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Facilitation of the flexor reflex in the cat by intrathecal injection of catecholamines

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Summary

1. Effects of some α- and β-adrenoceptor stimulants and antagonists were investigated on flexor reflex (FR) in chloralosed cats.

2. Noradrenaline (NA) produced facilitation of FR which was dose-dependent and reproducible and was blocked by α-adrenoceptor blocking agents.

3. Strychnine also produced facilitation of FR but the response was unaffected by α-adrenoceptor blocking agents.

4. Metaraminol and α-methyl-noradrenaline had little effect on FR but blocked the NA response.

5. β-adrenoceptor stimulants and antagonists had neither any effect on FR nor modified the NA response.

6. Vasopressin and histamine also failed to modify FR.

7. Possibility of α-adrenoceptors in the neurones integrating FR is suggested.

Introduction

Many workers have investigated the role of catecholamines on somatic reflexes and have suggested the possibility of their being released at various synapses in the central nervous system (Sigg, Ochs & Gerard, 1955 ; Carlson, 1959 ; McLennan, 1961, 1962). Biscoe & Curtis (1966), employing the iontophoretic technique, have shown that noradrenaline (NA) has an inhibitory action on spinal motor neurones and on Renshaw cells. More recently, Andén, Jukes, Lundberg & Vyklický (1966a) have recorded potentials from dorsal root and ascending pathways in spinal cats by micro-electrodes. They have observed that intravenous injection of 1-3-4-dihydroxyphenylalanine (dopa) consistently inhibits transmission from flexor reflex pathways. They have concluded that, " the effect of Dopa may be due to synthesis and outflow of transmitter from a descending nor-adrenergic pathway, which has an inhibitory effect on the spinal pathways in which transmission is depressed by Dopa ". The facilitation of flexor reflex after dopa was suggested to be due to long lasting discharge which was produced in the flexor motor neurones from the flexor reflex afferents.

Further studies by the same workers (Andén, Jukes & Lundberg, 1966b) on reserpinized cats, in cats pretreated with monoamine oxidase inhibitors and investigations with α- and β-adrenoceptor blocking agents supported the view that dopa produced its effect on the flexor reflex via noradrenaline and they suggested the presence of specific adrenoceptors in the spinal cord. The present study was under-

taken to obtain more direct evidence for this hypothesis by studying the effects of some α- and β-adrenoceptor stimulant and blocking agents on the flexor reflex in cats.

Methods

Male cats, weighing 3 to 4 kg, were anaesthetized with 80 mg/kg intravenous chloralose. The flexor reflex (FR) was elicited by electrical stimulation of the cut central end of the medial branch of the sciatic nerve (2–8 V, 30–40 ms at 12–15/min) and contraction of tibialis anterior muscle was recorded by connecting the terminal tendon through a system of pulleys to a spring-loaded lever writing on kymograph paper. The drugs were injected through a hypodermic needle inserted in the theca in the third or fourth lumbar space. Drug solutions were prepared in normal saline in such concentrations that the total volume injected never exceeded 0·5 ml. The effect of the same volume of solvent was also observed, to exclude any possible effects due to the volume of injection. The blood pressure was recorded via a mercury manometer from a cannula in the left common carotid artery (1 mmHg≡ 1·333 mbar). The drugs used are given in Table 1.

Results

Effect of noradrenaline

Intrathecal administration of 40–80 μg of noradrenaline led to marked facilitation of the flexor reflex within 3 to 5 min. The magnitude and duration of facilitation was dose dependent but generally lasted for 90 to 120 min. These doses of NA did not produce any change in blood pressure (Fig. 1). Repeated administration of the same dose produced almost equal facilitation of the reflex.

Effects of other catecholamines

To elucidate the mechanisms of NA-induced facilitation, several other related agents were tried. Neosynephrine (NS), which is predominantly a stimulant of

TABLE 1. *Drugs, doses and number or cats employed with each drug*

No.	Drugs tested	Abbreviations as used in text and figures	No. of cats	Doses (μg)
Adrenergic agonists				
1.	Noradrenaline	NA	17	40, 80, 200
2.	Neosynephrine	NS	2	400, 500
3.	Isoprenaline	Iso.	4	100, 200, 500
4.	Salbutamol	SB	3	200, 400
5.	Dopamine	Do.	2	50, 200
6.	α-methyl-noradrenaline	MN	2	200, 400
7.	Metaraminol	Metar.	2	200, 400
Adrenergic antagonists				
8.	Phentolamine	Phen.	4	100, 1,000
9.	Dibenamine	Diben.	4	100, 500
10.	Dichloroisopropylnoradrenaline	DCI	2	100, 200
11.	(±)-4-(2-isopropylamino-l-hydroxyethyl) methane-sulphonanilide HCl (MJ 1999)	MJ 1999	2	500, 1,000
Miscellaneous				
12.	Strychnine	Stry.	5	10, 50
13.	Lignocaine	Lign.	5	1,000, 2,000
14.	Vasopressin	Vaso.	4	60 mu.
15.	Histamine	Hist.	4	10. 60

α-adrenoceptors, produced similar but less marked facilitation of the response (Fig. 2). Isoprenaline and a non-catecholamine β-adrenoceptor stimulant, 2-*t*-butylamino-1-(4-hydroxy-3-hydroxymethyl) phenyl ethanol (Salbutamol), had no effect on the flexor response even in the highest doses tested (see Table 1). Dopamine (50–200 μg) was also without any effect.

Effect of α-adrenoceptor blocking agents

The facilitatory effect of NA and NS and the absence of facilitation with β-adrenoceptor stimulants suggested that the response may involve α-adrenoceptors. To investigate this possibility further two α-adrenoceptor blocking agents, dibenamine and phentolamine (Rogitine), were employed. Dibenamine at a dose of 200 μg completely blocked the facilitatory effect of the usual doses of NA, and even large doses of NA (200 μg) failed to produce any facilitation (Fig. 3). Another α-adrenoceptor blocking agent phentolamine (200 μg) also antagonized the NA (50 μg) induced facilitation of FR ; after administration of phentolamine even high doses of NA (200 μg) failed to produce any facilitatory effect on flexor reflex. To exclude the possibility of a non-specific effect of α-adrenoceptor blocking agents, their action on strychnine (10 μg intrathecally) induced facilitation of the flexor response was also studied. Dibenamine and phentolamine, however, failed to modify strychnine-induced facilitation even in large doses (500 μg and 1·0 mg respectively, Figs. 3 and 4).

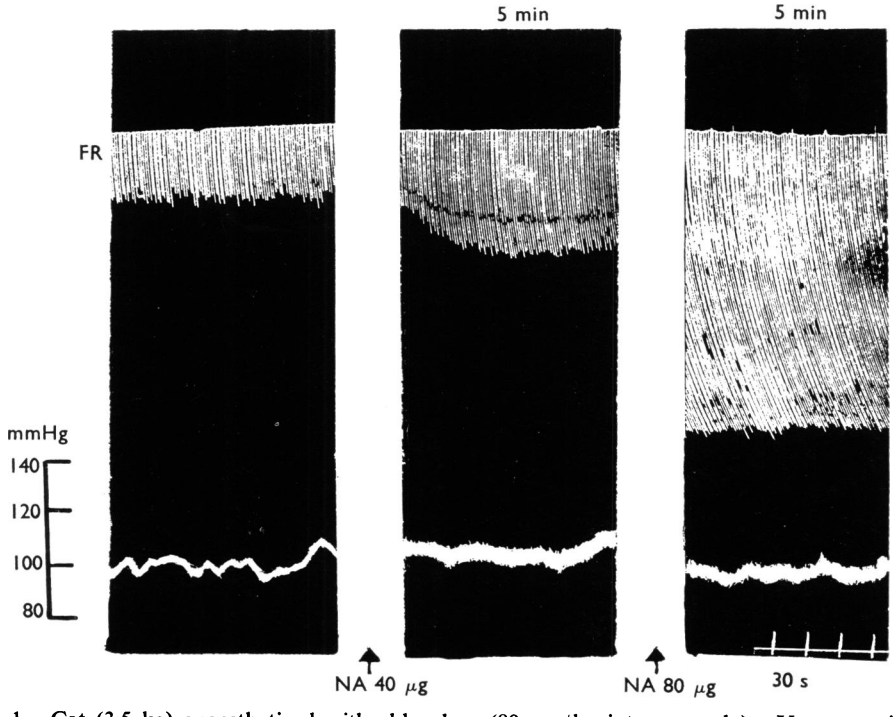

FIG. 1. Cat (3·5 kg) anaesthetized with chloralose (80 mg/kg intravenously). Upper tracing, flexor reflex ; lower tracing, blood pressure. Panel 1 shows the control flexor reflex (FR) and panel 2 facilitation of FR 5 min after 40 μg of intrathecal noradrenaline (NA). In panel 3, greater facilitation of FR has been produced by 80 μg of NA. These doses of NA have not produced any change in blood pressure level. Time marks 30 s. In this and the other figures, the time between adjacent records is indicated on the top of each.

Effect of local anaesthetics

It has been reported that α-adrenoceptor blocking agents possess local anaesthetic activity (Nickerson, 1949). To exclude the possibility of the blockade by dibenamine and phentolamine being due to such an effect, a local anaesthetic, lignocaine, was employed. Lignocaine (1–2 mg) also blocked NA-induced facilitation; smaller doses of lignocaine were ineffective. In contrast to α-adrenoceptor blocking agents, however, lignocaine also blocked strychnine-induced facilitation in the same doses (Fig. 5).

Effect of β-adrenoceptor blocking agents

In order further to exclude the possibility of involvement of β-adrenoceptors, two β-adrenoceptor blocking agents (±)-4-(2-isopropylaminohydroxyethyl) methanesulphonanilide HCl (MJ 1999) and dichloroisopropylnoradrenaline (DCI) were studied for the effects on NA-induced facilitation. Neither of them produced any effect. The results of two typical experiments are shown in Fig. 6.

Effect of metaraminol and α-methyl-noradrenaline

Investigations were also conducted with some pharmacological agents which have been reported to act on adrenoceptors and modify the response of catecholamines.

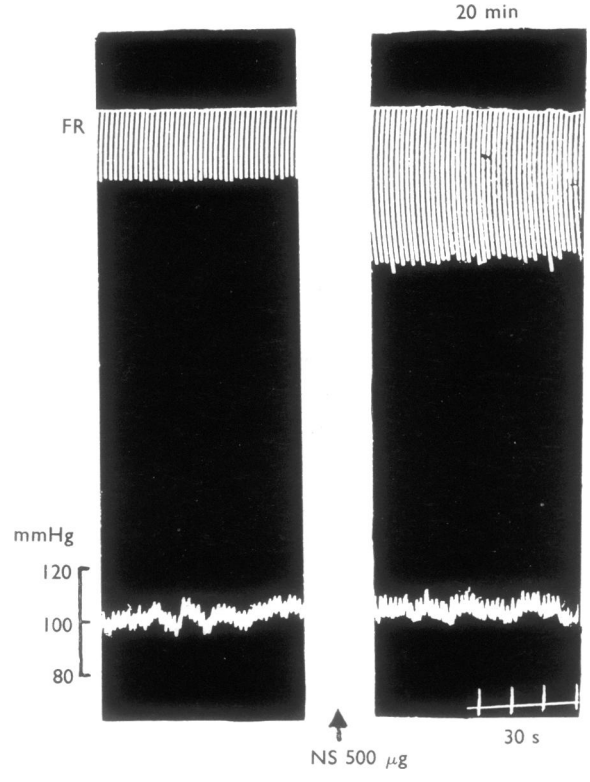

FIG. 2. Cat (3·0 kg) anaesthetized with chloralose 80 mg/kg intravenously. The record shows the facilitatory effect of 500 μg neosynephrine (NS) injected intrathecally on the flexor reflex (upper tracing) without any effect on blood pressure (lower tracing). Time marks 30 s.

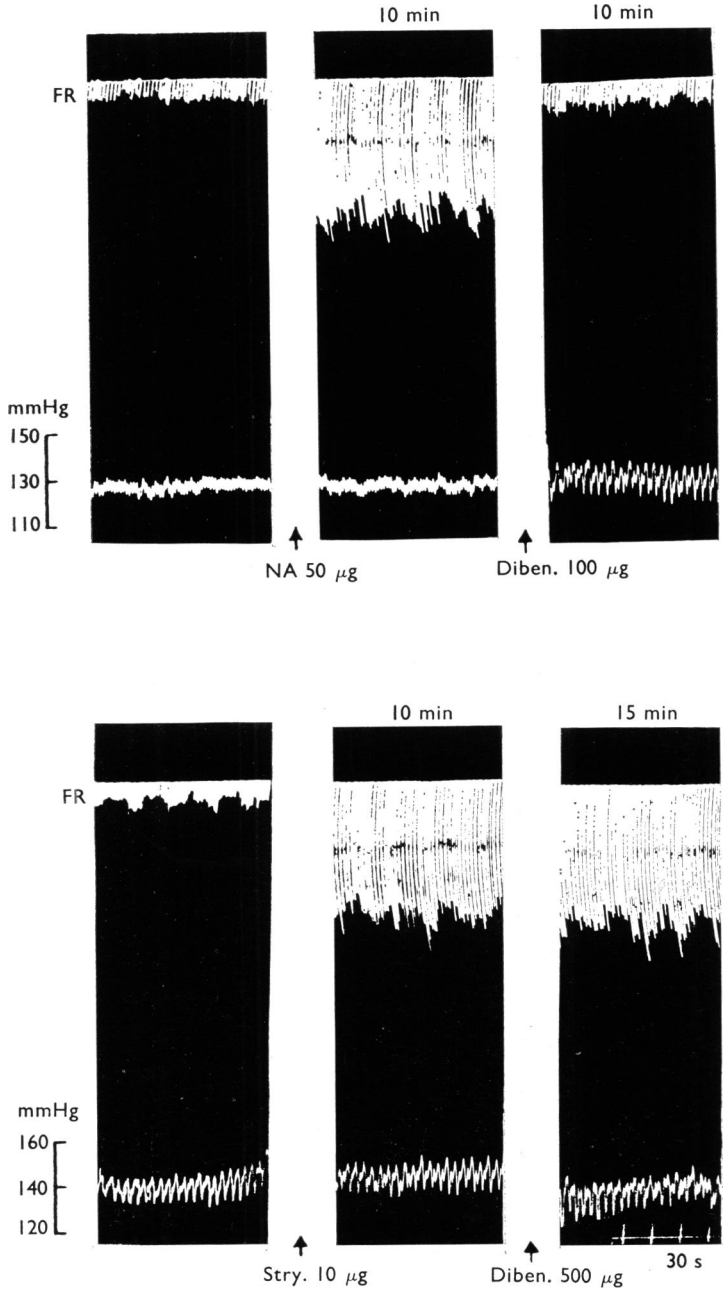

FIG. 3. Cat (2·8 kg) anaesthetized with chloralose (80 mg/kg intravenously). The upper panels show facilitation of FR (upper tracing) 10 min after 50 μg NA intrathecally without any effect on blood pressure (lower tracing) and its blockade by 100 μg of dibenamine. The lower panels show a similar facilitation of FR 10 min after 10 μg strychnine intrathecally and inability of dibenamine 500 μg intrathecally to block this. Note there is no significant change in blood pressure with strychnine. Time 30 s.

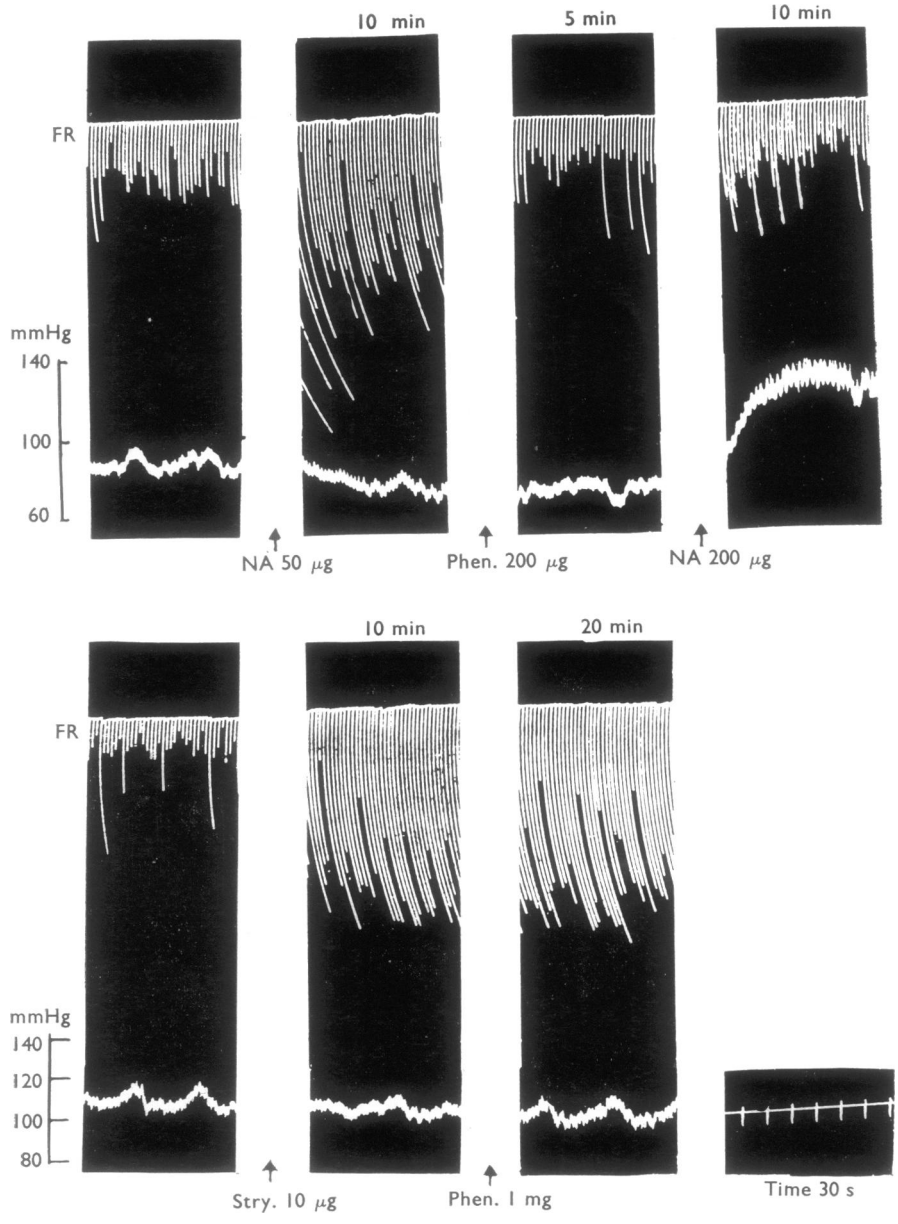

FIG. 4. Cat (3·0 kg) anaesthetized with chloralose 80 mg/kg intravenously. The upper panels show the facilitatory effects on FR of NA 50 μg intrathecally which is completely blocked by 200 μg phentolamine intrathecally in 5 min (panel 3). Subsequently even high doses of NA (200 μg intrathecally) are ineffective (panel 4) though a rise in blood pressure is produced, probably because of leakage into the systemic circulation. The lower set shows the inability of a much higher dose of phentolamine (panel 3) to block the facilitatory effect of strychnine (10 μg intrathecally) on FR (panel 2). Time mark 30 s applies to both sets.

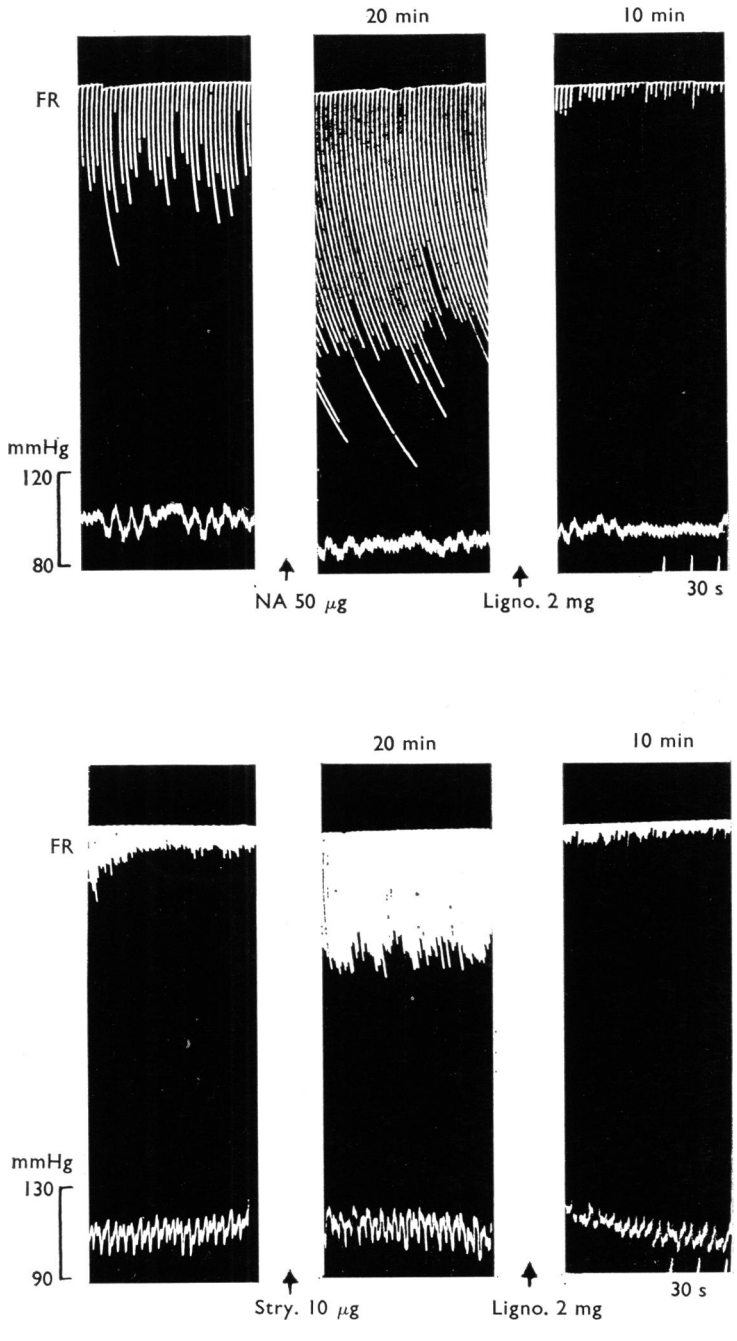

FIG. 5. Cat (4·0 kg) anaesthetized with chloralose (80 mg/kg intravenously). The last panel of the upper set shows the blocking effect of lignocaine (2 mg intrathecally) on NA (50 μg intrathecally) induced facilitation of FR (panel 2). In the lower set strychnine (10 μg intrathecally) induced facilitation of FR (panel 2) is also shown to be blocked by a similar dose of lignocaine (panel 3). (The time between panels 1 and 2 is 20 min and between 2 and 3 is 10 min in both sets.) The time mark applies to all the panels.

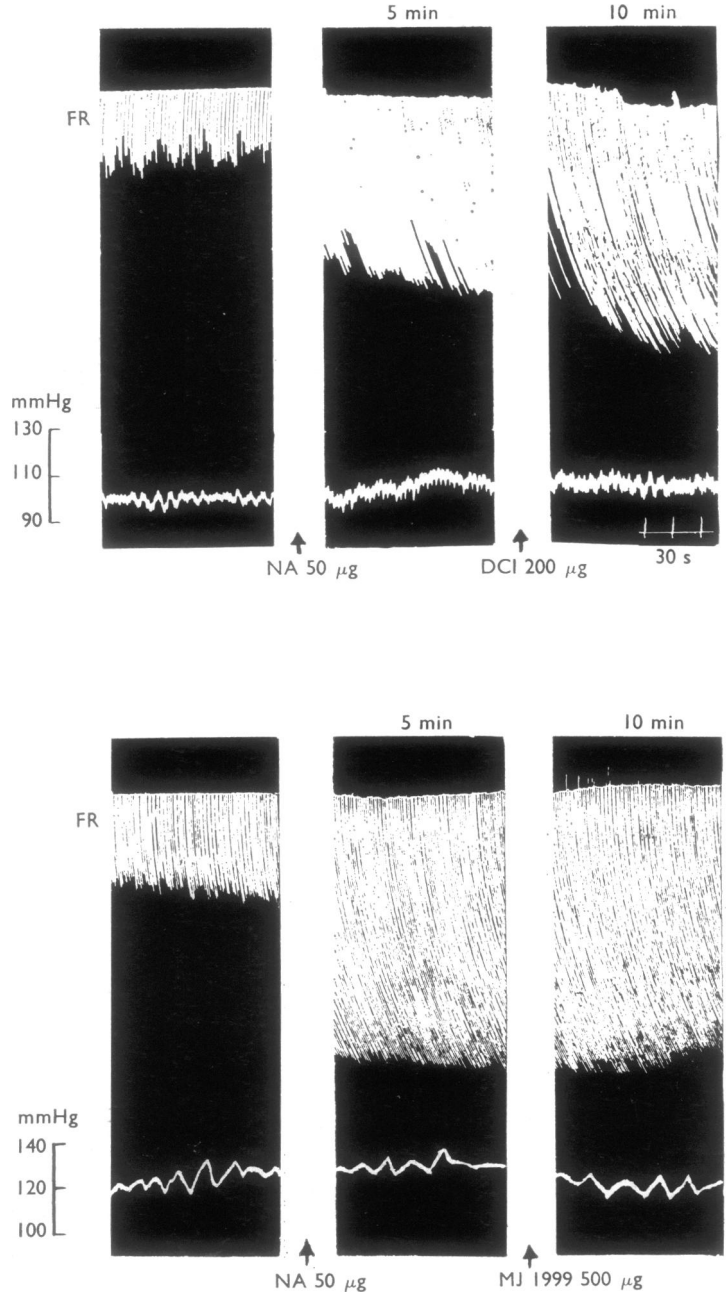

FIG. 6. Cat (3·8 kg) anaesthetized with chloralose (80 mg/kg intravenously). The upper set shows the facilitatory effect of NA (50 μg intrathecally) on FR (panel 2) which is unaffected (panel 3) by dichloroisopropylnoradrenaline (DCI, 200 μg intrathecally). The lower set similarly shows the ineffectiveness (panel 3) of MJ 1999 (500 μg intrathecally) in blocking the facilitatory response of the same dose of NA on FR (panel 2). (Panels 1 and 2 are separated by 5 min and 2 and 3 by 10 min in both the sets.)

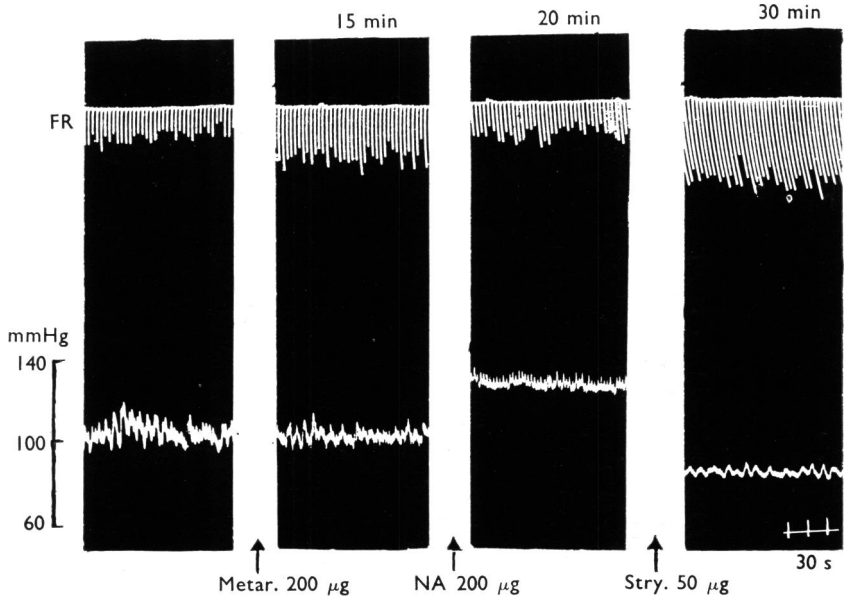

FIG. 7. Cat (3·0 kg) under chloralose anaesthesia (80 mg/kg intravenously). Metaraminol (200 μg intrathecally) produced a slight facilitation of FR (panel 2) but subsequently NA failed to produce any facilitatory effect (panel 3) though strychnine (50 μg intrathecally) is still effective (panel 4). Note also a rise in blood pressure following a large dose of NA (see Fig. 4).

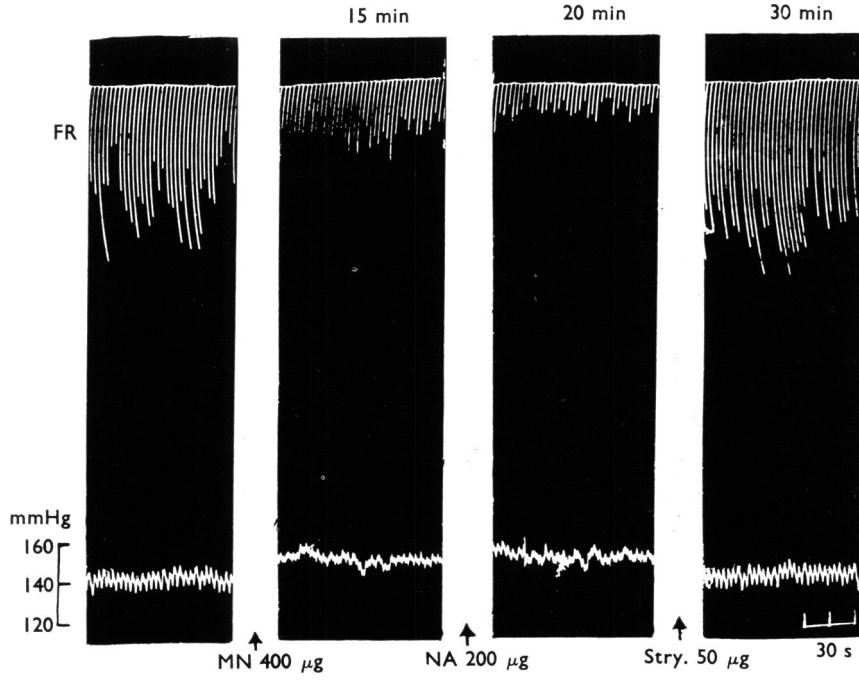

FIG. 8. Cat (3·5 kg) anaesthetized with chloralose (80 mg/kg intravenously) shows that α-methyl-noradrenaline (400 μg intrathecally) slightly depressed the FR (panel 2). Like metaraminol (Fig. 7), however, the facilitatory effect of NA (200 μg intrathecally) has been blocked (panel 3) while strychnine is still effective (panel 4).

Metaraminol and α-methyl-noradrenaline were used for this purpose. It was observed that metaraminol (200 μg) produced some facilitation of FR but after its administration, NA (up to 200 μg) failed to produce any effect, even though strychnine still elicited a facilitatory response (Fig. 7).

In contrast to metaraminol, α-methyl-noradrenaline (400 μg) had a slight depressant effect on FR. Nevertheless, as in the case of metaraminol, there was no effect of NA after administration of α-methyl-noradrenaline, while strychnine still produced a facilitatory effect (Fig. 8).

Effect of vasoconstriction and vasodilatation

Catecholamines produce marked vascular effects by acting on the α- (vasoconstrictor) and β- (vasodilator) adrenoceptors in blood vessels. To ensure that the effect of drugs on FR may not be due to vascular changes following their intrathecal administration, vasopressin (vasoconstrictor) and histamine (vasodilator) were administered intrathecally. It was observed that vasopressin (up to 60 mu.) and histamine (50 μg) did not show any effect on FR or on blood pressure (Fig. 9).

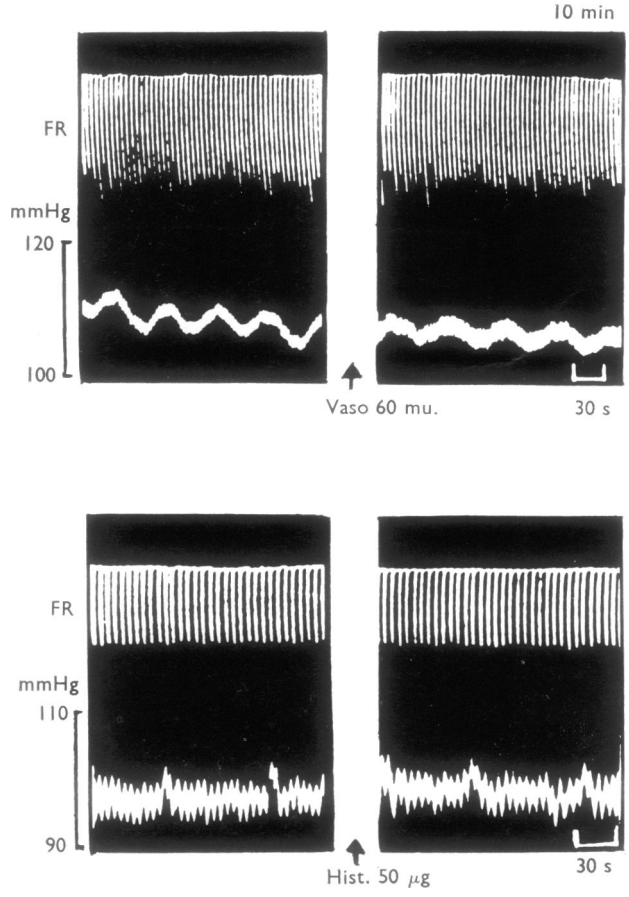

FIG. 9. Cat (4·0 kg) anaesthetized with (80 mg/kg intravenously) chloralose. The effect of a vasoconstrictor (vasopressin 60 mu. intrathecally) and a vasodilator (histamine 50 μg intrathecally) agent on FR. Vasopressin (upper set) and histamine (lower set) do not show any effect either on the FR (upper tracing) or on blood pressure (lower tracing).

When these agents were administered intravenously in the same doses, however, vasopressin produced a significant rise (40–60 mmHg) and histamine a steep fall in the blood pressure.

Discussion

Presence of α-adrenoceptors on spinal neurones was suggested by Andén *et al.* (1966a). These workers chiefly used dopa to support their hypothesis and concluded that when injected intravenously dopa acts by liberating transmitter, possibly NA, from a descending noradrenergic pathway, which acts on the α-adrenoceptors present on the spinal neurones.

In the present investigation it was found that among the adrenoceptor stimulants studied the adrenoceptor stimulant NA showed the most marked facilitatory effect on FR. It produced a significant and sustained enhancement of FR which was dose dependent (see Fig. 1). Neosynephrine, another α-adrenoceptor stimulant, also produced facilitation of FR. Further, the effect of NA was completely blocked by the α-adrenoceptor blocking agents, dibenamine and phentolamine. The blockade of NA response by α-adrenoceptor blocking agents was specific for a similar response to strychnine was unaffected (Fig. 4). Nor could this be due to their local anaesthetic activity because the local anaesthetic lignocaine blocked the response both to NA and to strychnine (see **Results**). McLennan (1961) reported effects of dopamine on the patellar reflex which, according to Andén *et al.* (1966a), could be due to the vasoconstrictor effect of dopamine. Since NA also produced local vasoconstriction it was considered important to exclude this possibility in the present experiments. As pointed out in **Results**, another vasoconstrictor agent, vasopressin, had no effect on FR. Furthermore, if facilitation was due to vasoconstriction, vasodilatation should have produced the opposite effect. This was not observed either with histamine or with the β-adrenoceptor stimulants, isoprenaline and salbutamol. It is therefore reasonable to conclude that the facilitation of FR by NA in the present experiments is due to a neuronal effect of NA and not a vascular effect. The inability of β-adrenoceptor stimulants to affect the FR and the ineffectiveness of β-adrenoceptor blocking agents to modify NA potentiation strongly suggest that β-adrenoceptors do not have a role in the neuronal integration of this reflex. Finally, the inability of α-adrenoceptor blocking agents to modify strychnine-induced facilitation suggest that NA and strychnine probably produce facilitation by different mechanisms.

Andén *et al.* (1966a) have reported facilitation of FR following intravenous administration of dopa. It is well known that dopa reaches the central nervous system and is metabolized into NA and dopamine. As pointed out earlier, intrathecal administration of dopamine has no influence on FR in the present investigation. It is logical to conclude, therefore, that facilitation obtained by Andén *et al.* (1966a) has been due to formation of NA from dopa.

Metaraminol and α-methyl-noradrenaline have been reported to act as false neurotransmitters (Crout & Shore, 1964 ; Day & Rand, 1963) peripherally in the adrenergic system. It is interesting to note that in the present study also, metaraminol could weakly simulate the effect of NA. Further, when given intrathecally both these substances could block the facilitatory effect of NA. These findings are in agreement with the recent observations of Boakes, Candy & Wolstencroft (1968),

who have applied α-methyl-noradrenaline electrophoretically to reticular neurones. They have reported that both excitatory and inhibitory effects of NA could be mimicked by α-methyl-noradrenaline and that the excitatory effect of NA could be blocked by α-methyl-noradrenaline. It appears that receptors for catecholamines in the spinal cord and in the brain stem resemble each other and behave differently from peripheral receptors. It would be interesting to study the effects of metaraminol on the NA sensitive neurones in the brain stem.

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