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A neuronal basis for the alerting action of (+)-amphetamine

R. J. BOAKES, P. B. BRADLEY AND J. M. CANDY

Medical Research Council, Neuropharmacology Unit, The Medical School, Birmingham B15 2TJ

Summary

1. (+)-Amphetamine mimicked the excitatory and inhibitory actions of (−)-noradrenaline on single neurones in the brain stem of acute halothane-anaesthetized rats when these compounds were applied by iontophoresis. (+)-Amphetamine had no actions on neurones unaffected by (−)-noradrenaline.

2. These mimicking actions of (+)-amphetamine could not be observed 20 h after treatment of the animals with reserpine 5 mg/kg.

3. The enzyme inhibitors α-methyl-*p*-tyrosine and FLA 63 also greatly reduced the number of (−)-noradrenaline-mimicking responses to (+)-amphetamine.

4. In animals pretreated with α-methyl-*p*-tyrosine, but not in those pretreated with FLA 63, excitatory actions of (+)-amphetamine on neurones excited by (−)-noradrenaline could be elicited 45–90 min after systemic injection of L-DOPA.

5. These results indicate that (+)-amphetamine can release noradrenaline from presynaptic sites in the brain stem, which may be a basis for its alerting actions.

Introduction

Since amphetamine passes the blood brain barrier relatively easily, it is generally considered that the increase in alertness which follows the administration of this drug in both man and animals is due to an action on the brain. However, whereas the peripheral actions of amphetamine are due to an indirect sympathomimetic action, the mechanism by which the central alerting is produced is not fully understood.

Early investigations into the central actions of amphetamine in this laboratory demonstrated that the alerting effect of the drug was dependent upon the integrity of ascending projections from the brain stem reticular formation to the cerebral cortex (Bradley & Elkes, 1953, 1957). On the basis of these findings and also from the results of experiments in which the effects of drugs on thresholds for arousal were measured (Bradley & Key, 1958), the hypothesis was formulated attributing the central alerting effect of amphetamine to a facilitatory action on brain stem mechanisms.

A relatively high proportion of neurones in the brain stem respond to iontophoretic application of noradrenaline, both excitation and inhibition of neuronal

activity being observed in the absence of anaesthesia (Bradley & Wolstencroft, 1962). A comparison of the actions of (−)-noradrenaline (NA) and (+)-amphetamine, applied by iontophoresis to the same neurones (Bradley, Hösli & Wolstencroft, see Bradley, 1968) has shown that (+)-amphetamine closely mimicked the effects of NA and did not have any independent actions. Reserpine causes depletion of brain monoamines, while α-methyl-*p*-tyrosine (AMPT) depletes catecholamines but not 5-hydroxytryptamine, by inhibiting tyrosine hydroxylase.

Recently, another substance FLA 63 has been reported to cause depletion of noradrenaline in central neurones, by blocking dopamine-β-hydroxylase (Svensson & Waldeck, 1969). Thus, if the actions of amphetamine are due to the release of noradrenaline from presynaptic nerve terminals, as has been suggested (Moore, 1963; Rech, 1964; Stein, 1964), depletion of amines, or more specifically, of noradrenaline, would be expected to abolish these effects. The present investigation was undertaken in order to determine whether (+)-amphetamine, applied iontophoretically to neurones in the brain stem, still had effects after pretreatment with reserpine, AMPT or FLA 63. A preliminary report on the abolition of the effects of (+)-amphetamine by reserpine has been published (Boakes, Bradley & Candy, 1971b). An attempt was made in some experiments to determine whether the actions of (+)-amphetamine could be restored in animals pretreated with AMPT or FLA 63 by subsequent administration of L-DOPA.

Methods

Four groups of rats of either sex weighing between 350 and 450 g were studied. One group received no pretreatment; a second was pretreated with 5 mg/kg reserpine (Halewood Chemicals Ltd.) injected intraperitoneally 20 h before recording. A third group was injected intraperitoneally 20 h before recording with either 500 mg/kg DL-α-methyl-*p*-tyrosine methylester (Kistner), calculated as the base and made up as a 10% solution in 0·9% saline, or with 500 mg/kg DL-α-methyl-*p*-tyrosine (Kistner), made up as a 7% suspension in a 30% solution of polyethene glycol in distilled water with one drop of Tween 80. Five rats in this group were injected systemically with 50 mg/kg L-DOPA (L-3,4-dihydroxyphenylalanine, BDH), whilst recording from a neurone. Solutions of L-DOPA were made up according to the method of Dahlström & Fuxe (1964). The fourth group of animals was injected intraperitoneally 4 h before recording with 25 mg/kg FLA 63 (bis-(1-methyl-4-homopiperazinyl-thiocarbonyl)-disulphide, Astra), made up by warming the appropriate amount in 2 ml 0·9% saline with an equal weight of ascorbic acid. Three rats in this group were injected systemically with 50 mg/kg L-DOPA whilst recording from a neurone.

Prior to recording, the animals were anaesthetized with halothane (Fluothane, ICI), a tracheal cannula inserted, and the cerebellum removed by suction. In some rats in the third and fourth groups a femoral vein was cannulated for injections. The halothane was delivered from a Fluotec Mk. 2 Vapouriser (Cyprane Ltd.). The oxygen flow rate during electrical recordings was 250 ml min^{-1} and the dial setting was 1·75. These settings produce a halothane concentration of less than 0·5% according to a calibration graph supplied by the manufacturers. Five-barrelled glass micropipettes were inserted through the exposed floor of the IVth ventricle; penetrations were made between 1 mm and 2·5 mm rostral to the

obex, and between 1·5 mm either side of the midline, avoiding the midline itself. The iontophoretic technique used was similar to that recently described by Boakes *et al.* (1971a). The following drugs were used in the micropipettes: (+)-amphetamine sulphate, 2%, pH 4·0–5·0 (Menley & James); (+)-amphetamine base, 2% in HCl, pH 4·5–5·5 (Aldrich); (−)-noradrenaline base, 5% or 10% in HCl, pH 4·5–5·5 (BDH). All iontophoretic applications were made with a current of 50 nA.

The iontophoretic release of (−)-noradrenaline from micropipettes similar to those used in this study has been determined (Bradley & Candy, 1970). The iontophoretic release of (+)-amphetamine from six micropipettes containing a 2% aqueous solution of (+)-amphetamine sulphate at pH 4·0, containing [G-3H]-(+)-amphetamine sulphate, specific activity 9·8 Ci/mM (New England Nuclear, Boston) was examined by the method of Bradley & Candy, 1970.

The density and fluorescence intensity of catecholamine-containing nerve terminals and cell bodies in operated and unoperated, control and drug-treated rats were investigated by means of the formaldehyde-induced fluorescence method (Falck & Owman, 1965). The tissue was freeze-dried for 5 days at −38° C and then allowed to warm to room temperature over a period of 2 days. It was then treated with 70% relative humidity paraformaldehyde for 1 h at 70° C and embedded in paraffin wax (54° C m.p.); 10 μm sections were cut and examined in a Vickers fluorescence microscope. Photographs were taken on TriX pan film (Kodak).

Results

Group 1 (control animals)

A total of 89 brain stem neurones was studied in 15 untreated rats. The actions of iontophoretically applied NA on these neurones were similar to those observed with NA on brain stem neurones in the decerebrate unanaesthetized cat (Bradley & Wolstencroft, 1962, 1964, 1965; Boakes *et al.*, 1971a). Short and long-lasting inhibitory effects, biphasic effects, i.e. short-lasting inhibition followed by excitation, and excitation were observed. (+)-Amphetamine sometimes caused depression of spike height during ejection and neurones showing marked spike depression were not included in the study. (+)-Amphetamine excited 31 of the 36 neurones excited by NA (Table 1); the excitatory response to (+)-amphetamine was usually shorter and smaller in amplitude than that to NA (Fig. 1A). The excitatory response to a second application of (+)-amphetamine was usually smaller than that to the first application, the decrease in the response being greater than the diminution of the NA response due to desensitization seen in these experiments. (+)-Amphetamine inhibited 19 of the 22 neurones inhibited by NA (Fig. 1B; Table 1). The inhibitory responses to the two amines were usually of the same magnitude, but long-lasting inhibition was not observed with (+)-amphetamine. Twenty-three neurones gave a biphasic response to NA; 15 of these showed a biphasic response to (+)-amphetamine, and 8 an inhibitory response. (+)-Amphetamine had no effect on the 8 neurones unaffected by NA.

The distribution and fluorescence intensity of the catecholamine-containing nerve terminals and cell bodies were in agreement with those described by Dahlström & Fuxe (1964) and Fuxe (1965). Fig. 5A shows the catecholamine-containing nerve

terminals in the region of the hypoglossal nucleus for comparison with those of the same region after drug treatment.

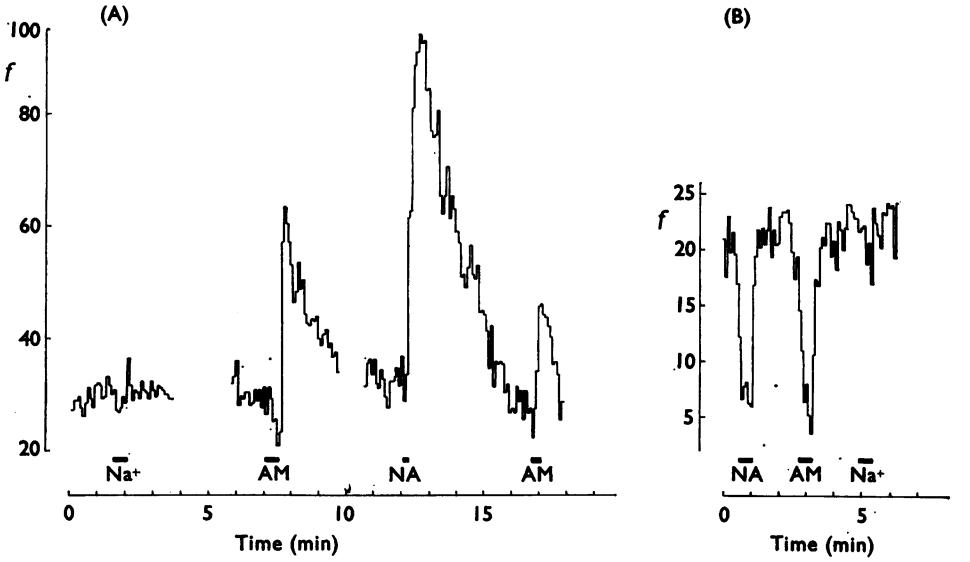

FIG. 1. Effects of noradrenaline and (+)-amphetamine on the spontaneous firing rates of two brain stem neurones. The mean firing rates in impulses sec^{-1} (f) in successive 5 s epochs are plotted against time in minutes. Iontophoretic applications of noradrenaline (NA), (+)-amphetamine (AM) and of a current control (Na+) are shown by horizontal bars. (A) Shows a neurone excited by noradrenaline and (+)-amphetamine. (B) Shows a neurone inhibited by noradrenaline and (+)-amphetamine.

TABLE 1. *Comparison of the effects of noradrenaline (NA) and (+) -amphetamine when applied to the same neurone in untreated rats*

		(+)-Amphetamine			
		+	—	0	Total no.
NA	+	31	2	3	36
	±	15	8	0	23
	—	2	19	1	22
	0	0	0	8	8

The figures represent the numbers of neurones showing excitation (+), a biphasic response (±), inhibition (—) or no effect (0).

Group 2 (animals pretreated with reserpine)

A total of 64 neurones was studied in the brain stem of 10 rats pretreated with 5 mg/kg reserpine. The excitatory actions of NA in these animals were much stronger and more prolonged than in the untreated animals (Fig. 2A). Thirty-nine neurones were excited by NA and 38 of these neurones were unaffected by (+)-amphetamine; one such neurone is shown in Fig. 2A, and the results are summarized in Table 2. Thirteen neurones were inhibited by NA and 2 of these were also inhibited by (+)-amphetamine. The inhibitory responses to NA were similar to those seen in the untreated animals (Fig. 2B). No biphasic responses either to NA or to (+)-amphetamine were seen in the reserpine-pretreated animals, and the 12 neurones unaffected by NA were unaffected by (+)-amphetamine.

No catecholamine-containing nerve terminals could be observed in the brain stem of animals pretreated with reserpine (Fig. 5B).

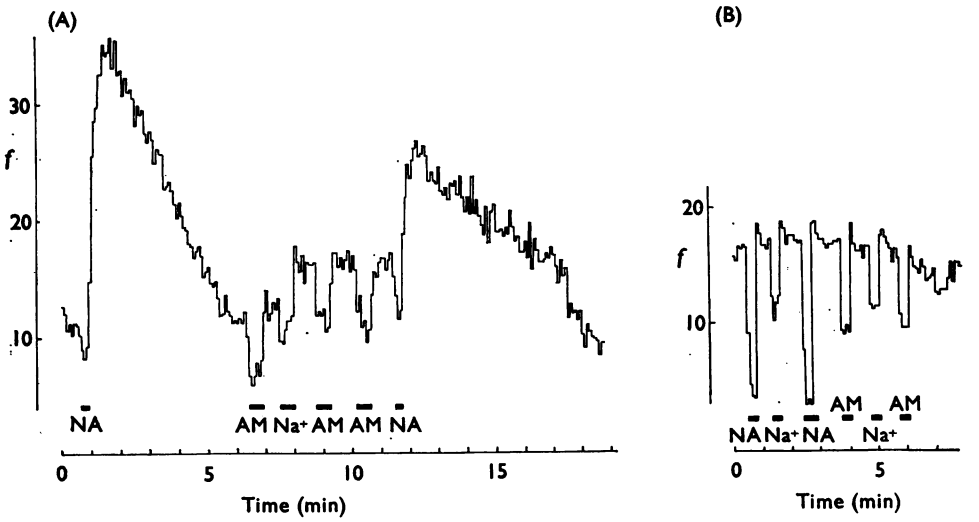

FIG. 2. Effects of noradrenaline (NA) and (+)-amphetamine (AM) on two brain stem neurones in rats pretreated with 5 mg/kg reserpine. (*f*) as in Fig. 1. (A) Shows a neurone strongly excited by noradrenaline whereas the action of (+)-amphetamine is comparable to that of current. (B) A neurone showing a short inhibitory response to noradrenaline, whilst the response to (+)-amphetamine is similar to that of current.

TABLE 2. *Comparison of the effects of noradrenaline (NA) and (+)-amphetamine when applied to the same neurone in rats pretreated with 5 mg/kg reserpine. Symbols as in Table 1*

		(+)-Amphetamine			
		+	—	0	Total no.
NA	+	1	0	38	39
	±	0	0	0	0
	—	0	2	11	13
	0	0	0	12	12

Group 3 (animals pretreated with AMPT)

Animals pretreated with 500 mg/kg AMPT as the methyl ester all died soon after surgery. However, the animals pretreated with a suspension of the base survived and 89 neurones were recorded from 16 animals. NA excited 44 of these neurones (Fig. 3A) and had a biphasic action on 6 neurones. (+)-Amphetamine had an excitatory action on 3 of these neurones and an inhibitory action on 6 neurones (Table 3). An example of a neurone excited by NA but not by (+)-amphetamine is shown in Fig. 3A. Twenty-one neurones were inhibited by NA and 13 of these were unaffected by (+)-amphetamine ; in the 4 that were excited by (+)-amphetamine, the response to NA was always of the long lasting type. Six neurones showed a biphasic response to NA and of these 3 were inhibited by (+)-amphetamine and 3 were unaffected. Neurones unaffected by NA were unaffected by (+)-amphetamine (Table 3).

Systemic injections of L-DOPA were made in 5 rats whilst recording from a neurone previously excited by NA but not by (+)-amphetamine. (+)-Amphetamine caused excitation of all 5 of these neurones, 45–90 min after the injection of L-DOPA (Fig. 3B).

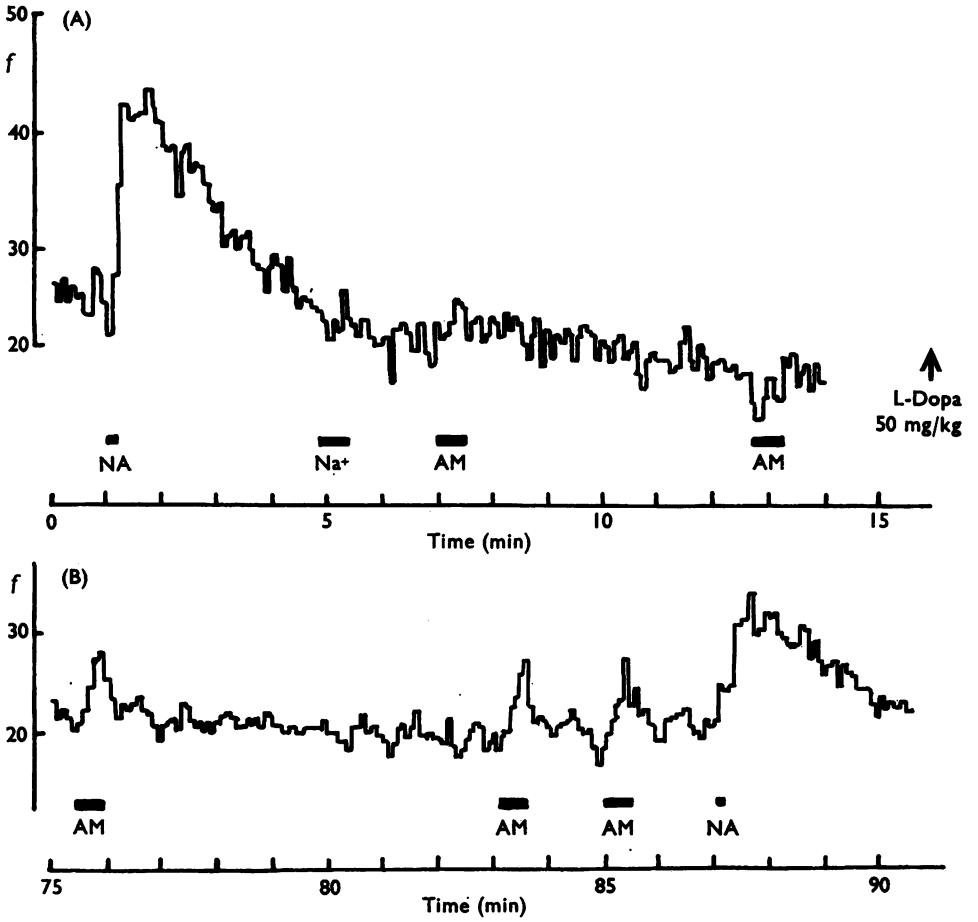

FIG. 3. (A) Lack of effect of (+)-amphetamine (AM) on a neurone excited by noradrenaline (NA) in an animal pretreated with 500 mg/kg AMPT. (B) The same neurone 60 min after systemic injection of L-DOPA, showing excitatory responses to (+)-amphetamine. The L-DOPA injection is shown by the vertical arrow. (*f*) as in Fig. 1.

TABLE 3. *Comparison of the effects of noradrenaline (NA) and (+)-amphetamine when applied to the same neurone in rats pretreated with 500 mg/kg AMPT. Symbols as in Table 1*

		(+)-Amphetamine			
		+	—	0	Total no.
NA	+	3	3	38	44
	±	0	3	3	6
	—	4	4	13	21
	0	0	0	18	18

A marked reduction in the number and intensity of the catecholamine containing nerve terminals was found after pretreatment with AMPT (Fig. 5C). The catecholamine-containing cell bodies also showed a decreased fluorescence intensity. After L-DOPA (1–1·5 h) the density and intensity of the catecholamine-containing nerve terminals (Fig. 5D) and cell bodies were comparable with control animals.

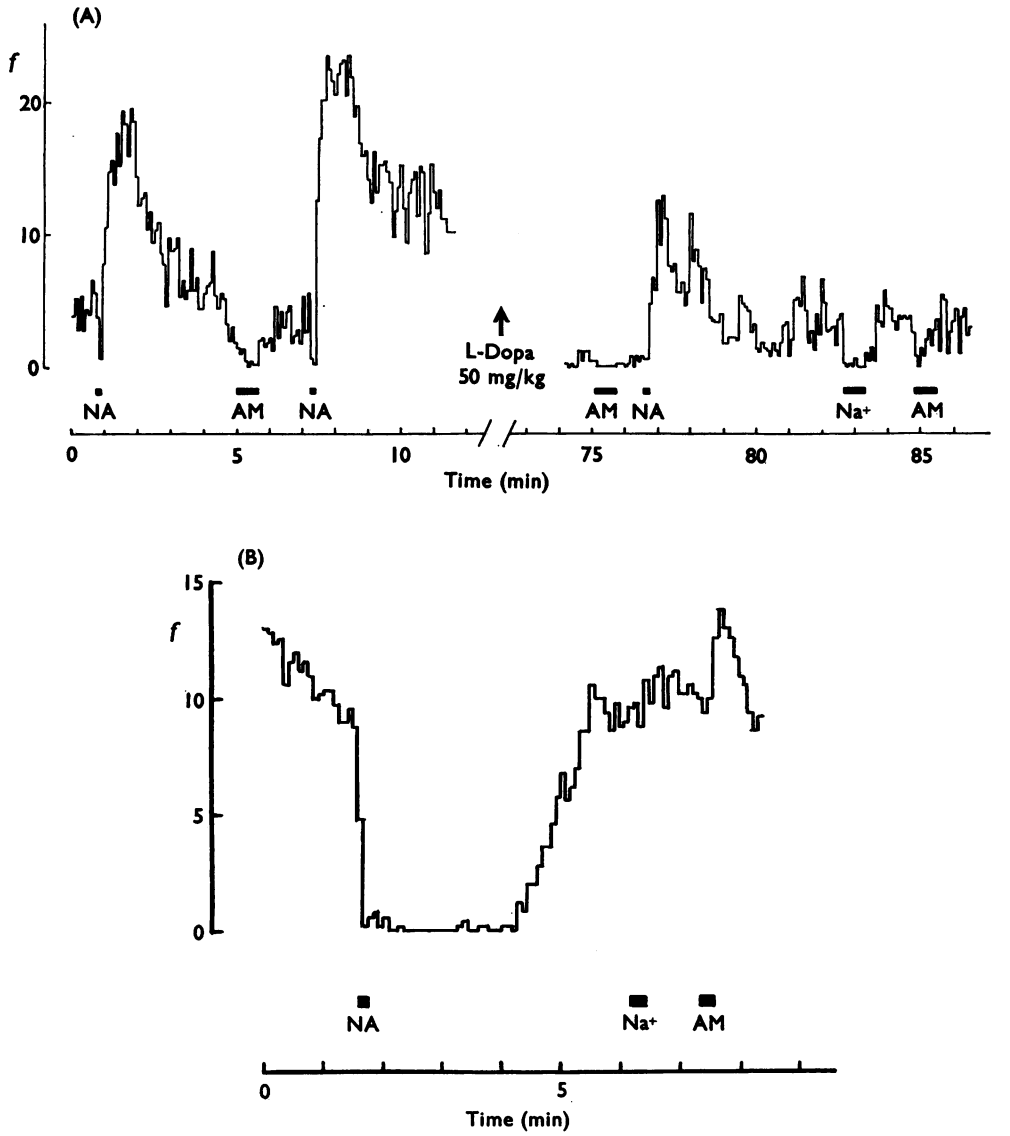

FIG. 4. (A) Lack of effect of (+)-amphetamine (AM) on a neurone excited by noradrenaline (NA) in an animal pretreated with 25 mg/kg FLA 63, before and after systemic injection of 50 mg/kg L-DOPA. The L-DOPA injection is shown by the vertical arrow. (B) A neurone recorded in a rat pretreated with FLA 63 showing a prolonged inhibitory response to noradrenaline and a weak excitatory response to (+)-amphetamine. (*f*) as in Fig. 1.

TABLE 4. *Comparison of the effects of noradrenaline (NA) and (+)-amphetamine when applied to the same neurone in rats pretreated with 25 mg/kg FLA 63. Symbols as in Table 1*

		(+)-Amphetamine			
		+	—	0	Total no.
NA	+	4	0	16	20
	±	0	2	2	4
	—	4	2	3	9
	0	0	0	7	7

Group 4 (animals pretreated with FLA 63)

Some of the animals pretreated with 25 mg/kg FLA 63 died soon after surgery but 40 neurones were successfully recorded in 10 pretreated animals which survived (Table 4). The actions of NA on neurones in these animals were similar to the actions of NA in the untreated group. (+)-Amphetamine had no effect on 16 of the 20 neurones excited by NA (Fig. 4A), but inhibited 2 of the 4 neurones which showed a biphasic response to NA and had no action on 7 neurones unaffected by NA. Eight neurones in this group showed prolonged inhibitory responses to NA, and 4 of these neurones were weakly excited by (+)-amphetamine (Fig. 4B). One neurone showed a short inhibitory response to NA which was not

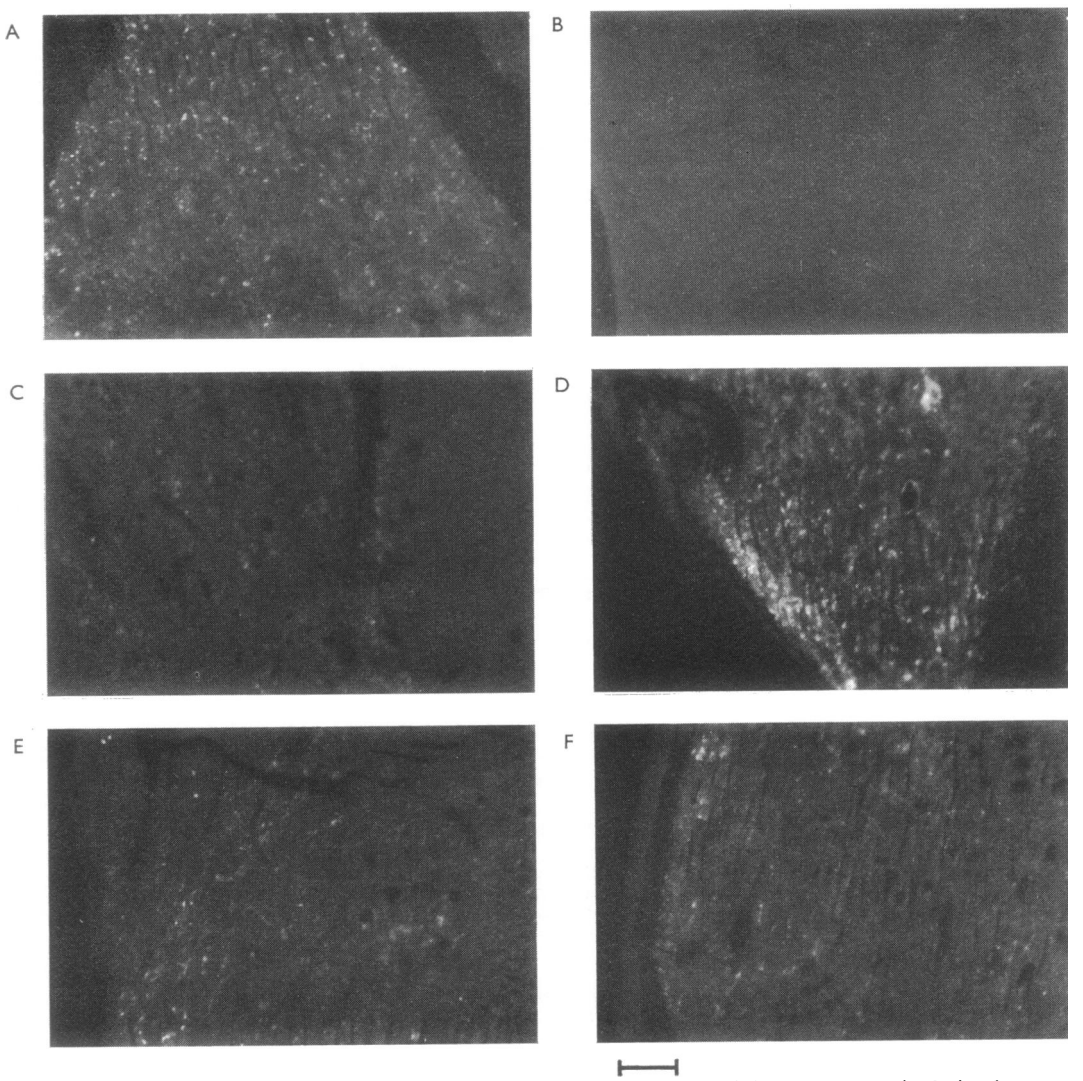

FIG. 5. Density and fluorescence intensity of catecholamine-containing nerve terminals in the region of the hypoglossal nucleus after (A) no pretreatment, (B) reserpine treatment 5 mg/kg, i.p. 20 h, (C) α-methyl-*p*-tyrosine treatment 500 mg/kg, i.p. 20 h, (D) α-methyl-*p*-tyrosine treatment 500 mg/kg, i.p. 20 h and L-DOPA treatment 50 mg/kg, i.p. 1 h, (E) FLA 63 treatment 25 mg/kg, i.p. 4 h and (F) FLA 63 treatment 25 mg/kg, i.p. 4 h and L-DOPA treatment 50 mg/kg, i.p. 1 h. Transverse sections, calibration bar 200 μm.

mimicked by (+)-amphetamine. Systemic injection of L-DOPA (3 neurones) failed to restore the excitatory response to (+)-amphetamine (Fig. 4A).

A marked reduction in the fluorescence intensity of both catecholamine-containing nerve terminals (Fig. 5E) and cell bodies was observed 4–8 h after FLA 63. After L-DOPA (1–1·5 h) the fluorescence intensity of the catecholamine-containing nerve terminals was still reduced (Fig. 5F) compared with control animals while there was a marked increase in the extra-neuronal fluorescence observed in the region of the catecholamine-containing cell bodies.

Release of (+)-amphetamine

The release of labelled (+)-amphetamine was linearly related to the charge passed through the micropipette barrel, both for individual micropipettes and for the micropipettes considered collectively. Regression lines were calculated for the data from each micropipette and transport numbers were derived from the slopes of these lines, expressed in moles coulomb^{-1}, multiplied by Faraday's number. The data and calculated regression lines for the micropipettes with highest and lowest transport numbers are given in Fig. 6. The transport numbers for these micropipettes were 0·036 and 0·014; the mean transport number for the six micropipettes was $0{\cdot}025 \pm 0{\cdot}01$ (S.D.). The regression lines for all the micropipettes were significant ($P<0{\cdot}05$). However, the deviations of the regression lines of five of the six micropipettes from the origin were significant ($P<0{\cdot}05$).

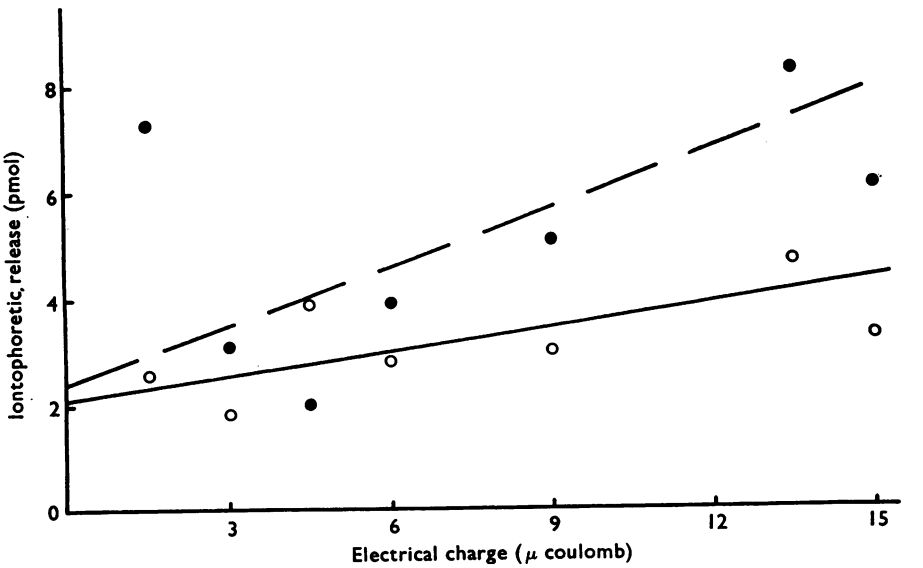

FIG. 6. Relationship between iontophoretic release (pmol) and the electrical charge passed (μ coulomb) for two micropipettes containing a 2% solution of [G-3H]-(+)-amphetamine sulphate. The data points for the micropipette with the highest transport number are shown by filled circles and the upper line is the calculated regression line for these data. The data points for the micropipette with the lowest transport number are shown by open circles and the lower line is the calculated regression line. Each data point is the mean of two observations.

Discussion

Bradley & Elkes (1953, 1957) and Schallek & Walz (1953) found that systemic administration of amphetamine in cats and dogs, respectively, caused behavioural and electroencephalographic arousal and from the results of experiments with acute preparations (*encéphale* and *cerveau isolé*), Bradley & Elkes (1953, 1957) suggested that amphetamine may activate receptors in the arousal system of the brain stem reticular formation. Bradley & Key (1958) found that doses of (+)-amphetamine less than 1·0 mg/kg caused a lowering of thresholds for behavioural and electroencephalographic arousal in the *encéphale isolé* cat preparation. Several theories have been proposed for the neuronal basis of the stimulant effect of amphetamine. A direct action of amphetamine on receptor sites has been suggested (Rossum, Schoot & Hurkmans, 1962 ; Smith, 1963). However, other studies suggest the mechanism of action of amphetamine is by release of endogenous catecholamines. Bradley, Hösli & Wolstencroft (see Bradley, 1968) found that iontophoretically applied (+)-amphetamine mimicked the actions of NA applied to the same neurones in the brain stem of the unanaesthetized decerebrate cat. The results presented here confirm and extend these observations to the brain stem of the halothane-anaesthetized rat. The presence of catecholamine-containing nerve terminals in the brain stem (Fuxe, 1965) and the demonstration by Carr & Moore (1970) that (+)-amphetamine can release NA from the cerebral ventricles supports the hypothesis that (+)-amphetamine releases NA from presynaptic sites but does not exclude entirely the possibility of a direct postsynaptic action.

Some biochemical studies have shown that high doses of amphetamine can lower the concentration of endogenous brain noradrenaline (McLean & McCartney, 1961 ; Moore & Lariviere, 1963 ; Baird & Lewis, 1964 ; Smith, 1965 ; Leonard & Shallice, 1971) but other studies have produced differing results (Sanan & Vogt, 1962 ; Breese, Kopin & Weise, 1970 ; Simon, Tillement, Larousse, Breteau, Guernet & Boissier, 1970). The significance of these findings is not clear as the doses of amphetamine used in these studies were in the lethal range (Stolk & Rech, 1968).

The study of the release of labelled (+)-amphetamine from micropipettes shows that the transport number of this compound is low compared to that of noradrenaline (Bradley & Candy, 1970). The applications of (+)-amphetamine and (−)-noradrenaline were made at the same iontophoretic current and for the same times. Therefore less (+)-amphetamine was released iontophoretically than (−)-noradrenaline in these experiments and this may partially explain the smaller excitatory responses elicited by (+)-amphetamine compared to those seen with (−)-noradrenaline.

Reserpine is an extremely potent depletor of brain monoamines. No catecholamine-containing nerve terminals were observed histochemically 24 h after reserpine, in agreement with the findings of Fuxe (1965). The lack of effect of iontophoretically applied (+)-amphetamine in the reserpine pretreated animals thus suggests that the effects of (+)-amphetamine in the untreated animals was due to release of NA from nerve terminals and not to a direct post-synaptic action, although the possibility remains that reserpine might directly antagonize post-synaptic actions of (+)-amphetamine but not of NA. These results indicate that the mechanism by which pretreatment with reserpine can increase some effects of (+)-amphetamine (Stein, 1964 ; Stolk & Rech, 1967) does not involve presynaptic release of NA. Svensson (1970) has suggested that dopamine is involved in (+)-

amphetamine-induced hypermotility in reserpine-pretreated animals which is consistent with results presented here.

After pretreatment with AMPT the behavioural effects of (+)-amphetamine are abolished (Weissman, Koe & Tenen, 1966). In our experiments (+)-amphetamine only rarely mimicked NA responses in animals pretreated with AMPT and histochemical studies showed a marked reduction in the number and intensity of catecholamine-containing nerve terminals, in agreement with the observations of Andén, Corrodi, Dahlström, Fuxe & Hökfelt (1966). This supports the concept of an NA-releasing action of (+)-amphetamine and demonstrates that the abolition of the effects of (+)-amphetamine in reserpine-pretreated animals is not due to post-synaptic antagonism. The importance of endogenous catecholamines in the effects of (+)-amphetamine was further demonstrated by the restoration of the actions of iontophoretically applied (+)-amphetamine in animals pretreated with AMPT by administration of L-DOPA. L-DOPA increased the fluorescence of the catecholamine-containing nerve terminals in rats pretreated with AMPT as reported by Corrodi, Fuxe & Hökfelt (1966). Furthermore, Hanson (1967) and Randrup & Munkvad (1966) have shown that the behavioural effects of (+)-amphetamine in animals pretreated with AMPT were restored by L-DOPA.

The marked reduction in the fluorescence intensity of the catecholamine-containing nerve terminals in the brain stem following pretreatment with FLA 63 is in agreement with the results of Corrodi, Fuxe, Hamberger & Ljungdahl (1970). These authors reported that FLA 63 does not affect dopamine levels and thus the results obtained in our experiments with FLA 63 indicate that (+)-amphetamine specifically releases NA in the brain stem. The significance of the weak excitatory effects of (+)-amphetamine on neurones which showed a long lasting inhibitory response to NA is not clear. Behavioural studies have shown that the stimulant actions of (+)-amphetamine are reduced by pretreatment with FLA 63 (Svensson, 1970). L-DOPA produced only a partial restoration of the fluorescence of terminals in the animals treated with FLA 63, as reported by Corrodi *et al.* (1970). The failure of L-DOPA to restore any action of (+)-amphetamine in animals pretreated with FLA 63 is further evidence that the actions of (+)-amphetamine after systemic injection of L-DOPA into animals pretreated with AMPT is due to release of NA and not dopamine.

Glowinski, Axelrod & Iversen (1966) found evidence that (+)-amphetamine can inhibit the uptake of exogenous NA by central nerve terminals, but Carlsson, Corrodi, Fuxe & Hökfelt (1969) found that (+)-amphetamine has a relatively weak inhibitory effect on uptake, while Fuxe & Ungerstedt (1968) demonstrated that (+)-amphetamine is a more potent releaser of NA than an inhibitor of NA uptake. (+)-Amphetamine did not prolong the actions of NA in the present experiments as might be expected if it blocks NA re-uptake, whereas desipramine and imipramine have been found to prolong the actions of iontophoretically applied NA (Avanzino, Ermirio & Zummo, 1971; Hoffer, Siggins & Bloom, 1971). Some inhibition of NA re-uptake by (+)-amphetamine cannot be precluded and Green (1970) has shown that low doses of (+)-amphetamine have some inhibitory effects on monoamine oxidase activity. It is possible that these two factors might increase the potency of (+)-amphetamine by increasing the amount of NA available for release and reduce its inactivation by re-uptake.

The stereotyped behaviour caused by amphetamine (Randrup, Munkvad &

Udsen, 1963) has been ascribed to an action on the dopaminergic nigroneostriatial tract. Carr & Moore (1970) found that high concentrations of (+)-amphetamine perfused through the cerebral ventricles increased the concentration of dopamine in the perfusate, and systemic injection of (+)-amphetamine into rats with unilateral lesions of the nigroneostriatial pathway caused turning behaviour similar to that produced by unilateral direct stimulation of the pathway (Anlezark, Arbuthnott, Christie & Crow, 1971). These behavioural effects of amphetamine are produced by doses of 2·0 mg/kg or more and appear to be only slightly stereospecific whereas the alerting effects of amphetamine are elicited by 1·0 mg/kg or less and are stereospecific, the (+)-isomer being ten times more potent than the (−)-isomer (Bradley & Elkes, 1957; Bradley & Key, 1958; Taylor & Snyder, 1970).

The findings presented here demonstrate that (+)-amphetamine acts by releasing endogenous NA onto neurones in the brain stem, thus providing a basis for the activation of the reticular arousal system proposed by Bradley & Elkes (1953, 1957).

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