Biochemical Pharmacology, Vol. 26, pp. 1877–1884. Pergamon Press, 1977. Printed in Great Britain.

NORADRENALINE-SENSITIVE ADENYLATE CYCLASE IN SLICES OF MOUSE LIMBIC FOREBRAIN: CHARACTERISATION AND EFFECT OF DOPAMINERGIC AGONISTS*

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(*Received* 11 *February* 1977; *accepted* 18 *March* 1977)

Abstract—The characteristics of the noradrenaline (NA)-sensitive adenylate cyclase in mouse limbic forebrain slices have been examined. The order of potency (EC_{50}) found for various adrenergic agonists was *l*-isoprenaline > *l*-adrenaline ≥ *l*-NA which corresponds to a β-adrenergic receptor. However, maximal stimulation was greatest for *l*-adrenaline, and smaller for *l*-NA and *l*-isoprenaline. The stimulation produced by *l*-isoprenaline was non-significantly enhanced by concomitant administration of the α-adrenergic agonists, *l*-phenylephrine and clonidine, but the stimulation produced by *l*-NA was not enhanced by *l*-isoprenaline. Various adrenergic antagonists inhibited the stimulation due to 10^{-5} M *l*-NA with IC_{50} values of order of potency phentolamine > phenoxybenzamine > *d,l*-propranolol. However, the maximal inhibition produced by *d,l*-propranolol was 95 per cent, whereas that produced by phentolamine was 82 per cent.

The results suggest the presence of a population in this region of at least two types of receptor which may be stimulated by *l*-NA. One has β-adrenergic properties and the other appears to correspond to a β-dependent α-adrenergic receptor. Desipramine also inhibited the NA-sensitive adenylate cyclase, suggesting some dependence of the adenylate cyclase on NA uptake mechanisms. The dopaminergic antagonist pimozide was also found to be a potent inhibitor of NA-sensitive adenylate cyclase with an IC_{50} of 1.3×10^{-7} M and a maximal inhibition at 10^{-6} M of 84 per cent. The effect of dopaminergic agonists was also studied on this system, which is not stimulated by dopamine itself at concentrations up to 10^{-4} M. The ergot alkaloids bromocryptine and ergocornine were potent inhibitors of the NA-sensitive adenylate cyclase, bromocryptine having an IC_{50} for inhibition of 9.4×10^{-9} M, similar to that of phentolamine.

The catecholamines dopamine (DA) and noradrenaline (NA) have been found to stimulate adenylate cyclase in tissue slices and cell-free preparations from several brain areas [1]. Both DA and NA stimulate cyclic AMP production in slices of rat caudate nucleus [2]. However, adenylate cyclase assayed in disrupted cell preparations from striatum [3, 4], mesolimbic areas [5] and substantia nigra [6] is stimulated more specifically by DA.

The existence of a NA-sensitive adenylate cyclase has recently been demonstrated in both slices [7] and vesicular homogenates [8] of rat limbic forebrain. It has not been found possible to stimulate this adenylate cyclase with DA.

The hypothesis has been put forward that catecholamine-sensitive adenylate cyclases form an integral part of the post-synaptically located receptors for catecholamines [9, 10]. The NA-sensitive adenylate cyclase in the limbic forebrain thus represents a means of investigating the effect of dopaminergic and adrenergic drugs on the NA receptor. The effect of various neuroleptic drugs has already been studied on this system [7, 8, 11].

The development of postsynaptic DA receptor agonists is important for the future of the chemotherapy of Parkinson's disease [12]. However, the pathology of this disease shows degeneration not only of the dopaminergic nigrostriatal tract but also of noradrenergic neurones in the nucleus locus coeruleus [13]. It is possible that loss of noradrenergic neurones also contributes to the aetiology of the disease [14], and that L-DOPA may act as a replacement therapy for central noradrenergic as well as dopaminergic functions [15].

Thus the investigation of the postsynaptic NA agonist and antagonist properties of dopaminergic agonists could be of use in understanding the mechanism of action of drugs such as 2-bromo-α-ergocryptine (bromocryptine) which is beneficial in the treatment of Parkinson's disease [16].

In this paper a characterisation of the NA-sensitive adenylate cyclase in slices of mouse limbic forebrain is presented, together with the effects of various dopaminergic agonists, including bromocryptine on this system.

MATERIALS AND METHODS

8-[^{3}H]adenosine 3′-5′ cyclic phosphate ammonium salt (specific activity: 27 Ci/μmole) was obtained from Radiochemicals, Amersham. The following drugs were used, dissolved in distilled water, except where

* A preliminary communication was presented at the 1st E.S.N. Meeting, Bath, U.K. September 1976.

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otherwise stated: *l*-noradrenaline (*l*-NA, *l*-arterenol HCl), *l*-adrenaline (dissolved in one drop of 2N HCl and diluted with distilled water), *l*-isoprenaline-D-bitartrate, *l*-phenylephrine HCl, dopamine (3-hydroxytyramine HCl) and yohimbine HCl (Sigma Inc.); dexamphetamine sulphate and phenoxybenzamine HCl (Smith, Kline and French Ltd); ET 495 methanesulphonate (piribedil) and S584 (Les Laboratoires Servier); bromocryptine (2-bromo-α-ergocornine methanesulphonate) and ergocornine hydrogen maleinate (Sandoz Ltd; both dissolved with an equal weight of tartaric acid in a small volume of 70% ethanol and then diluted in water); clonidine HCl and ST91-Cl (Boehringer, Ingelheim); phentolamine mesylate (Rogitine, Ciba); desmethylimipramine HCl (Geigy Pharmaceuticals); reserpine (dissolved in a small volume glacial acetic acid and diluted in water; Halewood Chemicals); apomorphine (dissolved in water with 5 mg of ascorbic acid, in the absence of light; Evans Medical Ltd); *d,l*-propranolol HCl (I.C.I. Ltd.); pimozide (dissolved by boiling in 1.5% tartaric acid; Janssen Ltd).

Male 'Swiss S' mice (20–25 g; Animal Suppliers Ltd) were used in all experiments. The animals were killed by cervical dislocation, decapitated, and the brains were dissected as follows:— the hypothalamus and cerebellum were removed, and a cut was made through the superior longitudinal fissure. The superficial cerebral cortex was drawn aside and removed, exposing the ventricular system and corpora striata. The striata were removed along their natural limits. A vertical coronal cut was made at the level of the optic chiasm and the caudal part was discarded. The remaining cortical areas were removed as far as possible. The resultant slice, referred to as the limbic forebrain (including hippocampus, olfactory tubercles, septal nuclei, the anterior part of the medial forebrain bundle, the amygdaloid nuclei and nucleus accumbens) was placed on a filter paper moistened with Krebs–Ringer buffer on the removable platform of the tissue chopper, which was cooled to 0°. The limbic forebrain areas from two mice were pooled and chopped into prisms of 0.36 mm^2 section using a McIlwain tissue chopper (H. Mickle Ltd). The tissue was transferred to a tube containing 20 ml Krebs–Ringer buffer and the slices were separated by brief mixing. The composition of the buffer was (in mM): NaCl 138; $CaCl_2$ 2.8; KH_2PO_4 1.3; $NaHCO_3$ 3.9; $MgSO_4$ 1.3; KCl 5.2; glucose 19.9; L-ascorbic acid 1.1; pH 7.4 adjusted after previous saturation with 95% O_2–5% CO_2. The slices were washed three times and pre-incubated at 37° for 10 min in 30 ml buffer using a shaking water bath. The medium was changed for fresh buffer (30 ml) and pre-incubation continued for a further 10 min. Generally three batches of tissue were prepared at 8 min intervals for each experiment. After the pre-incubation period, 25 ml of medium was removed from the tube, and 0.5 ml aliquots of medium containing about 15 mg of tissue slices were transferred at 2 min intervals to incubation tubes containing either a drug or its vehicle added in a volume of 0.1 ml. Incubation was continued for 15 min, then either NA or other drugs tested for their agonist activity (or their vehicles) were added in a volume of 0.1 ml. The final incubation volume was 30 ml.

Incubation was continued for 5 min. except when the time course of agonist activity was under investigation. At no time during the incubation periods from suspension of slices in buffer to termination of the reaction was the tissue allowed to remain without oxygenation. The reaction was stopped, after aspirating 29 ml of medium, by the addition of 1 ml ice-cold absolute ethanol and homogenising for 15 sec at 0° with an ultra Turrax homogeniser. The homogenates were centrifuged at 3000 *g* for 30 min. The supernatant, plus 1 ml distilled water with which the pellet was washed, were dried by rotary evaporation at 40°. The samples were resuspended in 1 ml 50 mM Tris buffer (pH 7.4) containing 8 mM theophylline and 6 mM α-mercaptoethanol. Cyclic AMP was determined immediately in duplicate or triplicate by the method of Brown *et al.* [17], using a final assay volume of 300 μl. The assay was linear between 0.5 and 50 pmoles of cyclic AMP per 50 μl of sample. Results were discarded unless binding of cyclic AMP to the protein was between 35 and 50 per cent. Recovery of cyclic AMP was determined by adding a tracer amount of [^{3}H]cyclic AMP (10^{-12} M) with the ethanol at the time of homogenisation, and counting an aliquot of the resuspended sample. Recovery was 85.3 ± 1.9 per cent from 23 determinations, thus the results were not corrected for recovery. The protein content of the pellet was determined by the method of Lowry *et al.* [18] and results are expressed as pmoles cyclic AMP per mg protein.

In each experiment the basal cyclic AMP level was determined at the same time as the effect of each agonist or antagonist. In order to take into account the variation in basal cyclic AMP level found between experiments, the percentage change from its respective basal level caused by the agonist or antagonist under study was calculated from each determination.

When the effects of agonists or antagonists were studied, the basal cyclic AMP level (B), the cyclic AMP level found after 5 min stimulation with the drug alone added as agonist (A), the cyclic AMP level after 5 min stimulation with *l*-NA (S), and the *l*-NA stimulated cyclic AMP production in the presence of the drug added 15 min before *l*-NA as an antagonist (AS) were estimated, using the same batch of prepared tissue slices. The result are given in pmoles cyclic AMP/mg protein, and all determinations are expressed as the mean ± 1 S.E.M. The significances of the changes in cyclic AMP formation with the different agonists and antagonists were determined by Student's *t* test.

The percentage inhibition of the *l*-NA stimulated cyclic AMP level was calculated as: AS/S × 100%. The percentage inhibition of the rise in cyclic AMP due to *l*-NA was calculated as: [(AS − B)/(S − B)] × 100%. The EC_{50} is the concentration of agonist required to produce 50 per cent of its maximal stimulation of adenylate cyclase. The IC_{50} is the concentration of antagonist required to produce 50 per cent inhibition of the stimulation of adenylate cyclase due to 10^{-5} M *l*-NA. Both these values were calculated graphically.

RESULTS

Effect of adrenergic agonists. The time curve for stimulation of adenylate cyclase in mouse limbic forebrain slices by *l*-NA (10^{-5} M) is shown in Fig. 1.

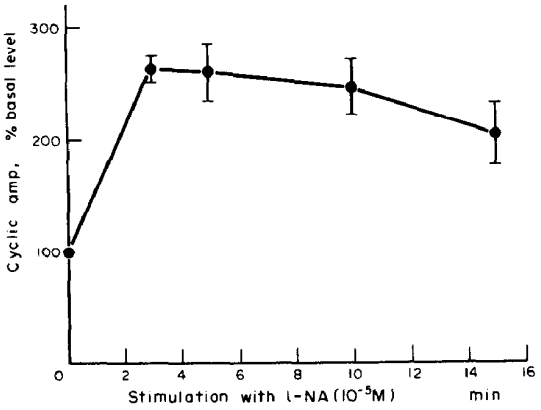

Fig. 1. Time curve for the stimulation of adenylate cyclase in mouse limbic forebrain slices by *l*-NA (10^{-5} M). Slices were preincubated for 20 min before aliquots of the slices were distributed and incubated for 15 min in individual tubes. L-NA was then added to a final concentration of 10^{-5} M and stimulation was allowed to continue for between 3 and 15 min, before the process was halted by aspiration of excess medium and homogenisation in 1 ml absolute alcohol. The samples were treated as described in the Methods and cyclic AMP was estimated according to the method of Brown *et al.* [17]. The cyclic AMP levels are given as percentage of control ± 1 S.E.M. Each value is the mean of at least four separate determinations estimated in duplicate. The basal cyclic AMP level was 9.1 ± 0.8 pmoles/mg protein from 16 results.

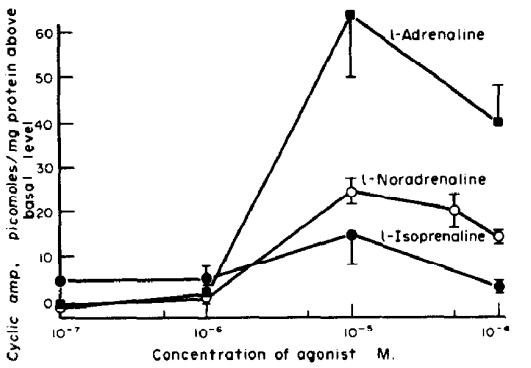

Fig. 2. Dose–response curve for the stimulation of adenylate cyclase in mouse limbic forebrain slices by *l*-NA, *l*-adrenaline, and *l*-isoprenaline. Preincubation and incubation of slices was carried out as in the legend to Fig. 1. The slices were exposed for 5 min to concentrations of the agonists between 10^{-7} M and 10^{-4} M. The stimulation of cyclic AMP formation produced by each concentration of agonist was calculated as described in Methods. The mean degree of stimulation ± 1 S.E.M. was determined from at least 6 independent estimations. The average basal cyclic AMP levels for all agonist concentrations were 9.9 ± 1.4 pmoles/mg protein for stimulation by *l*-adrenaline, 8.8 ± 0.8 pmoles/mg protein for stimulation by *l*-NA and 16.3 ± 2.0 pmoles/mg protein for stimulation by *l*-isoprenaline.

Maximal stimulation of 264 ± 12 per cent was achieved after 3 min and the level gradually declined with longer periods of stimulation. The stimulation produced after 15 min was significantly lower than that produced after 3 min exposure to *l*-NA (10^{-5} M) ($P < 0.05$). Basal cyclic AMP remained constant during the entire period (9.1 ± 0.8 pmoles/mg protein).

The concentration for half maximal stimulation of cyclic AMP production by *l*-NA (EC_{50}) was 3.2×10^{-6} M using a 5 min stimulation period (Fig. 2). Maximal stimulation of 278 ± 23 per cent ($P < 0.0005$) was obtained with 10^{-5} M *l*-NA and with greater concentrations the stimulation tended to decline. An incubation period of 5 min with 10^{-5} M *l*-NA was thus chosen for subsequent experiments.

The adenylate cyclase in limbic forebrain slices was also stimulated by *l*-adrenaline. Although the EC_{50} of 3.0×10^{-6} M was similar to that obtained with *l*-NA, the maximal stimulation at 10^{-5} M of 696 ± 155 per cent of basal cyclic AMP ($P < 0.0005$) was greater than that found for *l*-NA (10^{-5} M) ($P < 0.001$). The α-adrenergic agonist *l*-isoprenaline also stimulated the system with an EC_{50} of 1.6×10^{-6} M and a maximal stimulation at 10^{-5} M of 148 ± 29 per cent of basal level of cyclic AMP ($P < 0.05$), which was lower than that produced by *l*-NA (10^{-5} M) ($P < 0.001$). The α-adrenergic agonist *l*-phenylephrine had a slight but non-significant agonist effect at 10^{-5} M, producing a stimulation of 122 ± 42 per cent of the basal cyclic AMP level. This stimulation was not increased at 10^{-3} M (126 ± 37 per cent of basal cyclic AMP level). Neither clonidine nor its analogue ST 91 had any effect on basal cyclic AMP levels, in concentrations up to 10^{-4} M. Stimulation with clonidine (10^{-5} M) produced 92 ± 19 per cent of the basal cyclic AMP level in the absence of agonist.

L-Isoprenaline (10^{-5} M) and *l*-phenylephrine (10^{-5} M) together produced a greater stimulation of 178 ± 10 per cent ($P < 0.025$, compared to the basal cyclic AMP level) than either agonist alone, although

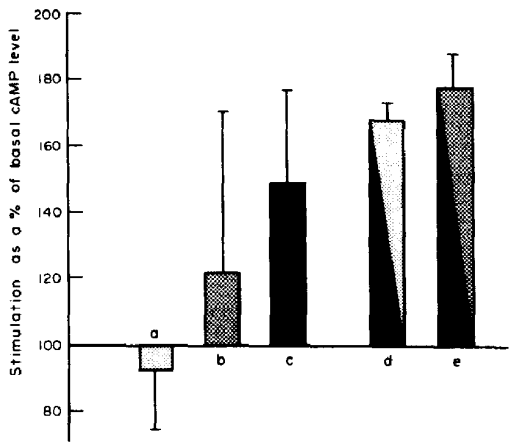

Fig. 3. The stimulation of mouse limbic forebrain adenylate cyclase produced by *l*-isoprenaline in combination with the α-adrenergic agonists clonidine and *l*-phenylephrine. L-isoprenaline, clonidine and *l*-phenylephrine were tested alone and in combination at concentrations of 10^{-5} M for their ability to stimulate adenylate cyclase. Experiments were performed and results calculated as described in Methods. A 5 min stimulation period was used for all agonists. a = clonidine (10^{-5} M); b = *l*-phenylephrine (10^{-5} M); c = *l*-isoprenaline (10^{-5} M); d = clonidine (10^{-5} M) + *l*-isoprenaline (10^{-5} M); e = *l*-phenylephrine (10^{-5} M) + *l*-isoprenaline (10^{-5} M). The significances between the results were: a vs d, $P < 0.01$; b vs e, c vs d, c vs e, $P > 0.05$.

Table 1. The effect of compounds with adrenergic and dopaminergic antagonist properties on the stimulation of adenylate cyclase by *l*-NA (10^{-5} M) in slices of mouse limbic forebrain

Antagonists	IC_{50} (M)	Concentration producing maximal inhibition (M)	Maximal inhibition %
Phentolamine	8.6×10^{-9}	10^{-5}	82 ± 8‡
Phenoxybenzamine	3.3×10^{-8}	10^{-6}	93 ± 7‡
Yohimbine	1.3×10^{-7}	10^{-6}	82 ± 11†
Pimozide	1.3×10^{-7}	10^{-6}	84 ± 19*
d,l-Propranolol	2.2×10^{-6}	10^{-5}	95 ± 8‡
Clonidine	2.2×10^{-6}	10^{-5}	70 ± 7‡
ST 91	—	10^{-5}	9 ± 32

Slices were incubated for 15 min with the antagonists prior to stimulation for 5 min with 10^{-5} M *l*-NA. The IC_{50} is the concentration of antagonist required to give 50 per cent inhibition of the stimulation of cyclic AMP produced by 10^{-5} M *l*-NA. These values were estimated graphically from the dose–response curves. Each point on the curve was the mean of 6 independent determinations each estimated in duplicate. Statistically significant differences compared to the stimulation gained with *l*-NA alone are indicated by—*$P < 0.05$; †$P < 0.0025$; ‡$P < 0.0005$.

The basal cyclic AMP level was 11.1 ± 0.6 pmoles/mg protein.

this stimulation was not significantly greater than that produced by *l*-isoprenaline (10^{-5} M) alone (Fig. 3). Despite the lack of effect of clonidine (10^{-5} M) on the basal cyclic AMP level, clonidine (10^{-5} M) together with *l*-isoprenaline (10^{-5} M) produced a 168 ± 6 per cent stimulation of adenylate cyclase ($P < 0.0005$, compared to the basal cyclic AMP level). However, this stimulation was not significantly greater than that produced by *l*-isoprenaline (10^{-5} M) alone (Fig. 3). In contrast, when the slices were stimulated with *l*-NA (10^{-5} M) together with either *l*-adrenaline (10^{-5} M), *l*-isoprenaline (10^{-5} M) or *l*-phenylephrine (10^{-5} M), the stimulation was less than that produced by the more potent agonist alone. Similarly clonidine, but not ST 91, also inhibited the stimulation of adenylate cyclase produced by *l*-NA (10^{-5} M) (Table 1). DA was inactive as an agonist or antagonist in this system at concentrations up to 10^{-4} M. At this concentration it produced a cyclic AMP level which was 95 ± 13 per cent of the basal level.

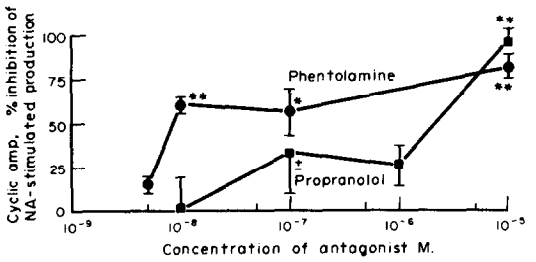

Fig. 4. Dose–response curves for the inhibition of *l*-NA (10^{-5} M) stimulated cyclic AMP production by the α-adrenergic antagonist phentolamine and the β-adrenergic antagonist *d,l*-propranolol. Slices were preincubated for 20 min, and then incubated for 15 min with concentrations of the two antagonists between 10^{-9} and 10^{-5} M, before stimulation of cyclic AMP formation for 5 min with *l*-NA (10^{-5} M). The per cent inhibition was calculated as described in Methods. The values given are the mean ± 1 S.E.M. of at least 6 independent determinations. The significances of the inhibitions produced at each concentration value are indicated by asterisks **$P < 0.0025$; *$P < 0.025$. The average basal cyclic AMP level was 11.3 ± 1.4 pmoles/mg protein for the propranolol dose–response curve and 10.1 ± 1.2 pmoles/mg protein for the phentolamine dose–response curve.

The effect of compounds with adrenergic and dopaminergic antagonist properties. The stimulation of cyclic AMP production by *l*-NA was inhibited both by the β-adrenergic antagonist *d,l*-propranolol and by the α-antagonist phentolamine (Fig. 4). The concentration of drug producing 50 per cent inhibition of the response to 10^{-5} M *l*-NA (IC_{50}) was 2.2×10^{-6} M for *d,l*-propranolol, and 8.4×10^{-9} M for phentolamine.

The effects of two other α-adrenergic antagonists, yohimbine and phenoxybenzamine and of the dopaminergic antagonist pimozide on NA-sensitive adenylate cyclase are shown in Table 1. At concentrations higher than the concentration required for maximal inhibition (see Table 1) both phenoxybenzamine and pimozide produced less than maximal inhibition.

The effect of compounds interfering with NA uptake and release. The NA uptake blocker desipramine (10^{-5} M) inhibited *l*-NA stimulated cyclic AMP production by 55 ± 14 per cent ($P < 0.01$), without affecting the basal cyclic AMP level. The residual stimulation was not affected by *d,l*-propranolol (10^{-5} M) but was non-significantly inhibited by a further 24 ± 18 per cent by phentolamine 10^{-5} M.

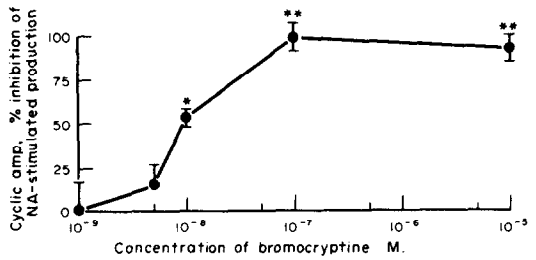

Fig. 5. Dose–response curve for the inhibition of *l*-NA (10^{-5} M) stimulated cyclic AMP production by bromocryptine. The dose–response curve was determined as in the legend to Fig. 3. Slices were preincubated for 15 min either with bromocryptine, or in control slices with its vehicle, containing ethanol and tartaric acid. The values given are the mean ± 1 S.E.M. of at least 6 independent determinations of the percentage inhibition. The significance of the inhibition produced at each concentration value is indicated by asterisks: **$P < 0.0025$; *$P < 0.025$. The average basal cyclic AMP level was 9.4 ± 0.8 pmoles/mg protein from 40 results.

Table 2. The effect of dopamine agonists on basal and NA-stimulated cyclic AMP levels in slices of mouse limbic forebrain

Drug	Concentration (M)	Basal cyclic AMP level % of control	NA-stimulated cyclic AMP level % of control NA stimulation	% Inhibition of rise in cyclic AMP due to NA
ET 495	5×10^{-5}	96 ± 8	63 ± 6†	32 ± 15
S584	5×10^{-5}	105 ± 9	67 ± 10†	31 ± 35
Apomorphine	5×10^{-5}	66 ± 7†	62 ± 4†	31 ± 8
Bromocryptine	10^{-5}	87 ± 6	38 ± 5‡	92 ± 8‡
Ergocornine	10^{-5}	122 ± 36	57 ± 11†	56 ± 29*

To determine the effect of the drugs on basal cyclic AMP levels the slices were incubated for 5 min with the drugs. To determine their ability to antagonise NA-stimulated cyclic AMP levels the drugs were added at the beginning of the 15 min incubation period and prior to stimulation with 10^{-5} M *l*-NA for 5 min. Apomorphine (5×10^{-5} M) produced a 49 ± 4 per cent ($P < 0.0005$) inhibition of the basal level of cyclic AMP when added alone for 15 min as a control for stimulation experiments. This was taken into account in the calculation of the percentage inhibition of the rise in cyclic AMP due to *l*-NA (10^{-5} M). The results are the means of 6 independent determinations each estimated in duplicate.

Details of calculation are given in the Methods. Statistical significances are indicated by—
* $P < 0.05$; † $P < 0.025$; ‡ $P < 0.0005$. The average basal cyclic AMP level was 16.6 ± 1.0 pmoles/mg protein.

Reserpine (10^{-5} M) and dexamphetamine (10^{-5} M) did not affect the basal cyclic AMP level or the stimulation produced by *l*-NA (10^{-5} M).

The effect of dopaminergic agonists. The effect of various compounds with dopaminergic agonist properties was studied on cyclic AMP production in limbic forebrain slices. Their effect on basal cyclic AMP levels and on *l*-NA stimulation is shown in Table 2. Only apomorphine (5×10^{-5} M) affected basal cyclic AMP producing a 34 ± 7 per cent inhibition when added as agonist ($P < 0.01$). All the DA agonists tested significantly reduced the absolute level of cyclic AMP found after stimulation with *l*-NA (10^{-5} M). However, only bromocryptine and ergocornine (10^{-5} M) significantly inhibited the rise in cyclic AMP solely attributable to NA stimulation. From a dose–response curve for the inhibition of NA-stimulated cyclic AMP formation by bromocryptine (Fig. 5), the IC_{50} was 9.4×10^{-9} M. Bromocryptine produced a maximal inhibition of 99 ± 12 per cent ($P < 0.0005$) at a concentration of 10^{-7} M.

DISCUSSION

The characteristics of the NA-sensitive adenylate cyclase in mouse limbic forebrain slices at present under investigation, are very similar to those found in rat limbic forebrain, using a similar incubation system for the slices [7, 11]. The basal cyclic AMP level and the degree of stimulation of adenylate cyclase by *l*-NA is also comparable to that found in vesicular cell-free preparations from rat limbic forebrain [8]. The incubation system, with continuous oxygenation and a high medium:tissue ratio of approximately 2000 during incubation was designed to reduce leakage of adenine nucleotides from the slices; for these increase basal cyclic AMP levels [19]. That the methodology used succeeded in this object is indicated by the low basal tissue cyclic AMP levels, which are similar to those found endogenously [20]. Common to this and previous reports of NA-sensitive adenylate cyclases in limbic forebrain [7, 8, 11] is the inability of DA to stimulate the system; although the DA content is high. In contrast, in limbic forebrain homogenates, a DA-sensitive adenylate cyclase is found when the tissue is prepared in a hypotonic medium in the absence of Ca^{2+} ions [5]. The relationship between these two adenylate cyclases has not yet been established [21, 31].

In the present system the potency of the adrenergic agonists for adenylate cyclase in terms of the concentration required for half-maximal stimulation (EC_{50}) was found to be isoprenaline > adrenaline ⩾ noradrenaline, which is the order of potency for classical β-receptors [22]. However, the maximal stimulation of adenylate cyclase achieved by each agonist at 10^{-5} M was greatest for adrenaline and lower for noradrenaline and isoprenaline. The ability of adrenaline to produce such a large stimulation of adenylate cyclase in limbic forebrain has not previously been reported in rodents [1]. However, the poor agonist ability of *l*-isoprenaline in mice is in confirmation of the results of Schultz and Daly [24], and the inability of the α-adrenergic agonist *l*-phenylephrine to stimulate adenylate cyclase has also been found previously [24].

These results suggest the presence in the limbic forebrain of a mixed population of receptors. Pure α-adrenergic receptors do not appear to be present, but the ability of *l*-adrenaline to produce a greater maximal stimulation than either *l*-NA or *l*-isoprenaline raises the possibility that it may be acting on a class of receptor which requires co-stimulation at two associated sites, one with α- and one with β-adrenergic properties. The presence in the brain of this type of receptor has previously been proposed by Skolnick and Daly [25]. These authors found that in rat cortex the adenylate cyclase stimulation produced by a sub-optimal concentration of *l*-isoprenaline was enhanced by the addition of the α-adrenergic agonist clonidine. From this evidence they postulated the existence of a β-adrenergic receptor which was associated with an α-adrenergic site for co-stimulation. Similarly, the presence in guinea-pig cortex of an α-adrenergic receptor whose activation may be enhanced by stimulation of an associated β-adrenergic receptor has been suggested by Sattin *et al.* [19].

In agreement with this hypothesis we observed a

tendency for synergism between the pure α- and β-adrenergic agonists *l*-phenylephrine and *l*-isoprenaline and also between clonidine in combination with *l*-isoprenaline in stimulation of cyclic AMP production. However, the ability of NA to produce any synergism with *l*-isoprenaline in its stimulation of adenylate cyclase and the failure of a combination of α- and β-adrenergic agonists to produce a maximal stimulation approaching that of *l*-adrenaline alone (Fig. 3) cannot be explained only by the presence of an adrenergic receptor which requires both α- and β-stimulation. It is possible that *l*-adrenaline may also be stimulating an independent adrenergic receptor associated with adenylate cyclase, which is in agreement with the finding that the limbic forebrain region is innervated by adrenergic neurones [26].

The existence of an α-receptor in rat cerebral cortex has previously been reported [27–29]. This was stimulated by NA in the presence of adenosine. It is unknown what relationship this might bear to the results of the present investigation, as attempts have been made to minimise the leakage of endogenous adenosine, and the effect of exogenous adenosine has not been studied.

From the IC_{50} values for the α- and β-adrenergic antagonists studied, phentolamine is a 250-fold more potent antagonist than propranolol, although both give maximal inhibitions of greater than 80 per cent at 10^{-5} M, suggesting that neither is acting on an entirely independent set of receptors. Palmer and Burks [30] concluded from similar results that the adrenergic receptors in rat cerebral cortex are relatively non-specific. However, that propranolol produced an almost 100 per cent inhibition of NA-sensitive adenylate cyclase at 10^{-5} M, would also lend support to the hypothesis that the noradrenergic receptors being studied are composed of two major groups: (i) a small population of independent β-receptors, which are phentolamine-insensitive; and (ii) a much larger population of β-dependent α-receptors, which is inhibited by both phentolamine and *d,l*-propranolol.

An alternative suggestion for similar results has been put forward by Bockaert *et al.* [31] that the α-adrenergic component of the NA-sensitive adenylate cyclase is a result of NA stimulation of the DA receptor, although the opposite conclusion is reached by Harris [21]. This is difficult to equate with present results as DA itself does not stimulate the system, and it is unlikely that the characteristics of the receptors have been altered in the sliced tissue preparation incubated in Krebs–Ringer buffer, used in the present study.

Of the other adrenergic antagonists studied, the α-antagonist phenoxybenzamine showed a similar potency to phentolamine, but with greater maximal inhibition. It has previously been found that at high concentrations phenoxybenzamine shows non-specific inhibitory properties, not only of adrenergic receptors but also of histaminergic and serotonergic systems [19, 32]. Yohimbine, reported to be a presynaptic α-receptor antagonist [33] had a slightly higher IC_{50} than the other α-antagonists, and produced a maximal inhibition of 82 ± 11 per cent. From lesion studies it has been suggested that the NA-sensitive adenylate cyclase is post-synaptically situated [11]. This result brings into question the ability of yohimbine to distinguish between pre- and post-synaptic receptors, especially at high concentrations.

Clonidine from behavioural and other studies, has been found to have the characteristics of a post-synaptic α-adrenergic agonist [34]. However, from biochemical and iontophoretic approaches it appears to have α-adrenergic antagonist properties [35, 38]. This may be due in part to preferential stimulation of presynaptically located α-adrenergic receptors by low concentrations of clonidine [36]. In the present results, neither clonidine, nor its structural analogue ST 91, which is 2.6-fold more potent as a peripheral α-receptor agonist [37] had any adenylate cyclase stimulant properties. This is in agreement with the hypothesis that the α-receptors in this system require co-activation by β-adrenergic agonists. Clonidine, but not ST 91, inhibited NA-sensitive adenylate cyclase with an IC_{50} similar to that of yohimbine. The relationship of these results to the behavioural effects of clonidine are unclear.

The effect of dopaminergic antagonists on NA-sensitive adenylate cyclase has been studied previously in slices of rat limbic forebrain and in homogenates derived from this area [7, 8, 11]. In the present study only the potency of the butyrophenone derivative pimozide has been investigated, and its IC_{50} of 1.3×10^{-7} M is similar to that found by Blumberg *et al.* [7], who gained an IC_{50} of 7.5×10^{-8} M, tested against a lower concentration of *l*-NA (5×10^{-6} M). However, Horn and Phillipson [8] found that 10^{-5} M pimozide produced less than 50 per cent inhibition of the stimulation of adenylate cyclase due to 5×10^{-5} M *l*-NA. This apparent discrepancy could be resolved, as in our hands, pimozide (10^{-5} M) produced lower than maximal inhibition (60 per cent) suggesting that non-specific membrane effects may be causing interference at this concentration level. These results indicate that pimozide is not a pure dopaminergic antagonist, although it has been used as such in many behavioural investigations.

Several studies have been performed to attempt to determine the subcellular localisation of adenylate cyclase, and it has been suggested that catecholamine-sensitive adenylate cyclases are associated with the post-synaptic receptor [39, 40] although this conclusion is not unopposed [41]. Present results indicate that desipramine (10^{-5} M) inhibits NA-sensitive adenylate cyclase by 55 per cent. Desipramine is a specific inhibitor of the neuronal uptake of *l*-NA [42], although it has been reported to have a weak post-synaptic α-adrenergic blocking effect, from iontophoretic data on cortical neurones [43]. A possible conclusion from the present results may be that part of the NA-sensitive adenylate cyclase depends on the prior uptake of NA and is located within the pre-synaptic neurone. This is in contrast to previous results [8]. However, it may reflect a difference between cyclic AMP formation in intact cells and in vesicular preparations.

An alternative explanation for this result might be that desipramine added during the incubation period leads to an accumulation of endogenous NA at the receptor, by inhibition of its reuptake. This might lead to rapid desensitization of the receptor to subsequent stimulation by added *l*-NA. The lack of effect of pro-

pranolol (10^{-5} M) on the residual stimulation suggests that it is primarily the β-receptor which has been desensitized, as has been reported previously [44].

Because DA has no effect on the adenylate cyclase under investigation, it forms a possible system in which to study the effects on NA receptors of drugs such as neuroleptics and dopaminergic agonists whose major effect is thought to be on cerebral dopaminergic systems. None of the dopaminergic agonists tested showed any stimulant properties, although apomorphine [2, 4], ET495 [45] and its active metabolite S584 [46] have all been shown to stimulate DA-sensitive adenylate cyclase systems.

All the dopaminergic agonists tested in the present system reduced the absolute level of tissue cyclic AMP found after *l*-NA stimulation, although for apomorphine this is partially accounted for by an inhibition of the basal cyclic AMP level. It has been reported previously that dopaminergic agonists such as apomorphine (Jenner, unpublished observation) and ET495, administered *in vivo*, increase cerebral NA turnover which may be through interaction with presynaptic NA receptors [47]. However, of all the DA agonists, only bromocryptine and ergocornine were found to produce a significant inhibition of the rise in cyclic AMP attributable solely to stimulation by *l*-NA. Further investigation of the potency of bromocryptine showed it to have an extremely low IC_{50} of 9.4×10^{-9} M for the inhibition of NA-stimulated adenylate cyclase, which is of the same order of magnitude as that of the α-blocker phentolamine (Table 1). Previous results have shown ergocornine and bromocryptine to have prolonged DA receptor stimulant properties [48, 49] and to cause some depletion of cerebral NA, either because of NA receptor blockade or as the result of a weak reserpine-like action [48, 50].

The effect of bromocryptine on DA-sensitive adenylate cyclase is as yet unclear [51]. There is some evidence that its mode of action on cerebral dopaminergic and noradrenergic systems may be biphasic, and depend on the dose of bromocryptine used [52], although no evidence for such a biphasic response has been obtained in the present results. Ergot alkaloids are known to be α-blocking agents and to inhibit NA-sensitive adenylate cyclase in the periphery [53]. The present results provide evidence for the ability of bromocryptine to produce extremely potent inhibition of NA-sensitive adenylate cyclase in the C.N.S. This noradrenergic receptor antagonist activity of bromocryptine should be taken into account in the attempts being made to investigate the pharmacological basis for the ability of bromocryptine to stimulate locomotor activity in animals, and its efficacy in the treatment of Parkinson's disease.

Acknowledgements—This work was supported by the research funds of the Bethlem Royal and Maudsley Hospitals, and of King's College Hospital, The Medical Research Council, The Wellcome Trust, and the National Fund for Research into Crippling Diseases. We would like to thank Professor H. McIlwain for loan of his tissue chopper and helpful advice, and I.C.I., S.K.F. Ltd, Les Laboratoires Servier, Sandoz Ltd., Boehringer Ingelheim, Ciba Ltd., Halewood Chemicals, and Janssen Ltd. for gifts of drugs. M.C.B.S. and A.D. would like to thank Dr. A. S. Horn and Dr. O. T. Phillipson for stimulating discussion of the results.

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