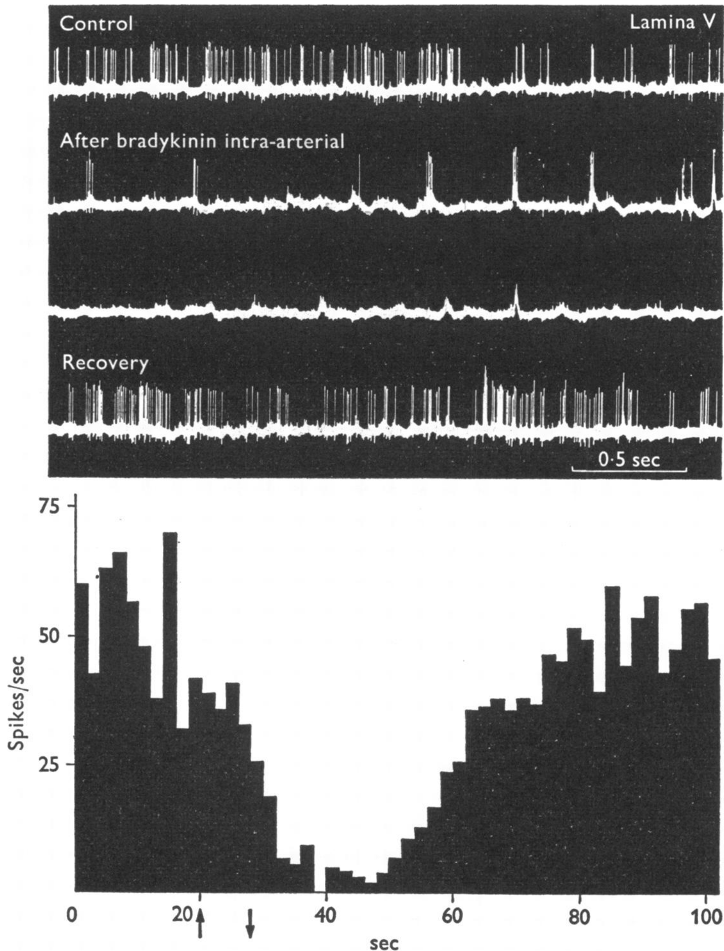

Fig. 7. Inhibition of a lamina V cell after intra-arterial injection of bradykinin. As described in the text, this unit had a wide inhibitory peripheral field.

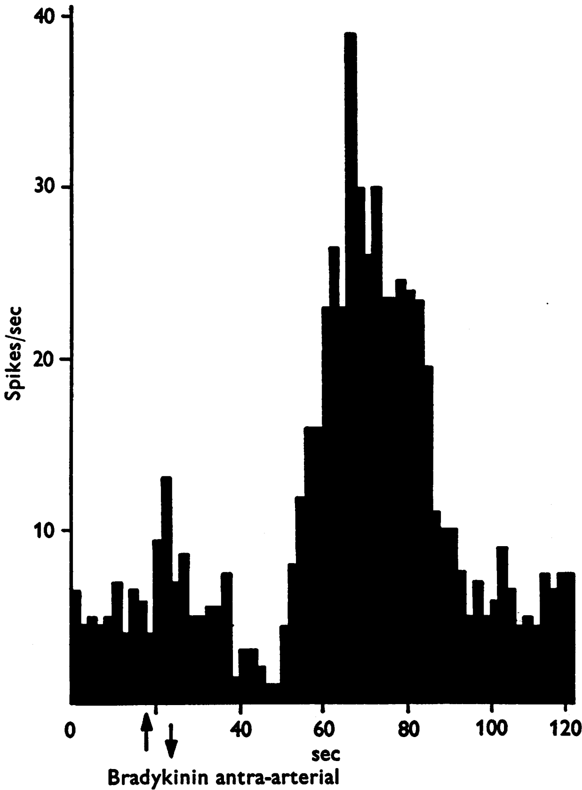

Fig. 8. A diphasic response of a lamina V cell. An inhibitory effect of short duration preceded the excitatory effect.

C. Lamina IV

The cells of lamina IV were activated by tactile stimulation of low intensity (movement of hair, light touch) from a small peripheral field. These cells were usually not modified by bradykinin injection (Fig. 9).

Four cells were activated, though to a lesser degree than those cells of lamina V.

We found one cell which was inhibited, but this effect is difficult to demonstrate since generally the units of the lamina IV have a low spontaneous activity.

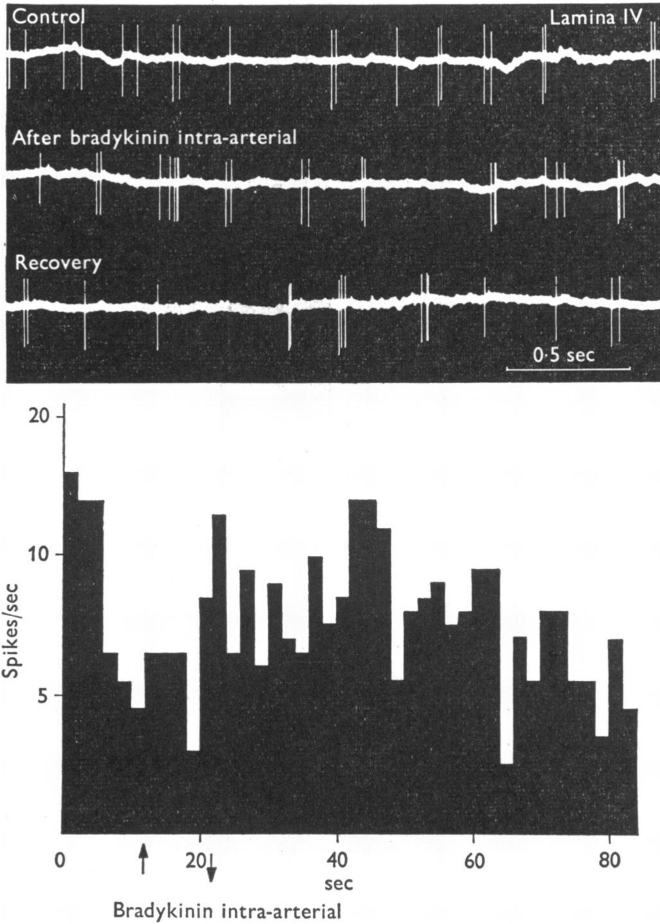

Fig. 9. The spontaneous activity of this lamina IV cell did not show significant modifications after intra-arterial injection of bradykinin.

DISCUSSION

From these results, it appears that intra-arterial injection of bradykinin primarily modifies the activity of the lamina V cells since about 77 % of them showed important variations, whereas only 17 % of the cells of lamina IV were affected.

The difference between the lamina IV and V cells is significant and cannot be due to experimental conditions. In fact, in order to overcome partially the circulatory problems produced by the cannulation of the popliteal artery, we limited our experiments to a few hours. Moreover, we

excluded some preparations, in which the irrigation of the limb extremity was visibly insufficiently indicated by: very slow recolouring of the small cushion after pressure, difficulty in characterization of receptive fields. We observed that the solution injected upstream in the popliteal artery passed mainly through the saphenous artery which is partially responsible for the irrigation of the limb extremity. However, this technique necessitates cutting off the circulation in the posterior tibial artery, which is also important for the limb irrigation.

The concentration of bradykinin at the periphery is also dependent on the injection speed and the systemic blood pressure. Therefore, we did not look systematically for a precise dose–effect relationship. On the other hand, considering the same cell and the same injection parameters (same dose, volume and injection speed), the effects were perfectly reproducible (Fig. 6). The circulatory problems cited above may explain the dispersion of the latency, duration and intensity of the effects between cells. In spite of these difficulties, this technique was very satisfactory since, when adequate circulatory conditions were secured, the results were relatively homogeneous.

From the physiological point of view, these results are in agreement with those of other authors, especially of Wall and co-workers. By using natural and electrical stimulation (Wall, 1967; Pomeranz, Wall & Weber, 1968; Mendell, 1966; Hillman & Wall, 1969) have shown that the lamina V cells receive afferents of thin fibres from skin, muscles and viscera. Similar results have been found again by Selzer & Spencer (1967, 1969*a*, *b*) on the cat, and by Wagman & Price (1969) on the monkey. Moreover, it is known that the thin afferent fibres are very likely to be involved in nociceptive processes, since some are exclusively activated by strong and noxious stimulations (Zotterman, 1939; Bishop, 1946; Hunt & McIntyre, 1960; Siminoff, 1965; Burgess & Perl, 1967; Iggo, 1968). Therefore, it is particularly interesting to notice that bradykinin intra-arterial injection, which is known to evoke specific pain and itch in man and nociceptive reactions in animals (Lim, 1968) produces large modifications in activity of the lamina V cells.

The results obtained in this study by the administration of bradykinin, a primarily noxious stimulus, appear to confirm that lamina V cells are involved in the transmission of nociceptive messages. This hypothesis is strengthened when the electrophysiological and pharmacological results concerning the visceral afferents are considered: it has been shown that the lamina V cells are activated by splanchnic nerve stimulation (Pomeranz *et al.* 1968; Selzer & Spencer, 1967, 1969*a*, *b*) and that bradykinin injection into the splenic artery provokes the appearance of action potentials in the splenic and splanchnic nerves (Lim *et al.* 1964).

Moreover, it is possible to compare our results with the observations in man and with the pseudo-affective reactions described in animals under the same conditions of stimulation (intra-arterial injection). With respect to the cells activated by bradykinin injection, the latency (20 sec) and the duration of activation (47 sec) are of the same order as those obtained by Guzman *et al.* (1964) in chronic dogs for the vocalization phenomena (latency 15 sec; duration 20–30 sec). In the same way Lim *et al.* (1964) have observed, in similar experimental conditions, identical activation durations in the splanchnic nerves with, however, a shorter latency than that of vocalization. The comparison between the neuronal activity and vocalization may be a gross one; however, from a psychophysiological point of view it is an interesting one.

Another interesting point concerns the inhibitory effects of bradykinin injection observed in 25% of the lamina V cells. These effects could be seen only in six cats out of fourteen. Moreover, in one animal, only inhibitory effects could be recorded (six cells) in spite of a total spinal section.

Each time this inhibition was observed, it was accompanied by the existence of a very wide inhibitory field (8–15 cm^2) explored by natural stimulations. The interruption of the spontaneous activity appeared with stimulations of very low intensity, and also with stronger mechanical stimulations applied at the peripheral field. Those cells, which were inhibited by bradykinin, showed a shorter latency of effect than those which were excited by the drug. This difference may be caused by a vascular difference. In fact, in this case the very large inhibitory fields, lying nearer than the excitatory ones, are likely to be reached earlier by bradykinin. The preponderance of excitatory effects can be explained by the fact that spinal preparations were used; Wall (1967), Hillman & Wall (1969), Brown (1970) showed in the decerebrate cat that the inhibitory fields of the lamina V cells were very much reduced when the spinal cord was disconnected from the brain stem structures by cooling or by section. Our classification of inhibitory and excitatory effects is relatively arbitrary, for the modification of the lamina V cell activity probably results from the predominance of one of the two effects. This seems to be confirmed by the fact that, in certain circumstances, an inhibition and an excitation were observed successively (Fig. 8). This observation suggests the existence of a gating mechanism, the aim of which would be to assure a modulation in the transmission of the nociceptive message to the upper centres (see Introduction).

In order to explain our observation of inhibitory effects after bradykinin administration with reference to physiological results from Hillman & Wall (1969), it must be considered that bradykinin does not activate the slow conducting fibres alone (fibres C and $A\delta$), but also the fast conducting

ones. However, as was shown in this study, bradykinin injection activates only a small percentage of the lamina IV cells, which are normally activated by large fibres after a light natural stimulation.

However, to explain the inhibitory effect, two hypotheses may be advanced:

(1) the small percentage of large diameter fibres which are activated may be compensated for by the wide area of the receptive inhibitory field;

(2) the bradykinin may exclusively activate high threshold fibres which may be either inhibitory or excitatory according to their origin in the peripheral receptive field of the cell.

The results of Lim *et al.* (1964) are very interesting but they do not make it possible for us to choose one of the two hypotheses since these authors have shown that intra-arterial injection of bradykinin into the splenic artery induces in the splanchnic nerve the activation of two groups of fibres: on the one hand, a group whose conduction speed is situated between 1·4 and 4·3 m/sec, and, on the other hand, a second group whose conduction speed is situated between 18 and 54 m/sec (75 % of the activated fibres have a speed lower than 36 m/sec). In order to resolve this problem, it seems necessary to consider again the effects of intra-arterial injection of bradykinin. We must mention the work of Burgess & Perl (1967) who, by applying bradykinin on 'abraded skin' or by injecting it into a 'skin cut' do not usually find any activation in fibres considered as nociceptive (6–37 m/sec). These negative results are probably due to the technique of bradykinin application which is very different from the one we used.

In conclusion, these results supply further evidence in support of the role played by the cells of the Rexed lamina V in the transmission of nociceptive messages. Moreover, they are compatible with the existence of a gate control which has been proposed by Wall and co-workers.

It would also be very interesting to apply this technique of nociceptive stimulation to two fields of study: on the one hand, from the physiological point of view, to study the subcortical and cortical structures implicated in nociceptive stimuli and, on the other hand, from the pharmacological point of view, to localize the locus of effect of analgesic drugs.

The synthetic bradykinin used in this study was kindly supplied by Sandoz Laboratories (Basel, Switzerland).

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