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The effect of bromocriptine on locomotor activity and cerebral catecholamines in rodents

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The locomotor activity in mice induced by bromocriptine was suppressed by drugs inhibiting both dopaminergic and noradrenergic pre- and post-synaptic actions. The onset of locomotor activity was preceded by a period of decreased activity which lengthened with increasing dose. Both increased and decreased turnover of noradrenaline and decreased turnover of dopamine was shown by measurement of the dopamine metabolites homovanillic acid, dihydroxyphenylacetic acid (DOPAC) and the noradrenaline metabolite MOPEG-SO_4 and following pretreatment of animals with α-methyl-*p*-tyrosine. The increased activity caused by bromocriptine did not correlate with a consistent biochemical change, but the period of behavioural suppression appeared to be associated with an increased noradrenaline turnover. Bromocriptine potently inhibited the noradrenaline-stimulated adenylate cyclase system from mouse limbic forebrain suggesting that the increased *in vivo* turnover of noradrenaline may be due to a post-synaptic receptor blockade. The involvement of cerebral dopamine receptors was substantiated by the ability of bromocriptine to displace [^{3}H]haloperidol from binding sites in rat striatal preparations. The findings are interpreted as indicating a complex involvement of both noradrenaline and dopamine pre- and post-synaptic components in the locomotor activity produced by bromocriptine, possibly due to the involvement of a partial agonist action or an active metabolite.

Bromocriptine (2-bromo-α-ergocryptine; CB 154) is of value in the treatment of Parkinson's disease (Calne, Leigh & others, 1974a; Calne, Teychenne & others, 1974b; Parkes, Marsden & others, 1976). This therapeutic action has been attributed to a direct stimulation of cerebral dopamine receptors, for bromocriptine mimics apomorphine in causing contralateral turning in rodents with unilateral nigrostriatal lesions (Corrodi, Fuxe & others, 1973; Fuxe, Corrodi & others, 1974; Pieri, Pieri & others, 1975; Anlezark, Pycock & Meldrum, 1976; Johnson, Loew & Vigouret, 1976). Such dopamine receptor stimulation is believed to be reflected by a reduction in cerebral dopamine turnover in the presence of α-methyl-*p*-tyrosine (α-MT) induced synthesis blockade (Hokfelt & Fuxe, 1972; Corrodi & others, 1973; Fuxe & others, 1974).

However, a simple post-synaptic action is difficult to reconcile with the observation that reserpine or α-MT inhibit bromocriptine-induced circling (Corrodi & others, 1973; Fuxe & others, 1974) and prevent the locomotor activity produced by bromocriptine (Johnson & others, 1976). This dependence on intact presynaptic events differentiates bromocriptine from classical dopamine agonists. A further difference is apparent in the considerable delay before bromocriptine increases locomotor activity, during which behavioural suppression has been observed.

The current study therefore further investigates the mechanism by which bromocriptine alters locomotor activity in rodents by examining the roles of both cerebral dopamine and noradrenaline using a combination of behavioural and biochemical assessments.

MATERIALS AND METHODS

Locomotor activity experiments

Locomotor activity was measured in 4 sets of Animex activity meters (LKB Farad Ltd) using batches of 3 'Swiss S' or 'P' strain male mice (20–25 g; Animal Suppliers Ltd). Animals were housed in clear Plexiglass cages with similar lids so that behavioural observations could be made during the experiments which were carried out under conditions of standard laboratory lighting and temperature; food and water were withdrawn during the test period. All control and drug-treated animals were examined in parallel over the same time period, on the same occasions, using mice of the same batch and by interdigitating sets of Animex apparatus. Activity was measured as the mean number of

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counts per 10 min or 1 h intervals (±1 s.e.), or as the mean total number of counts (±1 s.e.) registered during the experiment. Each experiment incorporated at least 4 batches of animals whose activity was monitored over 9 or 24 h.

Animals received bromocriptine mesylate (Sandoz Products Ltd; 1–160 mg kg^{-1}, i.p.) dissolved in a minimum quantity of 70% ethanol using an equal quantity of tartaric acid. The amount of ethanol administered necessarily increased with the dose; the maximum quantity received was 0·1 ml of 3·5% ethanol. In one experiment the animals receiving bromocriptine (5 mg kg^{-1}, i.p.) also received the maximum quantity of ethanol.

In other experiments animals receiving bromocriptine mesylate (10 mg kg^{-1}, i.p.) were pretreated with 0·9% saline (0·1 ml, i.p.), phenoxybenzamine (SKF Ltd; 20 mg kg^{-1}, i.p.), propranolol (ICI Ltd; 20 mg kg^{-1}, i.p.), FLA-63 (bis-[1-methyl-4-homopiperazinylthiocarbonyl]disulphide, Labkemi AB; 25 mg kg^{-1}, i.p.), α-methyl-*p*-tyrosine methyl ester HCl (α-MT; Sigma Chemical Co.; 200 mg kg^{-1}, i.p.), all 1 h previously; pimozide (Janssen Pharmaceuticals; 1 mg kg^{-1}, i.p.) 3 h previously or reserpine (Halewood Chemicals Ltd; 10 mg kg^{-1}) 18–24 h previously.

Other animals receiving bromocriptine (5 or 40 mg kg^{-1}) also received apomorphine (Evans Medical Ltd; 0·5 mg kg^{-1}, s.c.) or clonidine (Boehringer Ingelheim Ltd; 2 mg kg^{-1}, i.p.) 2 or 4 h after drug administration.

Catecholamine turnover studies

'Swiss S' or 'P' strain male mice (20–25 g) were pretreated with α-MT (200 mg kg^{-1}, i.p.) 2 h before death. Bromocriptine mesylate (5–80 mg kg^{-1}, i.p.) or vehicle was administered 2, 5·5 or 10 h before death. Animals were killed by cervical dislocation and decapitation, the brains were rapidly removed and cooled to −20°. The brains from two animals were pooled for each sample. Catecholamine separation was performed by the method of Atack (1973). Dopamine was assayed according to this method and noradrenaline by the method of Weil-Malherbe & Bigelow (1968).

Determination of homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) concentrations in the mouse

Male mice (20–25 g) received bromocriptine mesylate (5–80 mg kg^{-1}) 2, 5·5 or 10 h before death. The brain was removed as described above and forebrain concentrations of HVA and DOPAC were determined according to Westerink & Korf (1976).

Determination of 3-methoxy-4-hydroxyphenylglycol sulphate (MOPEG-SO_4) concentrations in rats

Male Wistar rats (225–275 g; Animal Suppliers Ltd) received bromocriptine mesylate (5–160 mg kg^{-1}, i.p.) 2, 5·5 or 10 h before death. Animals were decapitated and the brains rapidly removed and cooled to −20°. Whole brain concentrations of MOPEG-SO_4 were subsequently determined by the technique of Meek & Neff (1972). A behavioural assessment of the animals was made immediately before death.

This determination was carried out in rats because the sulphate conjugate is not formed in mice (Caesar, Hague & others, 1974; Howlett, Jenner & Nahorski, 1975) and fluorimetric techniques are not available for the determination of the free glycol.

Determination of mouse limbic forebrain noradrenaline sensitive adenylate cyclase

Noradrenaline sensitive adenylate cyclase was assayed by a modification (Sawaya, Dolphin & others, 1977) of the method of Blumberg, Taylor & Sulser (1975) in slices of mouse (20–25 g) limbic forebrain (including hippocampus, olfactory tubercles, septal nuclei, the anterior part of the medial forebrain bundle, the amygdaloid nuclei and nucleus accumbens) dissected as previously described (Sawaya & others, 1977). Stimulation with (−)-noradrenaline (10^{-5} M) for 5 min produced an approximate three-fold increase in cyclic (c)AMP production. Bromocriptine (10^{-9}–10^{-5} M as the base) or vehicle was added as an antagonist 15 min before the stimulation period.

Determination of rat striatal dopamine receptor binding

Dopamine receptor binding was assayed in the striatum of female Wistar rats (150 g) by a modification (Leysens & Laduron, 1977) of the technique of Creese, Burt & Snyder (1976). [^{3}H]Haloperidol (Janssen Pharmaceutica; 10 Ci $mmol^{-1}$) 2×10^{-9} M was used to label the striatal preparation. Bromocriptine (10^{-10}–10^{-6} M as the base) or vehicle was incorporated into the system. Incubations containing either (+)- or (−)-butaclamol (2×10^{-6} and 2×10^{-7} M respectively; Ayerst Laboratories) were also included to distinguish specific and non-specific [^{3}H]haloperidol binding.

Statistics

All data was analysed by Student's *t*-test and were considered significant when $2P < 0.05$.

RESULTS

General behavioural observations

Apart from the changes in locomotor response detailed below, few other behavioural changes were observed. The period before the onset of locomotor activity was characterized by the animals remaining quiet but alert and responding to sensory stimuli. During periods of enhanced activity stereotyped sniffing was apparent and in rat experiments licking but not compulsive gnawing was observed.

Locomotor activity studies

Bromocriptine (1–160 mg kg^{-1}) produced variable changes in locomotor activity in normal mice (Fig. 1, Table 1). A low dose (1 mg kg^{-1}) produced

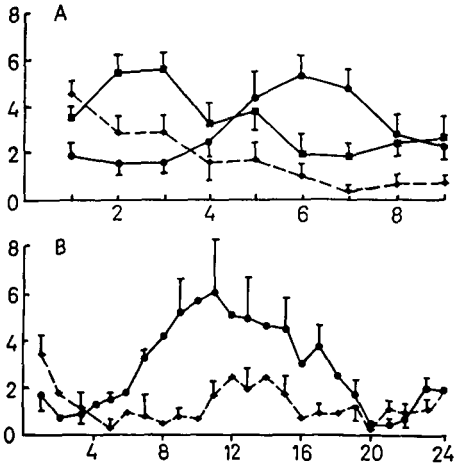

FIG. 1. The effect of bromocriptine (A) 5 mg kg^{-1} (—■—) or 40 mg kg^{-1} (—●—) and (B) 80 mg kg^{-1} (—●—) on locomotor activity compared to vehicle treated animals (– –◆– –) as judged using groups of three mice in Animex activity meters over 9 or 24 h. Ordinate—Mean Animex counts h^{-1} ± 1 s.e.m. (× 10^3). Abscissa—Time (h) after bromocriptine administration.

a decrease in locomotor activity compared with control animals. Higher doses (10–160 mg kg^{-1}) produced an initial period of behavioural suppression. This lag phase was followed by a period of enhanced locomotor activity. The degree of reduction of locomotor activity during the initial lag phase, and the duration of the lag phase, were dose dependent (Table 1). The intensity of the subsequent period of hyperactivity with doses of 2·5–160 mg kg^{-1} was not related to the dose, but the time of

Table 1. *Bromocriptine-induced changes in locomotor activity in mice as judged using Animex activity meters.* I—Counts during 1st hour as % controls (± 1 s.e.). II—Duration of lag phase (h). III—Time of peak hyperactivity (h). IV—Maximum activity as % control values during same 1 h period. V—Counts during recording period as % controls (1 s.e.).

Dose mg kg^{-1}	I	II	III	IV	V
1·0	59 ± 4*	–	–	–	65 ± 9
2·5	87 ± 15	0	2	138	161 ± 20*
5·0	77 ± 10	0·5	2·5	195	187 ± 26*
10·0	52 ± 11*	1	4	314	180 ± 37*
20·0	47 ± 6*	2	5	554	173 ± 24*
40·0	43 ± 7*	3	6	486	170 ± 28*
80·0	34 ± 6*	3	11	361	232 ± 38*
160·0	18 ± 5*	5	15	558	215 ± 24*

* $P < 0.05$ compared to control values.
Activity was measured over 9 h (1–40 mg kg^{-1}) or 24 h (80 and 160 mg kg^{-1}). Each value is the mean of at least 6 observations. Control animals registered 4588 ± 523 Animex counts during the 1st hour of recording and 16654 ± 3432 or 29368 ± 3316 during the 9 or 24 h periods respectively.

peak activity was delayed as the dose increased (Table 1).

The locomotor activity recorded after administration of bromocriptine (5 mg kg^{-1}) in the maximum quantity of ethanol (0·1 ml of 3·5%) was not different from that recorded after the normal administration of bromocriptine (5 mg kg^{-1}).

The increased locomotor activity observed during the measurement period after bromocriptine (10 mg kg^{-1}) was inhibited by the administration of pimozide (1 mg kg^{-1}), FLA-63 (25 mg kg^{-1}), phen-

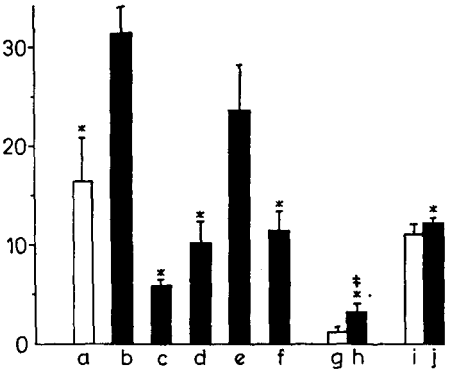

FIG. 2. The effect of drugs altering dopamine and noradrenaline mediated neuronal transmission on the locomotor response of groups of three mice to bromocriptine (10 mg kg^{-1} closed columns) over 9 h as judged using Animex activity meters. Ordinate—Mean Animex counts 9 h ± 1 s.e.m. (× 10^3). a, vehicle, b, bromocriptine (bct); c, bct + pimozide; d, bct + phenoxybenzamine; e, bct + propranolol; f, bct + FLA63; g, reserpine; h, reserpine + bct; i, α-MT; j, α-MT + bct. * $P < 0.01$ *vs* bct; ‡ $P < 0.01$ *vs* reserpine.

oxybenzamine (20 mg kg^{-1}), reserpine (10 mg kg^{-1}) or α-MT (200 mg kg^{-1}) (Fig. 2). Propranolol (20 mg kg^{-1}) was without effect.

Clonidine (2 mg kg^{-1}) did not alter bromocriptine (5 mg kg^{-1}) induced locomotor activity when administered 2 and 4 h after this dose (during periods of enhanced locomotor responses) (Table 2).

Administration of apomorphine (0·5 mg kg^{-1}) during the period of inhibition of locomotor activity 2 h after bromocriptine (40 mg kg^{-1}) produced a period of locomotor activity of 60–90 min duration that was greater than that seen after the administration of apomorphine to normal animals (P <0·05, Table 2). The administration of clonidine (2 mg kg^{-1}) at that time produced an immediate increase of locomotor activity that continued until the end of the measurement period (Table 2).

Table 2. *The effect of bromocriptine (5 or 40 mg kg^{-1}) in combination with clonidine (2 mg kg^{-1}) or apomorphine (0·5 mg kg^{-1}) on locomotor activity in mice as judged using Animex activity meters.*

Drug administered	Total Animex counts during recording period*
Bromocriptine 5 mg kg^{-1} + saline at 2 and 4 h	31 178 ± 4261 (6)
Bromocriptine 5 mg kg^{-1} + clonidine 2 mg kg^{-1} at 2 and 4 h	27 365 ± 2865 (6) P >0·05
Bromocriptine 40 mg kg^{-1} + saline at 2 h	26 399 ± 3853 (8)
Bromocriptine 40 mg kg^{-1} + clonidine 2 mg kg^{-1} at 2 h	49 627 ± 4827 (4) P <0·005
Apomorphine 0·5 mg kg^{-1} in normal animals	4255 ± 441 (4)
Apomorphine 0·5 mg kg^{-1} 2 h after bromocriptine 40 mg kg^{-1}	6096 ± 580 (4) P <0·05

* Activity was recorded over 9 h in experiments involving clonidine. Apomorphine was administered 2 h after bromocriptine (40 mg kg^{-1}) during the period of behavioural suppression. Activity was recorded during the 1·5 h of enhanced activity produced by apomorphine that occurred before the onset of bromocriptine-induced hyperactivity.

Catecholamine turnover studies

The administration of α-MT (200 mg kg^{-1}) to normal animals 2 h before death caused a 40% depletion of cerebral noradrenaline (control animals 527 ± 38 ng g^{-1} n = 6; α-MT treated animals 316 ± 19 ng g^{-1} n = 18; P <0·0005) and 42% depletion of cerebral dopamine (control animals 814 ± 42 ng g^{-1} n = 6; α-MT treated animals 472 ± 28 ng g^{-1} n = 18; P <0·0005). The depletion of dopamine and noradrenaline 2 h after α-MT was assessed at different time intervals after a range of doses of bromocriptine (Fig. 3).

Bromocriptine (5 mg kg^{-1}) reduced the depletion of both noradrenaline and dopamine at 2 and 5·5 h after its administration (P <0·01). Bromocriptine (10 mg kg^{-1}) produced a similar reduction in dopamine depletion at 2 h (P <0·01) but not at 5·5 h (P >0·05) and had no effect on noradrenaline depletion.

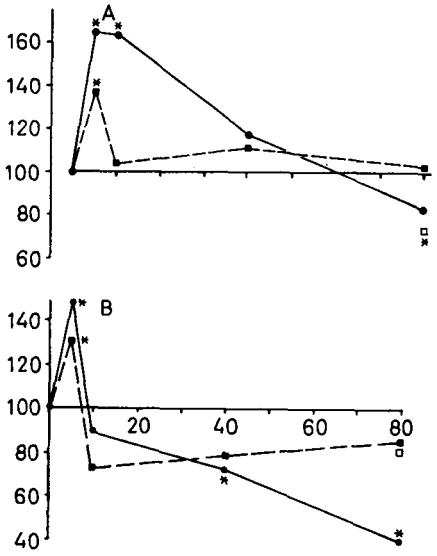

FIG. 3. Alterations in (A) dopamine and (B) noradrenaline turnover 2 h (—●—), 5·5 h (- -■- -) and 10 h (□) after the administration of bromocriptine (5–80 mg kg^{-1}) to mice pretreated with α-MT (200 mg kg^{-1}) 2 h before death. Ordinates A—Dopamine concentration as % α-MT-treated controls. B—Noradrenaline concentration as % α-MT treated controls. Abscissa—Bromocriptine (mg kg^{-1}). * P <0·05.

Bromocriptine (40 or 80 mg kg^{-1}) had no effect on dopamine depletion at either time interval, but enhanced noradrenaline depletion 2 h after administration (P>0·05 and P<0·01 respectively). Bromocriptine (80 mg kg^{-1}) had no effect on noradrenaline depletion 10 h after its administration but enhanced the depletion of dopamine (P<0·05).

Determination of cerebral HVA and DOPAC concentrations in the mouse

The forebrain concentrations of both HVA and DOPAC were decreased 2 h following the administration of bromocriptine 5–80 mg kg^{-1} (Fig. 4). At 5·5 h, however, no change was observed. Similarly 10 h after bromocriptine (80 mg kg^{-1}) the

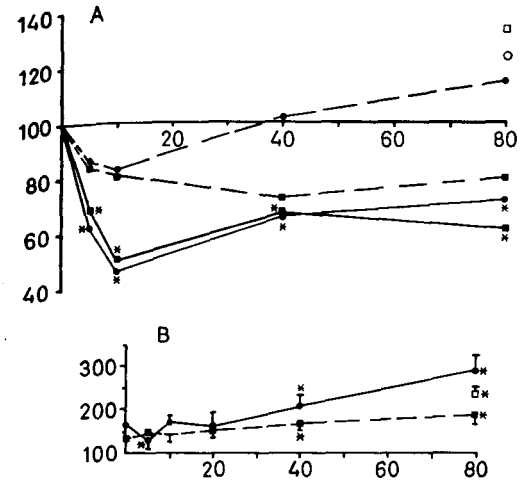

FIG. 4. Alterations in (A) HVA (■) and DOPAC (●) concentrations in mice 2 h (——), 5·5 h (– – –) and 10 h (□ HVA; ○ DOPAC) after the administration of bromocriptine (5–80 mg kg^{-1}) and (B) MOPEG-SO_4 concentrations in rats 2 h (—●—), 5·5 h (– –■– –) and 10 h (□) after the administration of bromocriptine (5–80 mg kg^{-1}). Ordinates—A—HVA or DOPAC concentrations as % control values. B—MOPEG SO_4 (ng g^{-1}). Abscissa—Bromocriptine (mg kg^{-1}). * P <0·05.

concentrations of HVA and DOPAC were unaffected.

Determination of cerebral MOPEG-SO_4 concentrations in rats

Bromocriptine (5 mg kg^{-1}) decreased whole brain concentrations of MOPEG-SO_4 2 h after administration (P<0·05) but had no effect at 5·5 h (Fig. 4). Bromocriptine (10 and 20 mg kg^{-1}) had no effect on MOPEG-SO_4 concentrations 2 h or 5·5 h after administration (P>0·05) but at higher doses (40 and 80 mg kg^{-1}) increased concentrations of this metabolite were observed at both time intervals (P<0·05). 10 h after bromocriptine (80 mg kg^{-1}) MOPEG-SO_4 concentrations remained elevated (P<0·001).

Control concentrations of MOPEG-SO_4 in animals receiving vehicle alone were higher at 2 h (162 ± ng g^{-1} n = 20) than at 5·5 h (133 ± 4 ng g^{-1} n = 16). This effect did not vary with the amount of alcohol administered.

Behavioural observations made immediately before death showed the animals to exhibit activity identical to that observed in mice.

Determination of mouse limbic forebrain noradrenaline sensitive adenylate cyclase

Noradrenaline (10^{-5} M) stimulated adenylate cyclase activity approximately three-fold. Basal concentrations were 9·3 ± 0·9 pmol cAMP mg^{-1} protein. In the presence of 10^{-5} M noradrenaline activity was 33·7 ± 3·1 pmol cAMP mg^{-1} protein. Bromocriptine 10^{-9} to 10^{-5} M produced marked inhibition of this stimulation; maximal inhibition of 99 ± 12% was observed at 10^{-7} M (P<0·0005). The IC50 value was calculated to be 9·4 × 10^{-9} M (compared to 8·6 × 10^{-9} M for the α-blocker phentolamine—unpublished data).

Determination of rat striatal dopamine receptor binding

Specific binding of [^{3}H]haloperidol was defined as that bound in the presence of (−)-butaclamol (2 × 10^{-7} M) minus that bound in the presence of (+)-butaclamol (2 × 10^{-6} M). These isomers in the concentrations used were previously shown to produce minimal and maximal displacement (IC50 >10^{-6} and 4·5 × 10^{-9} M respectively). Bromocriptine (10^{-10}–10^{-6} M) incorporation produced a marked inhibition of [^{3}H]haloperidol binding. The IC50 value was calculated to be 4·3 × 10^{-8} M (compared with 1·6 × 10^{-7} M for apomorphine; Laduron personal communication).

A theoretical consideration of the molecular resemblance between bromocriptine and tyrosine

From conformational studies of lysergic acid diethylamide (Bailey & Grey, 1972; Baker, Chothia & others, 1972) a model of the lysergic acid moiety of bromocriptine can be constructed using Drieding molecular models. This shows that the basic nitrogen atom is at a distance of *ca* 5·1 A° from the centroid of the phenyl ring and *ca* 0·5 A° above its plane (Fig. 5). L-Tyrosine in solution adopts conformations, as again evidenced in Dreiding models, that show similarities with the relevant portion of

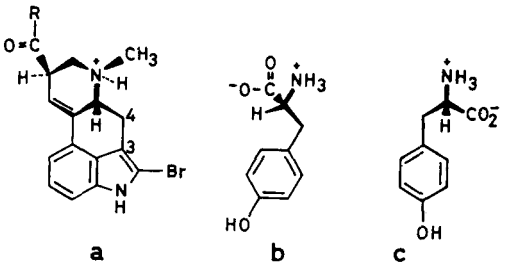

FIG. 5. Structural formulae to show the resemblance between (a) the lysergyl moiety of bromocriptine, (b) L-tyrosine in the conformation τ_1 = 20°; τ_2 = −170° and (c) L-tyrosine in the conformation τ_1 = −20°; τ_2 = 170°.

lysergic acid; the basic nitrogen occuping the same position relative to the phenyl ring. One of these likely conformations has τ_1 (phenyl-C–C) and τ_2 (C phenyl –C–C–N) dihedral angle values of *ca* 20° and −170° respectively with the carbon atom of the carboxylic group 2·2 A° above the plane of the phenyl ring (Fig. 5). Another interesting conformation is with τ_1 and τ_2 −20° and 170° respectively and with the carbon atom of the carboxylic group close to the plane of the phenyl ring (Fig. 5). Of possible significance in the latter conformer is the fact that the electron-rich carboxylic group corresponds spatially to C–3 and C–4 of bromocriptine, a region of the molecule which quantum calculations (Kumber & Sankar, 1973) show to be considerably enriched in electron density by the 2-bromo substituent.

DISCUSSION

The action of bromocriptine most studied in animals has been the increase in locomotor activity caused by some doses of the drug, for this is of relevance to its dopamine agonist activity which is of clinical importance. The increased activity generated appears to involve not only cerebral dopamine pathways, but also noradrenaline neuronal systems since the present study has shown that drugs blocking both types of catecholamine receptor reduce its action. Also, the activity of bromocriptine would seem to involve both pre- and post-synaptic actions at these catecholaminergic synapses, since its action is reduced by drugs inhibiting tyrosine hydroxylase, dopamine-β-hydroxylase and amine storage in agreement with previous findings (Corrodi & others, 1973; Fuxe & others, 1974; Johnson & others, 1976). In these respects bromocriptine differs from apomorphine; the locomotor activity produced by the latter drug is virtually unaffected by blockade of noradrenergic function or by inhibition of pre-synaptic events (see Dolphin, Jenner & Marsden, 1976 and references quoted therein).

The requirement for intact pre-synaptic events in bromocriptine-induced locomotor activity has previously been noted in relation to circling behaviour (Corrodi & others, 1973; Fuxe & others, 1974). Animals with unilateral nigrostriatal lesions receiving bromocriptine rotate in a direction which would indicate a direct action on striatal dopamine receptors rather than an indirect pre-synaptic action. It has, however, recently been demonstrated that while striatal events may determine the direction of turning, the nucleus accumbens controls the rate of rotation (Kelly & Moore, 1976). It is, therefore, feasible that it is a pre-synaptic action of bromocriptine in the intact nucleus accumbens combined with a weak post-synaptic action on denervated striatal dopamine receptors that results in the circling response seen with this drug. If this is correct then a pre-synaptic action in the nucleus accumbens may also be involved in the locomotor response observed in the present study.

The effect of bromocriptine on locomotor activity in rodents is, however, complex, for it does not always increase activity. Low doses of bromocriptine (1 mg kg^{-1} and less) appear to be inhibitory on motor function. Such an action has previously been attributed to a preferential action of the drug on pre-synaptic dopamine autoreceptors (Snider, Hutt & others, 1976). Similar effects have also been described for low doses of apomorphine (Strombom, 1976). Intermediate doses of bromocriptine (1–5 mg kg^{-1}) produce increased locomotor activity. This excitatory action of the drug is preceded by a lag phase of up to 1 h as previously reported (Johnson & others, 1976; Snider & others, 1976). High doses of bromocriptine (10–160 mg kg^{-1}), however, produce a biphasic response. This consists of a period of inhibitory activity, when the motor response is suppressed compared to control animals, and is subsequently followed by a period of enhanced activity.

The inhibitory action of high doses of bromocriptine on motor function is rapid in onset being clearly discerned in the first hour following drug administration. The extent of the behavioural suppression caused and the duration of the period of inhibition is dose dependent between 10 and 80 mg kg^{-1}. Based on turnover studies using α-MT this inhibitory action was correlated with a *relative* dose-dependent increase in both dopamine and noradrenaline turnover when compared with the action of a small dose of bromocriptine (5 mg kg^{-1}) as shown by a decline in dopamine and noradrenaline concentrations. Indeed, the profound and prolonged inhibitory action of the highest doses used (40 and 80 mg kg^{-1}) was associated with an *absolute* increase in noradrenaline turnover, although absolute changes in dopamine turnover were not apparent.

Using metabolite data to interpret the inhibitory action of high doses of bromocriptine the involvement of noradrenaline is still apparent. Thus, the concentration of MOPEG-SO_4 increased, supporting the α-MT findings which suggested increased noradrenaline turnover. The concentrations of the dopamine metabolites HVA and DOPAC, however

were decreased by high doses of bromocriptine, suggesting an action associated with agonist activity. We would therefore conclude on the present evidence that this inhibitory action of the drug is due to a blockade of post-synaptic noradrenaline receptors associated with increased noradrenaline turnover as previously suggested (Fuxe & others, 1974). This conclusion is strengthened by the ability of the α-agonist clonidine to cause arousal when administered during the period of behavioural suppression, since this compound does not increase locomotor activity when administered alone but enhances dopamine induced motor responses (Andén, Corrodi & others, 1970; Maj, Sowinska & others, 1972; Andén, Strömbom & Svensson, 1973; Andén & Strömbom, 1974). Similarly, the marked inhibitory action of bromocriptine on noradrenaline stimulated adenylate cyclase would again support this hypothesis.

The almost pure excitatory effects caused by intermediate doses of bromocriptine (5–10 mg kg^{-1}) was associated with a decrease in dopamine turnover as judged by both α-MT and metabolite data. This suggests that the drug in these doses is producing a motor response in a manner analogous to classical dopamine agonists such as apomorphine, namely by a direct action on post-synaptic dopamine receptors leading, in turn, to a reduction in turnover via a feedback mechanism. Noradrenaline turnover also was reduced by bromocriptine 5 mg kg^{-1} although not by 10 mg kg^{-1}. This may indicate that low doses of the drug also stimulate noradrenaline receptors which might augment the dopamine mediated activity.

However, during the phase of excitation produced at later times by higher doses of bromocriptine, these biochemical changes were not so clear cut. For example, 5·5 h after the administration of the drug (40 mg kg^{-1}), immediately before the peak locomotor response, dopamine turnover was not decreased as judged by α-MT studies or by the measurement of HVA and DOPAC. Similarly, noradrenaline turnover was, if anything, increased as judged by the concentration of MOPEG-SO_4 although α-MT studies showed no significant change. There is at present no clear explanation for these findings. We can only suggest that the earlier period of inhibition in some way distorts the effects of subsequent excitation on the turnover of transmitters. It should be remembered also that α-MT inhibited the production of enhanced locomotor activity following bromocriptine administration which might suggest that the biochemical changes observed in the presence of α-MT may not be those responsible for motor hyperactivity.

Clearly the increase in activity produced by bromocriptine is increasingly delayed in onset with increasing dose. A number of explanations can be put forward to explain this observation. Thus, the data might be explained if high concentrations of bromocriptine were inhibitory on motor function. The increasing lag phase before the onset of enhanced activity might be an indication of the time necessary for drug concentrations to fall to a point where the agonist activity of bromocriptine is manifest. This idea finds support in the actions of bromocriptine in displacing [^{3}H]haloperidol and [^{3}H]dopamine from their binding sites in rat striatal preparations (Burt, Creese & Snyder, 1976; and this study). The ratio of their potency in displacing these ligands suggests mixed agonist-antagonist properties. Also, while *in vivo* bromocriptine causes a rapid but short lived increase in cAMP, *in vitro* non-competitive inhibition of the striatal adenylate cyclase system is observed (Trabucchi, Spano & others, 1976).

Alternatively, the lag phase may represent the necessity for formation of an active metabolite. It may be that the necessity for intact pre-synaptic events is involved in the formation of such a pharmacologically active moiety. The close similarity of a portion of the bromocriptine molecule to L-tyrosine makes it feasible that bromocriptine or one of its peripheral metabolites may be acted on by tyrosine hydroxylase to produce an active function. Further, the accumulation of bromocriptine pre-synaptically may occur by utilization of tyrosine uptake mechanisms. In this respect the intra-neuronal accumulation of the related ergot alkaloid dihydroergotoxine is of interest (Meier-Ruge & Iwagnoff, 1976). However, there is as yet no direct evidence for the formation of an active metabolite of bromocriptine and this would not explain the increasing length of the inhibitory phase.

Bromocriptine is the only member of the second generation of dopamine agonists to prove as clinically effective as L-dopa in the treatment of Parkinson's disease. The data presented in this work serve to illustrate the complexity of the behavioural and biochemical actions of bromocriptine. We have neglected the previously demonstrated involvement of cerebral 5-hydroxytryptamine pathways in its action (Snider, Hutt & others, 1975) which adds to the complexity. Our difficulty in interpreting the data also may be related to the recent interest in

the presence of different populations of dopamine receptors within the brain. Indeed, the related ergot alkaloid ergometrine has been shown to specifically inhibit one population of these receptors and it is proposed that it is this inhibition that results in the production of the locomotor response to this drug (Cools, Honig & others, 1976).

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REFERENCES

ANDÉN, N.-E., CORRODI, H., FUXE, K., HOKFELT, B., HOKFELT, T., RYDIN, C. & SVENSSON, T. (1970). *Life Sci.*, **9**, 513–523.

ANDÉN, N.-E., STRÖMBOM, U. & SVENSSON, T. (1973). *Psychopharmac. (Berl.)*, **29**, 289–298.

ANDÉN, N.-E. & STRÖMBOM, U. (1974). *Ibid.*, **38**, 91–103.

ANLEZARK, G., PYCOCK, C. & MELDRUM, B. (1976). *Eur. J. Pharmac.*, **37**, 295–302.

ATACK, C. (1973). *Br. J. Pharmac.*, **48**, 699–714.

BAILEY, K. & GREY, A. A. (1972). *Can. J. Chem.*, **50**, 3876–3885.

BAKER, R. W., CHOTHIA, C., PAULING, P. & WEBER, H. P. (1972). *Science*, **178**, 614–615.

BLUMBERG, J. B., TAYLOR, R. E. & SULSER, F. (1975). *J. Pharm. Pharmac.*, **27**, 125–128.

BURT, D. R., CREESE, I. & SNYDER, S. H. (1976). *Molec. Pharmac.*, **12**, 800–812.

CAESAR, P. M., HAGUE, P., SHARMAN, D. F. & WERDINIUS, B. (1974). *Br. J. Pharmac.*, **51**, 187–195.

CALNE, D. B., LEIGH, P. N., TEYCHENNE, P. F., BAMJI, A. N. & GREENACRE, J. K. (1974a), *Lancet*, **2**, 1355–1356.

CALNE, D. B., TEYCHENNE, P. F., CLAVERIA, L. E., EASTMAN, R., GREENACRE, J. K. & PETRIE, A. (1974b). *Br. med. J.*, **4**, 442–44.

COOLS, A. R., HONIG, W. M. M., PIJNENBURG, A. J. J. & VAN ROSSUM, J. M. (1976). *Neurosci. Lett.*, **3**, 335–339.

CORRODI, H., FUXE, K., HOKFELT, T., LIDBRINK, P. & UNGERSTEDT, U. (1973). *J. Pharm. Pharmac.*, **25**, 409–410.

CREESE, I., BURT, D. R. & SNYDER, S. H. (1976). *Life Sci.*, **17**, 1715–1720.

DOLPHIN, A. C., JENNER, P. & MARSDEN, C. D. (1976). *Pharmac. Biochem. Behav.*, **4**, 661–670.

FUXE, K., CORRODI, H., HOKFELT, T., LIDBRINK, P. & UNGERSTEDT, U. (1974). *Med. Biol.*, **52**, 121–132.

HOKFELT, T. & FUXE, K. (1972). In: *Brain Endocrine Interactions.* Editors: Knigge, K. M., Scott, D. E. & Weindl, A. Basle: Karger.

HOWLETT, D. R., JENNER, F. A. & NAHORSKI, S. R. (1975). *J. Pharm. Pharmac.*, **27**, 447–449.

JOHNSON, A. M., LOEW, D. M. & VIGOURET, J. M. (1976). *Br. J. Pharmac.*, **56**, 59–68.

KELLY, P. H. & MOORE, K. E. (1976). *Nature*, **263**, 695–696.

KUMBAR, M. & SANKAR, D. V. S. (1973). *Res. Commun. Chem. Path. Pharmac.*, **6**, 65–100.

LEYSENS, J. & LADURON, P. (1977). *Life Sci.*, **20**, 281–288.

MAJ, J., SOWINSKA, H., BARAN, L. & KAPTURKIEWICZ, Z. (1972). *Ibid.*, **11**, Part I, 483–491.

MEEK, J. L. & NEFF, N. H. (1972). *Br. J. Pharmac.*, **45**, 435–441.

MEIER-RUGE, W. & IWAGNOFF, P. (1976). *Postgrad. med. J.*, **52**, *Suppl.* 1, 47–54.

PARKES, J. D., MARSDEN, C. D., DONALDSON, I., GALEA-DEBONO, A., WALTERS, J., KENNEDY, G. & ASSELMAN, P. (1976). *J. Neurol. Neurosurg. Psychiat.*, **39**, 184–193.

PIERI, M., PIERI, L., SANER, A., DA PRADA, M. & HAEFELY, W. (1975). *Archs int. Pharmacodyn. Thér.*, **217**, 118–130.

SAWAYA, M. C. B., DOLPHIN, A., JENNER, P., MARSDEN, C. D. & MELDRUM, B. S. (1977). *Biochem. Pharmac.*, **26**, 1877–1884.

SNIDER, S. R., HUTT, C., STEIN, B. & FAHN, S. (1975). *Neurosci. Lett.*, **1**, 237–241.

SNIDER, S. R., HUTT, C., STEIN, B., PRASAD, A. L. N. & FAHN, S. (1976). *J. Pharm. Pharmac.*, **28**, 563–566.

STROMBOM, U. (1976). *Naunyn-Schmiedebergs Arch. Pharmac.*, **292**, 167–176.

TRABUCCHI, M., SPANO, P. F., TONO, G. C. & FRATTOLA, L. (1976). *Life Sci.*, **19**, 225–232.

WEIL-MALHERBE, H. & BIGELOW, L. B. (1968). *Analyt. Biochem.*, **22**, 321–334.

WESTERINK, B. H. C. & KORF, J. (1976). *Eur. J. Pharmac.*, **38**, 281–291.