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Actions of noradrenaline, other sympathomimetic amines and antagonists on neurones in the brain stem of the cat

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Summary

1. The effects of (−)-noradrenaline ((−)-NA) and related compounds on brain stem neurones in decerebrate unanaesthetized cats have been investigated using the technique of iontophoretic application from micropipettes.

2. Four types of response to (−)-NA have been described. These were short lasting inhibition, long lasting inhibition, excitation, and a biphasic response consisting of short lasting inhibition followed by excitation. A variable amount of desensitization of the excitatory response, but not of inhibitory responses, was observed.

3. Experiments in which small currents were used to pass (−)-NA from pipettes with smaller tips did not lead to any appreciable change in the proportions of neurones excited or inhibited.

4. A variety of sympathomimetic agonists was tested. Short lasting inhibition was less sensitive than excitation to changes in molecular structure. Long lasting inhibition was more sensitive to molecular change and was not mimicked by some of the agonists which mimicked short lasting inhibition.

5. Although agonists without one ring hydroxyl had weaker effects than those with both, compounds in which both ring hydroxyl groups were absent (β-hydroxyphenylethylamine, ephedrine and amphetamine) mimicked excitation strongly. It is possible that the compounds without both ring hydroxyl groups had some effect other than simple agonistic activity.

6. A dissociation was observed between responses to dopamine and (−)-NA. *p*-Tyramine mimicked dopamine, rather than (−)-NA.

7. Neither the α-agonist, phenylephrine nor the β-agonist, isoprenaline mimicked neuronal responses to (−)-NA. The α-antagonists phentolamine and phenoxybenzamine and the β-antagonists dichloroisoprenaline, propranolol and D(−)-INPEA and combinations of propranolol with phentolamine or phenoxybenzamine were ineffective in blocking either excitation or inhibition. Thus, the central receptors appear to be different from peripheral α- and β-receptors.

8. The most effective antagonist of excitation was (−)-α-methylnoradrenaline. Metaraminol and dihydroergotamine also had some antagonistic activity. None

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of the compounds tested blocked inhibition. The effects of (−)-α-methylnoradrenaline have been discussed in relation to the hypotensive action of α-methyldopa.

Introduction

Noradrenaline has long been considered a possible synaptic transmitter in the central nervous system. Vogt (1954) showed that both adrenaline and noradrenaline are present in the mammalian brain, the noradrenaline content being much greater than that of adrenaline, and that the distribution of both amines is uneven, the concentrations being higher in the hypothalamus and medulla than in other parts of the brain. The technique of fluorescence histochemistry has demonstrated the presence of catecholamine-containing cells and also diffuse nerve terminals ending on cell bodies in the medulla (Dahlström & Fuxe, 1964 ; Fuxe, 1965). These terminals appear to be derived from neurones in the brain stem (Andén, Dahlström, Fuxe, Olson & Ungerstedt, 1966).

The effects observed with systemic administration of catecholamines have not provided any clear indication as to their role in the central nervous system. Both activation (Bonvallet, Dell & Hiebel, 1954) and depression (Feldberg & Sherwood, 1954) have been observed. The direction of the effect obtained appears to depend upon the route of administration and the presence of an intact blood brain barrier (Key & Marley, 1962 ; Marley, 1966). It has been suggested (Baust & Niemczyk, 1964) that the central excitation produced by systemically injected catecholamines is due entirely to the peripheral hypertension which acts reflexly on brain stem arousal mechanisms, but there are some observations which cannot be explained by this hypothesis (Aldridge, 1965). Certainly, it appears that the effects of catecholamines, administered by the usual routes, are not the same as those of the endogenously released substances. The fact that many drugs which affect mental function modify catecholamine metabolism in the brain supports the concept that these substances play an important role.

Effects on the activity of single neurones in the brain stem of decerebrate cats with systemic injections of adrenaline and noradrenaline were first shown by Bonvallet, Dell & Hugelin (1954) and Bradley & Mollica (1958), both excitation and inhibition being observed. However, these effects could have been due to indirect actions of the amines. The technique of microiontophoresis has made it possible to apply active substances locally to neurones whilst recording their electrical activity, and this technique has been widely used in studies on the central nervous system. In early studies, noradrenaline produced inhibitory effects in many regions (see reviews by Bradley, 1968 ; Hebb, 1970 ; McLennan, 1970). Other studies, particularly those in which the use of barbiturate anaesthetized preparations was avoided, have shown that excitatory responses to catecholamines can be observed (Bradley & Wolstencroft, 1962, 1964a, 1965). Furthermore, the actions of certain centrally active drugs, such as chlorpromazine (Bradley, Wolstencroft, Hösli & Avanzino, 1966b) have been related to noradrenaline sensitive neurones in the brain stem.

Our study represents a detailed analysis of the action of (−)-noradrenaline ((−)-NA) in terms of the types of responses observed and the actions of agonists and antagonists. The pharmacological characteristics of responses to noradrenaline

in the brain stem are discussed in relation to those found in other regions of the brain and at peripheral receptors. The results have been reported in preliminary communications (Boakes, Bradley, Brookes & Wolstencroft, 1968 ; Boakes, Bradley, Brookes, Candy & Wolstencroft, 1969).

Methods

Adult cats of either sex were decerebrated under halothane anaesthesia, and 1–2 h after withdrawal of the anaesthetic, micropipettes were inserted through the ventral surface of the medulla and pons, or through the dorsal surface after removal of the medial portions of the cerebellum (Bradley, Dhawan & Wolstencroft, 1966a).

Glass multibarrelled micropipettes were used to record neuronal activity and to eject drugs into the vicinity of the cells. Only spontaneously active neurones were studied, most of which were located in the region 3·0–6·5 mm rostral to the obex and 0·5–1·5 mm lateral to the midline. Unit activity was amplified and counted as described by Bradley & Wolstencroft (1964b). Firing rates were plotted as mean frequencies, in spikes s^{-1}, during successive 5 s epochs. The micropipettes normally had four or five barrels, with an overall tip diameter of 5–10 μm, but in some experiments three barrelled electrodes with 3–5 μm tips were used. The recording barrel contained 4 M NaCl and one barrel always contained approximately 1 M NaCl to determine the effects of the current. The other barrels contained an appropriate selection of the following drugs in aqueous solution: (−)-noradrenaline base, (−)-noradrenaline bitartrate (B.D.H.) ; (+)-noradrenaline bitartrate (Sterling-Winthrop) ; (−)-noradrenaline bitartrate, *p*-tyramine hydrochloride, dopamine hydrochloride, (−)-phenylephrine hydrochloride, *p*-Sympatol tartrate, (−)-ephedrine hydrochloride (Koch-Light) ; 2-aminoheptane sulphate, β-hydroxyphenylethylamine (K & K Laboratories) ; (−)-isoprenaline bitartrate (John Wyeth) ; (−)-α-methylnoradrenaline (Corbasil, Hoechst) ; metaraminol tartrate (Merck, Sharp & Dohme) ; phentolamine hydrochloride (Rogitine, CIBA) ; dihydroergotamine methanesulphonate (Sandoz) ; phenoxybenzamine hydrochloride (Dibenyline, Smith, Kline & French) ; dichloroisoprenaline hydrochloride (Ralph N. Emmanuel) ; propranolol hydrochloride (Inderal, I.C.I.) ; D(−)-INPEA (2-isopropylamino-l-[*p*-nitrophenyl] ethanol hydrochloride) (Selvi & Co.).

Most drugs were used as 10% solutions adjusted to pH 5·0–6·0. Compounds with peripheral adrenergic blocking effects, which were often applied for long periods, were used as more dilute solutions (1–5%) to reduce fluctuations in electrode resistance during ejection. The micropipettes were filled 48 h before use and stored at 4° C in the dark. All compounds were expelled iontophoretically as cations. (−)-NA and agonists were ejected with currents of 10–100 nA, usually 50 nA, for 30 s periods, but compounds with possible blocking effects were often applied for longer periods. Low currents were used to expel compounds from three-barrelled electrodes with small tips. A retaining current of 15 nA was used to prevent unwanted diffusion of compounds from the electrode. Bradley & Candy (1970) have shown that this retaining current prevents detectable leakage of labelled NA from micropipettes similar to those used in these experiments. It has also been shown that the amount released is directly proportional to the electrical charge passed, within the range normally used for microiontophoretic studies (Bradley & Candy, 1970).

Results

Response to (−)-noradrenaline

The effect of iontophoretically applied (−)-NA was observed on the spontaneous firing rate of 620 neurones in the lower brain stem. (−)-NA was usually applied for 30 s periods with currents between 10 and 100 nA. No effect was observed on 216 (35%) of the neurones studied. The firing rate of 25 (4%) was changed to a new level by the first application of (−)-NA, but no recovery was seen and these neurones were unresponsive to further applications. Amongst the neurones which were sensitive to (−)-NA, both inhibition and excitation were observed, in accordance with earlier findings in the brain stem (Bradley & Wolstencroft, 1962, 1964a, 1965). However, in this study it was possible to distinguish four types of response: excitation, two types of inhibition and excitation preceded by an inhibitory phase.

Inhibition

Inhibitory responses were shown by 158 (25%) of the neurones studied and in most cases (110 neurones, 17·5%) the inhibition was of the short lasting type.

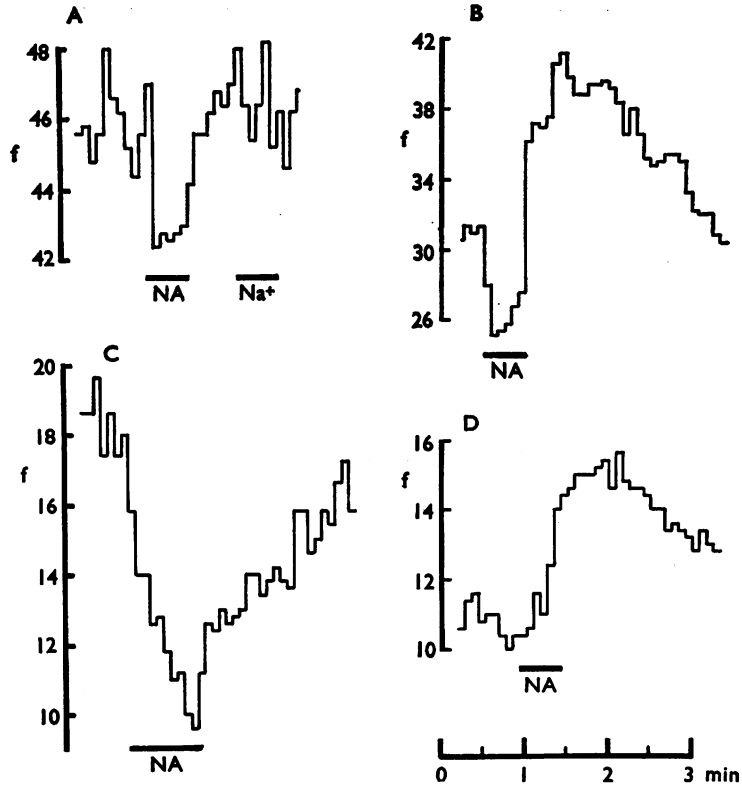

FIG. 1. Effects of (−)-NA on the spontaneous firing rates of four neurones in the brain stem. The mean firing rate in impulses s^{-1} (f) in successive 5 s epochs is plotted against time in minutes. Iontophoretic applications of (−)-NA (NA), or of a current control (Na^+), are shown by horizontal bars. The short lasting and long lasting inhibitory responses are shown in A and C. A simple excitatory response is shown in D, and excitation preceded by inhibition in B. All these responses were obtained using micropipettes of small tip size (3–5 μm) and small iontophoretic currents (10–25 nA). The responses shown in A, B and C were obtained in one penetration.

Short lasting inhibition began within the first 5 s after the (−)-NA application and ended within 5 s after the current had been switched off (Figs. 1A, 3A). The extent of inhibition often increased slightly during the application but the greater part of the depression of firing rate occurred within the first 15 seconds. The degree of

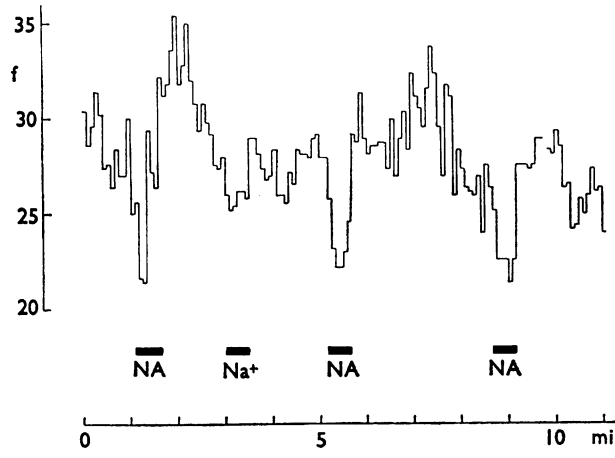

FIG. 2. Desensitization of the excitatory response with repeated applications of (−)-NA. The decrease in size of the excitation reveals a short lasting inhibitory effect.

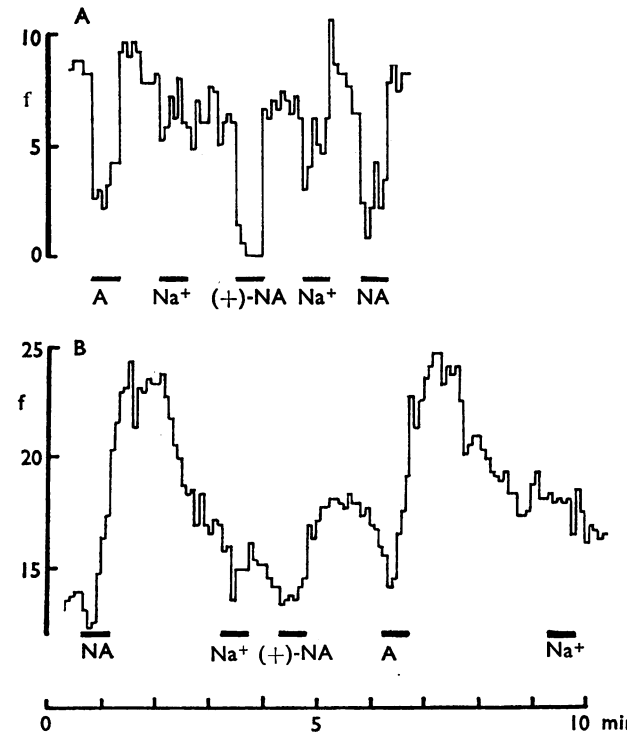

FIG. 3. A, Inhibitory effects of (+)-NA and of (−)-adrenaline (A), compared with those of (−)-NA (NA). B, Excitatory effects of (+)-NA and (−)-adrenaline compared with that of (−)-NA.

reduction of firing rate varied considerably. Care was taken to differentiate between short lasting inhibition by (−)-NA and that caused by an identical current passed through the control barrel which contained sodium chloride solution.

An inhibitory response of much longer duration was observed with 48 neurones (7·5%) (Figs. 1C, 4A). Long lasting inhibition was always clearly distinguishable from the short lasting type, and on no occasion was there any doubt about the classification. The particular characteristic of the long lasting response was that spike frequency returned very gradually towards its previous level, or to a lower one, over a period of 2·5 min or more after the current was switched off. The latency of onset was not more than 10 seconds. The time taken to reach peak value of depression was long, about 60 s after switching on the current. Thus, the peak was often not reached until after the current had been switched off.

Excitation

Excitatory responses were shown by 221 (36%) of the neurones. When excitation was not preceded by a transient inhibition, the latency of the response was variable but usually of the order of 20 seconds. The rate of firing reached a peak 10–30 s after the application had been terminated and then gradually subsided over a further period of 0·5–3·5 min depending on the degree of excitation (Figs. 1D, 3B).

When a transient inhibitory phase preceded the excitatory response (63 neurones, (10%), the latency of excitation varied between 10 and 45 s (Fig. 1B). Peak excitation was reached 5–65 s after the end of a 30 s application and the spike frequency then fell over a further period of up to 4 minutes.

When an excitatory response was observed, irrespective of whether it was preceded by inhibition, subsequent applications of (−)-NA often resulted in a reduced

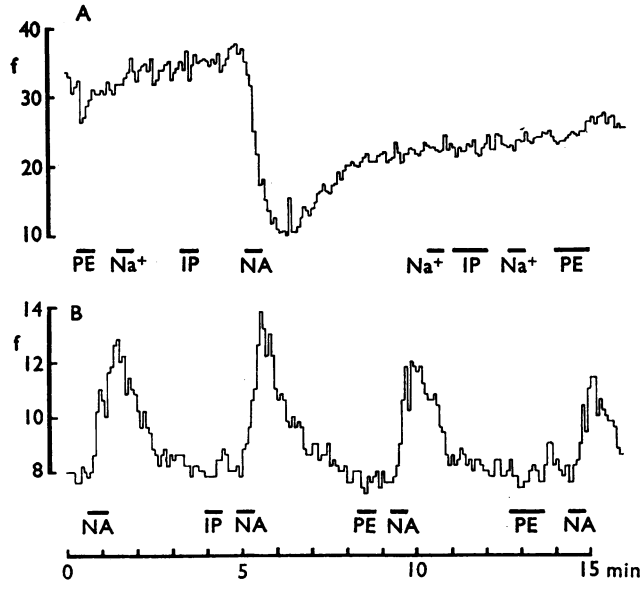

FIG. 4. Actions of (−)-isoprenaline (IP) and (−)-phenylephrine (PE), compared with A inhibition, and B excitation by (−)-NA. The excitatory response to (−)-NA shown in B shows slight desensitization.

response. The degree of this desensitization was very variable. Thus, marked desensitization is shown in Fig. 2, while the effect is only slight in Fig. 4B. Desensitization was more evident when the interval between applications of (−)-NA was short. An extreme example of desensitization may be represented by the few

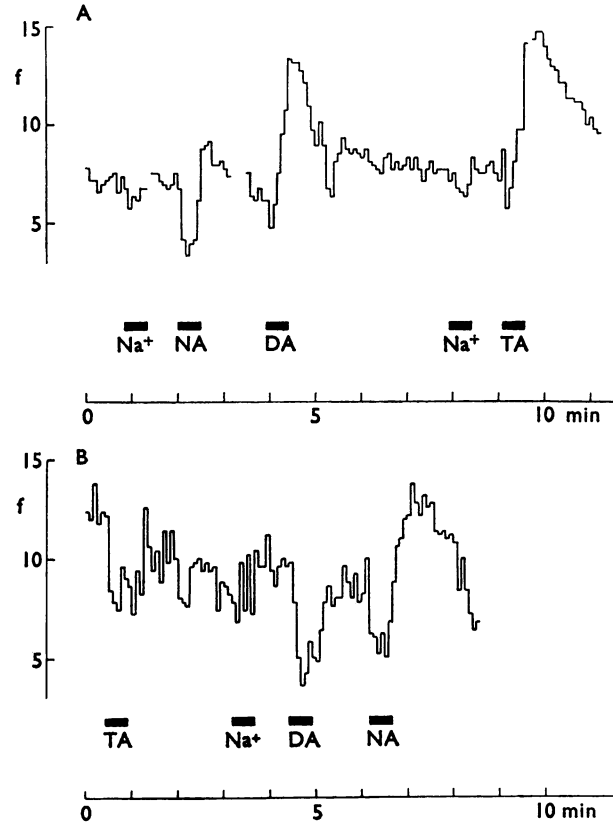

FIG. 5. A, Excitatory effects of dopamine (DA) and *p*-tyramine (TA) on a neurone inhibited by (−)-NA. B, Inhibitory effects of DA and TA on a neurone excited by (−)-NA.

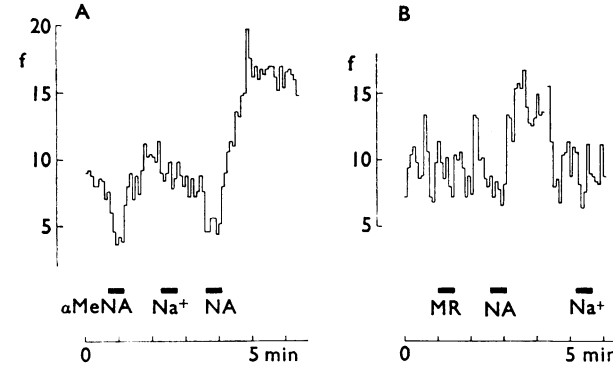

FIG. 6. A, Biphasic responses to (−)-α-methylnoradrenaline (α-MeNA) and (−)-NA. Note that the excitatory effect of α-MeNA is less than that of (−)-NA. B, Lack of effect of metaraminol (MR) on a neurone excited by (−)-NA.

neurones which were excited by the first application of (−)-NA but unaffected by subsequent applications.

When the excitatory component of a biphasic response was desensitized (Fig. 2), or blocked by an antagonist (Figs. 8A, 9B), the preceding inhibitory phase was found to be of the short lasting type. Occasionally, blockade of an excitatory response revealed a short lasting inhibitory component, which was not previously observed (Fig. 9B).

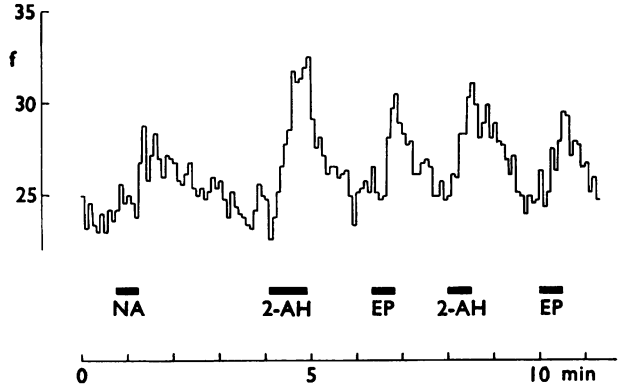

FIG. 7. Excitatory effects of ephedrine (EP) and 2-aminoheptane (2-AH) on a neurone weakly excited by (−)-NA. Excitation by these amines was of shorter latency and duration than excitation by (−)-NA.

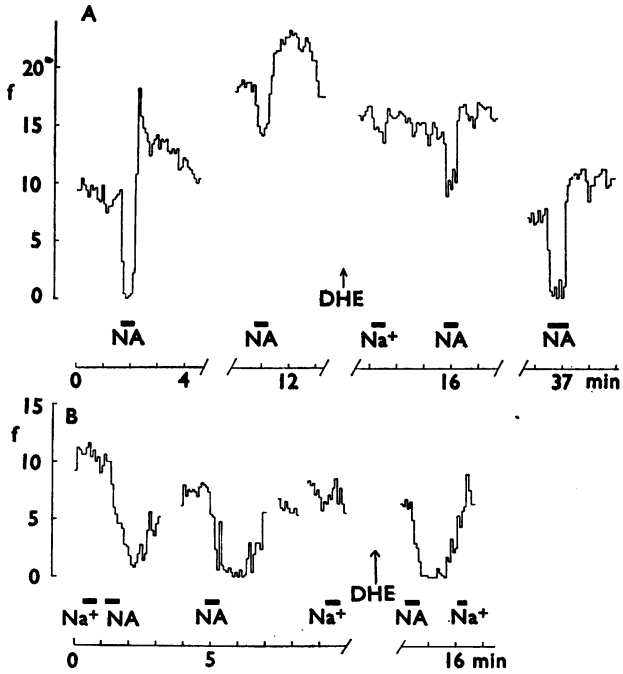

FIG. 8. A, Antagonism by dihydroergotamine (DHE) of the excitatory action of (−)-NA. Two applications of (−)-NA at 2 min and 11 min elicited excitation. After applying DHE for 5 min at 100 nA, the excitatory response to (−)-NA was reduced. Fifteen minutes after the end of the application of DHE, the excitatory response showed recovery. B, Absence of antagonism by DHE to a prolonged inhibitory effect of (−)-NA. Note that the short lasting inhibition preceding the excitation shown in A was not blocked by DHE.

Reduction of pipette size and current strength

The possibility must be considered that, of the effects observed, one (for example, inhibition) might be a direct effect and the other (excitation), indirect, or *vice versa.* Thus, (−)-NA might facilitate or inhibit a small neurone, functionally connected and adjacent to the one being recorded. It would then be expected that a reduction in the iontophoretic current, combined with the use of micropipettes with smaller tips, which would allow smaller neurones to be studied, might result in a change in the proportions in the different types of responses.

The four types of responses were still observed when small currents (10–25 nA) were used to expel (−)-NA from micropipettes of tip sizes 3–5 μm (Fig. 1). The responses shown in Fig. 1A, B and C were observed during one penetration with a

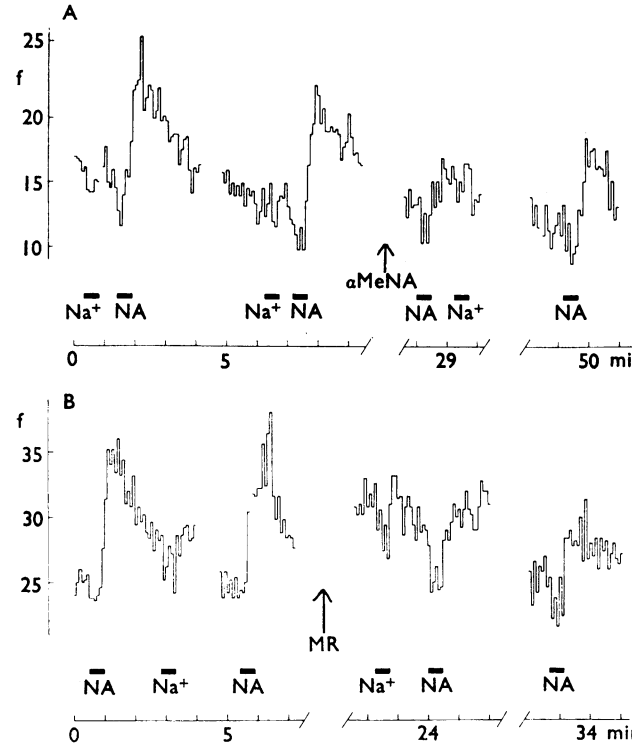

FIG. 9. Antagonism by α-methylated amines to excitatory actions of (−)-NA. A, (−)-α-Methylnoradrenaline: two applications of (−)-NA produced consistent excitation. One minute after applying α-MeNA for 5 min (25–50 nA), the excitatory response to (−)-NA was markedly reduced. Recovery was seen 21 min after the end of the application of α-MeNA. B, Metaraminol (MR) was applied for 11 min (50–75 nA). (−)-NA excitation was blocked 5 min after the end of the application of MR, and recovered 9 min later. Blockade of the excitatory response to (−)-NA revealed a short lasting inhibitory response.

TABLE 1. *Proportions of responses to* (−)*-NA with electrodes of different tip size*

	(—)-Noradrenaline			
Tip size	+	−	○	No. of neurones
3– 5 μm	44%	18%	38%	71
6–10 μm	38%	26%	36%	280

The figures represent the numbers of neurones showing the responses indicated: +, excitation; −, inhibition; ○, no effect.

pipette of overall tip size 5 μm. Table 1 compares the proportion of excitation and inhibition obtained with pipettes of tip size 3–5 μm and 6–10 μm. For the purpose of this comparison, biphasic effects have been classed as excitation and both types of inhibition combined. Reduction in tip size and current strength did not change appreciably the proportions of excitatory and inhibitory responses.

Actions of structurally related amines

In these experiments (−)-NA was always present in one of the barrels of the micropipette and its action compared with the actions of related amines on each neurone. Some of the salts used were tartrates but it was established in control experiments that tartrate ions did not modify either excitatory or inhibitory actions of (−)-NA.

The actions of the (+)-isomer of noradrenaline ((+)-NA) were compared with those of (−)-NA on fifty-nine neurones (Table 2A). The short lasting inhibitory responses to (+)-NA were similar to those of (−)-NA, but its excitatory effects were feeble or absent (Fig. 3). (−)-Adrenaline mimicked the actions of (−)-NA more closely (Table 2B), although excitatory responses were usually smaller (Fig. 3B). Neither (+)-noradrenaline nor (−)-adrenaline had any actions on neurones unaffected by (−)-NA (Table 2A and 2B).

(−)-Isoprenaline is an agonist at peripheral β-receptors, and the peripheral effects of (−)-phenylephrine are largely specific to α-receptors. Both of these substances

TABLE 2. *Comparison of the effects of (+)-noradrenaline and (−)-adrenaline with those of (−)-NA when applied to the same neurone*

A		(+)-Noradrenaline			
		+	−	○	Total No.
	+	8	1	12	21
(−)-NA	−	0	12	4	16
	○	0	0	22	22

B		(−)-Adrenaline			
		+	−	○	Total No.
	+	12	1	3	16
(−)-NA	−	0	10	1	11
	○	0	0	17	17

The figures represent the numbers of neurones showing the responses indicated: +, excitation; −, inhibition; ○, no effect.

TABLE 3. *Comparison of the effects of (−)-isoprenaline, (−)-phenylephrine and* p-*Sympatol, with those of (−)-NA when applied to the same neurone*

A		(−)-Isoprenaline			
		+	−	○	Total No.
	+	0	1	8	9
(−)-NA	−	0	3	13	16
	○	0	0	18	18

B		(−)-Phenylephrine			
		+	−	○	Total No.
	+	2	0	11	13
(−)-NA	−	4	4	9	17
	○	1	0	21	22

C		*p*-Sympatol			
		+	−	○	Total No.
	+	0	0	3	3
(−)-NA	−	0	6	3	9
	○	0	0	14	14

were relatively ineffective on neurones which responded to (−)-NA, even when applied with a current of 50 nA for 1 min or more (Table 3A & 3B, Fig. 4). Whereas (−)-isoprenaline had no excitatory effects, (−)-phenylephrine occasionally produced excitation but this was not necessarily related to excitation by (−)-NA.

p-Sympatol is a racemic mixture, and otherwise differs from (−)-phenylephrine only in that the phenolic hydroxyl group is in the *p*-position. No excitatory responses to *p*-Sympatol were observed but it usually mimicked short lasting inhibition by (−)-NA (Table 3C).

A dissociation was found between responses to dopamine and (−)-NA, the actions of which were compared on 74 neurones (Table 4A, Fig. 5). Dopamine sometimes mimicked the actions of (−)-NA, but short lasting inhibitory effects were often produced on neurones which gave excitatory responses to (−)-NA (Fig. 5B). Excitation by dopamine when (−)-NA inhibited was rare (Table 4A). No neurones were found which were affected by dopamine and not by (−)-NA, although 21% of neurones which responded to (−)-NA were unaffected by dopamine. The actions of *p*-tyramine and (−)-NA are compared in Table 4B and Fig. 5. *p*-Tyramine mimicked the effects of dopamine (Table 4C) rather than those of (−)-NA.

Two α-methylated amines, (−)-α-methylnoradrenaline and metaraminol, were studied. Both mimicked the excitatory and short lasting inhibitory actions of (−)-NA, but excitatory effects were always weaker, particularly in the case of metaraminol, which often failed to excite at all (Table 5, Fig. 6). When applied for longer periods, these substances antagonized excitatory effects of (−)-NA (see below).

TABLE 4. *Comparison of the effects of dopamine and* p-*tyramine with those of* (−)-*NA applied to the same neurone, and* p-*tyramine and dopamine applied to the same neurone*

A		Dopamine			
		+	−	○	Total No.
	+	13	11	3	27
(−)-NA	−	3	21	10	34
	○	0	0	13	13

B		*p*-Tyramine			
		+	−	○	Total No.
	+	6	5	4	15
(−)-NA	−	3	8	6	17
	○	0	0	7	7

C		*p*-Tyramine			
		+	−	○	Total No.
	+	4	1	1	6
Dopamine	−	0	10	1	11
	○	0	0	6	6

TABLE 5. *Comparison of the effects of α-methylnoradrenaline and metaraminol with those of* (−)-*NA when applied to the same neurone*

A		α-Methylnoradrenaline			
		+	−	○	Total No.
	+	11	2	4	17
(−)-NA	−	0	11	1	12
	○	0	0	1	1

B		Metaraminol			
		+	−	○	Total No.
	+	2	2	9	13
(−)-NA	−	0	8	0	8
	○	0	0	14	14

Ephedrine and β-hydroxyphenylethylamine, which have no phenolic hydroxyl groups, and 2-aminoheptane, an aliphatic amine, all mimicked the excitatory and short lasting inhibitory actions of (−)-NA (Table 6, Fig. 7). This group of substances sometimes produced powerful excitatory effects, frequently accompanied by a decrease in spike amplitude. Ephedrine often produced a purely excitatory response when the response to (−)-NA was biphasic.

The following agonists, (−)-isoprenaline, (−)-phenylephrine, *p*-Sympatol, dopamine and tyramine never produced long lasting inhibition of neurones which gave this response to (−)-NA.

TABLE 6. *Comparison of the effects of ephedrine, α-hydroxyphenylethylamine and 2-aminoheptane with those of (−)-NA when applied to the same neurone*

A — Ephedrine

		+	−	○	Total No.
(−)-NA	+	7	0	2	9
	−	1	4	2	7
	○	0	0	8	8

B — β-Hydroxyphenylethylamine

		+	−	○	Total No.
(−)-NA	+	3	0	0	3
	−	0	4	1	5
	○	0	0	7	7

C — 2-Aminoheptane

		+	−	○	Total No.
(−)-NA	+	4	0	3	7
	−	0	2	2	4
	○	0	0	2	2

With regard to structure-activity relationships, the following generalizations can be made, based on the data summarized in Table 7.

1. The excitatory effects of noradrenaline are stereo-isomerically specific, but the inhibitory effects are not. In general, the short lasting inhibitory action was less sensitive to changes in structure.

TABLE 7. *Ability of each agonist tested to mimic the actions of (−)-NA*

Compound	Molecular change: Ring	Molecular change: Side chain	Excitatory actions	Inhibitory actions*
(−)-NA			+++++	−−−−−
(−)-Adrenaline		N-CH_3	++++	−−−−−
(+)-Noradrenaline		Stereoisomer	++	−−−−−
(−)-Isoprenaline		N-C_3H_7	0	−
(−)-α-Methylnor-adrenaline		α-CH_3	++	−−−−−
Dopamine		No β-OH	+++	−−−
(−)-Phenylephrine	No *p*-OH	N-CH_3	++	−−−
Metaraminol	No *p*-OH	α-CH_3	+	−−−
p-Sympatol	No *m*-OH	N-CH_3	0	−−−
p-Tyramine	No *m*-OH	No β-OH	++	−−−
β-Hydroxyphenyl-ethylamine	No *p*-, *m*-OH		++++	−−−
(−)-Amphetamine†	No *p*-, *m*-OH	No β-OH,α-CH_3	++++	−−−−
Ephedrine	No *p*-, *m*-OH	N-CH_3,α-CH_3	++++	−−−
2-Aminoheptane	$CH_3(CH_2)_4CH(NH_2)CH_3$		+++	−−

* Includes both short lasting and prolonged inhibition. †, Data from Bradley, Hösli & Wolstencroft (unpublished observations). The number of symbols allotted to each compound ((−)-NA=5) is based on a comparison of the effects of the compound with those of (−)-NA (Tables 2–6) and on the potencies of the mimicking substances. Responses opposite to (−)-NA actions are not included.

2. All the related amines in which there was N-substitution, or one of the ring hydroxyl groups was absent, had less excitatory activity than (−)-NA. The amines without a β-hydroxyl group also had less excitatory activity than (−)-NA but on some neurones they had opposite effects.
3. Two amines which were α-methylated were less excitatory than (−)-NA and had a component of antagonistic activity.
4. The amines in which both ring hydroxyl groups were absent in general mimicked the actions of (−)-NA and sometimes were more strongly excitatory.

Actions of antagonists

Several substances were found to be effective antagonists of (−)-NA excitation, whereas none of the compounds tested showed any clear antagonism towards the inhibitory effects (see for example Fig. 8B). The actions of chlorpromazine in blocking the excitatory effects of (−)-NA, but not those of acetylcholine, 5-hydroxytryptamine or glutamate, have already been described (Bradley *et al.*, 1966b) and the antagonistic action of (−)-α-methylnoradrenaline has been the subject of a preliminary report (Boakes, Candy & Wolstencroft, 1968). Care was taken in these experiments to ensure that antagonism was not confused with desensitization. (−)-NA was applied at least twice before the antagonist to test whether the excitatory response was consistently present, and those neurones which showed marked desensitization were not studied. In addition, recovery of excitation was regularly observed under conditions which should have favoured desensitization, by repeated applications of (−)-NA.

The α-adrenoceptor blocking agents, phentolamine and phenoxybenzamine, and the β-adrenoceptor blocking agents dichloroisoprenaline, propranolol and D(−)-INPEA, were ineffective as antagonists although they were applied to neurones excited or inhibited by (−)-NA for periods of up to 9 minutes. However, D(−)-INPEA had marked effects on spike amplitude and firing rates. Blockade was not observed when propranolol was applied in combination with phentolamine or phenoxybenzamine. Dihydroergotamine showed some antagonistic activity. It produced complete or partial block of the excitatory action of (−)-NA on five out of eleven neurones on which it was tested and an example of this is shown in Fig. 8A. The excitatory action of 5-HT on this neurone was unaffected by dihydroergotamine.

(−)-α-Methylnoradrenaline was the most effective compound in antagonizing (−)-NA excitation. The excitatory response to (−)-NA was blocked in fourteen out of fifteen neurones tested and recovery was regularly observed within 25 min after the end of the application (Fig. 9A). (−)-α-Methylnoradrenaline had no effect on excitation (six neurones) or inhibition (three neurones) by 5-hydroxytryptamine. Metaraminol antagonized the excitatory action of (−)-NA in three out of six neurones on which it was tested (Fig. 9B).

Discussion

The results presented here demonstrate that four different kinds of effect can be observed when (−)-NA is applied iontophoretically to brain stem neurones, in the absence of anaesthesia. These effects are (1) short lasting inhibition, (2) prolonged inhibition, (3) excitation, and (4) a biphasic action, consisting of short lasting inhibition

followed by excitation. It has been suggested (Yamamoto, 1967) that the excitatory effect of (−)-NA on brain stem neurones could be an indirect action, due to inhibition of the activity of small inhibitory neurones. From the results of the present experiments, this possibility seems most unlikely, since the proportions of neurones excited or inhibited by (−)-NA were not appreciably different when micropipettes with small tips were used to record neuronal activity, and small currents used to eject (−)-NA (Table 1). Also, the existence of specific antagonists of (−)-NA excitation, which do not affect (−)-NA inhibition, indicates that excitation is not an indirect result of inhibition.

It should be emphasized that the proportions of the different responses observed are likely to be dependent upon the location of the micropipette and upon the bias involved in the selection of neurones for study. It is probable that such data are only significant when related to an homogeneous population of neurones which can be defined anatomically or physiologically. Thus, neurones in the paramedian reticular nucleus were uniformly inhibited by (−)-NA (Avanzino, Bradley & Wolstencroft, 1966).

Excitation of single neurones by iontophoretic application of (−)-NA was observed by Bradley & Wolstencroft (1962) and has since been found in other regions of the central nervous system (see reviews by Curtis & Crawford, 1969, McLennan, 1970). In all cases excitation by (−)-NA was of long latency and duration, as reported here. It is possible that the long latency of this effect is due to diffusion of (−)-NA to distant receptors, for example on dendrites. If this was so then a reduction in latency might be expected occasionally after movement of the micropipette, but no such reduction was ever observed in the present experiments. Johnson, Roberts, Sobieszek & Straughan (1969) concluded that the long latency of excitation of cortical neurones by (−)-NA is not due to diffusion to distant receptors since they found an inverse relationship between latency and firing rate. No such relationship was apparent in this study. The absence of an effective extracellular enzyme for inactivation of noradrenaline, as there is for acetylcholine, probably contributes to the prolonged action of this substance. The mechanism by which noradrenaline is removed from adrenergic synapses is by reuptake into presynaptic terminals, a process which occurs peripherally and centrally (Hertting & Axelrod, 1961 ; Wolfe, Potter, Richardson & Axelrod, 1962 ; Aghajanian & Bloom, 1966) and which can be saturated (Iversen, 1967). Candy (unpublished observations) has shown histochemically that iontophoretically released (−)-NA is taken up into nerve terminals in the brain stem. It is possible that uptake by nerve terminals initially reduces the concentration of iontophoretically released (−)-NA at the excitatory receptors, thus delaying its effects ; saturation of the uptake process may then result in slower removal of the (−)-NA, prolonging its effect ; and subsequent release of excess (−)-NA from the terminals might also contribute to the prolonged action. A similar explanation might account for prolonged inhibition.

The varying degree of desensitization could not be correlated with any other property of the neurones. Desensitization of excitation of thalamic neurones by (−)-NA has been observed (Phillis & Tebēcis, 1967), and desensitization of excitation by 5-hydroxytryptamine and acetylcholine has also been reported (Roberts & Straughan, 1967 ; Boakes, Bradley, Briggs & Dray, 1970 ; Tebēcis, 1970).

The failure of the β-agonist isoprenaline to mimic (−)-NA excitation, and of the β-antagonists tested to block (−)-NA excitation indicates that the receptors in the

brain stem are not analogous to peripheral β-receptors. The α-agonist phenylephrine mimicked the excitatory actions of (−)-NA on only a few neurones, and of the α-antagonists studied, dihydroergotamine was partially effective whilst the others were ineffective. Chlorpromazine specifically antagonizes (−)-NA excitation of brain stem neurones (Bradley *et al.*, 1966b) but is only a weak α-antagonist peripherally. Thus, the receptors mediating (−)-NA excitation in the brain stem may have some properties in common with peripheral α-receptors, but are quite unlike β-receptors. The antagonism of (−)-NA excitation by (−)-α-methylnoradrenaline and by metaraminol, neither of which have been found to be antagonists at peripheral adrenergic receptors, is further evidence that the receptors involved are different from peripheral (−)-NA receptors. There also appear to be differences between the (−)-NA excitatory receptors in the brain stem and those in the cortex and thalamus. Thus, Johnson *et al.* (1969) found that (−)-NA excitation of cortical neurones was mimicked by (−)-isoprenaline and blocked by both α- and β-blockers. Phillis & Tebēcis (1967) reported that (−)-NA excitation of thalamic neurones could be mimicked by (−)-isoprenaline and sometimes depressed by D(−)-INPEA. Yamamoto (1967) found that (−)-NA excitation of neurones in Deiters' nucleus could be blocked by dichloroisoprenaline, but this effect was only observed in two neurones out of eight, and these two neurones could not be excited by (−)-isoprenaline. In the hypothalamus microinjection of (−)-NA causes behavioural changes which can be antagonized by phenoxybenzamine or phentolamine, indicating that there may be (−)-NA receptors similar to α-receptors in this region (Marley & Stephenson, 1969 ; Margules, 1969).

No antagonism of short lasting inhibition was seen in these experiments, and the effect was mimicked by a wide range of agonists (Table 7). The pharmacological characteristics of this response in the brain stem appear to be similar to those of (−)-NA inhibition in the spinal cord (Engberg & Ryall, 1966 ; Biscoe, Curtis & Ryall, 1966). Johnson *et al.* (1969) found (−)-NA inhibition of cortical neurones to be resistant to the action of adrenergic antagonists and concluded that the effect may be non-specific. However, the inhibition of cerebellar neurones by (−)-NA, but not by γ-aminobutyrate, can be antagonized by prostaglandins (Hoffer, Siggins & Bloom, 1969) and Biscoe *et al.* (1966) found that (−)-NA inhibition of spinal neurones was accompanied by hyperpolarization.

The biphasic response to (−)-NA appears to consist of a short lasting inhibitory effect, followed by excitation. Excitation followed by inhibition, as described by Krnjević & Phillis (1963) was never seen. Biphasic responses similar to those observed in the present study have been seen in the spinal cord (Weight & Salmoiraghi, 1966), in Deiter's nucleus (Yamamoto, 1967) and rarely in the cortex (Johnson *et al.*, 1969). The relative proportions of the two phases in our experiments did not depend upon the iontophoretic current used, increased current producing increased amplitude of both phases, in contrast to the observations of Krnjević & Phillis (1963) and Phillis, Tebēcis & York (1968).

The prolonged inhibitory response to (−)-NA was not frequently seen. The pharmacology of this response appeared to differ from that of short lasting inhibition in that it was not mimicked by any of the agonists tested and thus appeared to be much more sensitive to changes in structure than short lasting inhibition. (−)-NA inhibition of long duration was observed by Weight & Salmoiraghi (1966) and

Yamamoto (1967). Yamamoto also found that glutamate excitation was not depressed during the period of inhibition.

The absence of any action by dopamine on neurones unaffected by (−)-NA is consistent with the lack of evidence that dopamine has any physiological function in the brain stem. However, the low correlation between the effects of dopamine and (−)-NA is surprising. The effects of tyramine were similar to those of dopamine (Table 4C) and this suggests that tyramine does not release (−)-NA from central adrenergic terminals. Collins & West (1968) have shown that tyramine can release dopamine from sympathetic nerve terminals.

The ability of (−)-α-methylnoradrenaline and metaraminol to antagonize the excitant actions of (−)-NA may be relevant to an understanding of the hypotensive actions of α-methyldopa and α-methyl-*m*-tyrosine, which are converted into α-methylnoradrenaline and metaraminol (Carlsson & Lindqvist, 1962). The false transmitter theory of Day & Rand (1963) explains this hypotensive action of α-methyldopa as being due to replacement of noradrenaline by α-methylnoradrenaline in adrenergic terminals, followed by the release of α-methylnoradrenaline, which has a weaker action, by nerve stimulation (see review by Kopin, 1968). Results which are not entirely explained by the false transmitter hypothesis have been obtained by Haefely, Hürlimann & Theonen (1967) who found a complete lack of correlation between replacement of the physiological transmitter by α-methylnoradrenaline and the impairment of the effects of sympathetic nerve stimulation in peripheral organs. Henning (1969) has produced evidence that the hypotensive effect of α-methyldopa is centrally mediated but not correlated with depletion of brain noradrenaline. The possibility that the effects of α-methyldopa are related to antagonism by α-methylnoradrenaline at central adrenergic excitatory synapses would appear to require further consideration.

The existence of specific antagonists of (−)-NA excitation, and the specific antagonism of 5-HT excitation in the brain by D-lysergic acid diethylamide (Boakes *et al.*, 1970) indicates that the suggestion that there is a common receptor for indoleamines and catecholamines (Dewhurst, 1968) is incorrect.

The lack of correspondence between the receptors investigated in our study and peripheral α- and β-adrenoceptors has already been pointed out. The investigation of a range of agonists has shown the effects of some changes in molecular structure. Substitution on the side chain, or removal of the β-hydroxyl, or removal of one ring hydroxyl, all reduced activity. However, the removal of both ring hydroxyls restored the excitatory action. It is possible that this action is not a direct agonistic effect (Bradley, 1968 ; Boakes, Bradley & Candy, 1971).

To conclude, the excitatory actions of (−)-NA on neurones in the brain stem appear to be mediated by a specific mechanism. The response can be specifically blocked and is reduced by structural differences in the agonists, indicating the existence of a stereo-specific receptor. The prolonged inhibitory effect is also sensitive to structural differences in the agonists. Further studies of the short lasting inhibitory effect are needed to clarify its significance.

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