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Correlation of behavioural inhibition or excitation produced by bromocriptine with changes in brain catecholamine turnover

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The dopamine agonist, bromocriptine, produced either inhibition or stimulation of motor behaviour in rats depending upon the dose and time after administration. Stimulation of motor activity occurred only with high doses after a 1–2 h delay. Both inhibition and stimulation were associated with decreased turnover of dopamine in the brain. Release of noradrenaline in brain and noradrenaline plus adrenaline in adrenal varied with motor activity. It is suggested that low doses of bromocriptine inhibit behaviour by activating an inhibitory presynaptic receptor, resulting in reduced synthesis and release of dopamine, whilst high doses cause behavioural excitation by activating the post-synaptic dopamine receptor.

The ergot alkaloid, bromocriptine, shares many of the dopamine receptor stimulating properties of the classic dopamine agonist, apomorphine (Fuxe, Corrodi & others, 1974). However, there are substantial differences in the behaviour elicited. Bromocriptine has a slower onset and longer duration of action in nigra-lesioned rats (Fuxe & others, 1974). Unlike apomorphine, it does not cause behavioural excitation in intact baboons (Anlezark, Meldrum & Trimble, 1975) or increased motility in mice (Puech, Fichelle & others, 1975). These data suggest that bromocriptine, as other ergot drugs (Pijnenburg, Woodruff & van Rossum, 1973; Struyker Boudier, Gielen & others, 1974) may differ from apomorphine in its action on dopamine receptors. We have examined the relation between bromocriptine-induced changes in brain catecholamine metabolism and two behavioural events, locomotor activity and sympathetic activation.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 250 g, were kept in the animal quarters for at least two days on a night-dark, day-light cycle. All experiments were done between 900 and 1300 h, the day following an overnight fast.

Rats were decapitated 1, 2 or 4 h after injection of bromocriptine or diluent. Brain and adrenal glands were rapidly removed and homogenized in iced 0·4 N perchloric acid. One adrenal pair was used for one set of biochemical determinations.

Amino acids and amines were extracted and separated on a strong cation exchange resin, Bio-Rad AG 50W-X8, according to the method of Prasad & Fahn (1973). Dopa, dopamine and noradrenaline together with adrenaline were sequentially eluted (Prasad & Fahn, 1973) and the eluted samples were stored at −20° until chemical analyses could be made, usually within 4 days. Dopa was analysed by the method of Fahn, Prasad & Delesie (1972), brain noradrenaline according to Laverty & Taylor (1968) and dopamine according to Atack (1973) as modified by Prasad & Fahn (1974). Adrenal noradrenaline and adrenaline were assayed by native fluorescence against a noradrenaline standard. γ-Aminobutyric acid (GABA) was eluted from the cation exchange resin along with dopa and was assayed by a fluorometric modification of the enzymatic procedure of Fahn & Coté (1968).

In some experiments, an inhibitor of tyrosine hydroxylase, α-methyl-*p*-tyrosine methylester HCl (AMPT), or of L-aromatic amino acid decarboxylase, 3-hydroxybenzylhydrazine HCl (NSD-1015), was used to determine catecholamine release or synthesis (Carlsson, Davis & others, 1972). AMPT was purchased from Sigma Chemical Co., St. Louis, and NSD-1015 was obtained from Dr. Per Martinson, Göteborg. Bromocriptine (2-bromo-α-ergocryptine, CB-154) was generously supplied by Sandoz Pharmaceuticals, Hanover, New Jersey. Haloperidol injection, 5 mg ml^{-1} (McNeil Laboratories, Pennsylvania), was used. Other drugs were dissolved in water except for bromocriptine which was dissolved in 5% ethanol. The amount of

* Correspondence.

ethanol administered was below that affecting catecholamine metabolism (Carlsson & Lindqvist, 1973) or behaviour (Carlsson, Engel & others, 1974).

Motor activity was measured with an electronic activity meter (Columbus Instruments, Columbus, Ohio). One rat a time was transferred to the activity cage and activity was measured for 4 min in each hour for 4 consecutive hours. Handling was by one person and experimental and control animals were matched for litter, diet and body weight.

RESULTS

Motor behaviour

Motor behaviour of rats treated with bromocriptine showed a biphasic response (Fig. 1, Table 1). There was an initial inhibition followed by excitation. As the dose of bromocriptine was increased, the period of inhibition was less pronounced and excitation occurred earlier. Motor inhibition was accompanied by ptosis and sedation whilst increased activity was associated with hyperreactivity to stimuli. No stereotypy was seen. Haloperidol, 0·5 mg kg^{-1}, given along with bromocriptine, 0·5 mg kg^{-1} or 5·0 mg kg^{-1}, resulted in a marked inhibition of activity and blocked the motor excitation at 5·0 mg kg^{-1} (Table 1). Activity values of control animals did not vary significantly in 4 h.

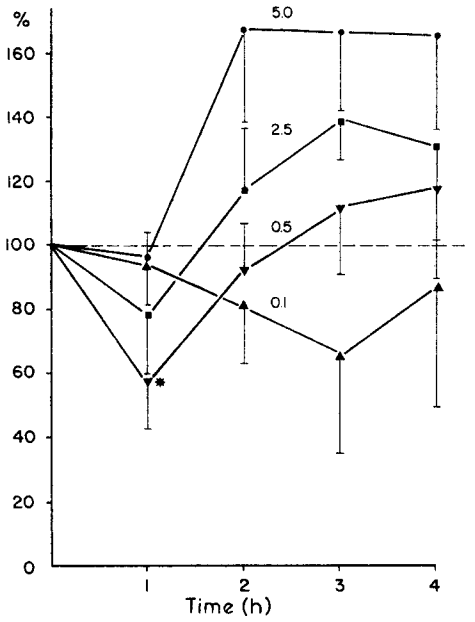

FIG. 1. Motor activity after bromocriptine. 0·1 (▲), 0·5 (▼), 2·5 (■) or 5·0 (●) mg kg^{-1}, was given to groups of 4 rats and activity measurements were made on single rats for 4 min 1, 2, 3 and 4 h after bromocriptine. Control rats were measured in parallel. Each point represents the mean ± s.e. of 4 values expressed as % of respective control. Detailed analysis of two of the points (0·5 mg kg^{-1}, 1 h; 5·0 mg kg^{-1}, 2 h) is in Tables 1–3. * $P<0·05$ (matched pairs).

Table 1. *Motor activity after bromocriptine (Bc).*

Bc dose mg kg^{-1}	n	h after Bc inj	Motor activity Control counts min^{-1}	Bc % control
0·5	4	1	76 ± 19	57 ± 14%*
5	7	2	77 ± 19	142 ± 8%*
5†	3	2	18 ± 8	103 ± 44%

Mean ± s.e. in counts min^{-1} (activity of control rats) or % of respective control value (activity of Bc treated rats). † Plus haloperidol, 0·5 mg kg^{-1}, intraperitoneally, 2 h before death.
* $P<0·05$ *vs* respective control value (statistics by matched pairs).

Catecholamine turnover during inhibition of motor behaviour

To determine the biochemical changes associated with behavioural inhibition, catecholamine metabolism at a point where motor activity was significantly reduced, 1 h after 0·5 mg kg^{-1} bromocriptine, was examined. Whole brain catecholamine synthesis was decreased as indicated by the rate of dopa accumulation after decarboxylase inhibition with NSD-1015. Dopa in bromocriptine-treated animals was 66 ± 12% of control (n = 4, control = 3·11 ± 0·65 nmol g^{-1}, $P<0·05$).

Disappearance of dopamine and noradrenaline after synthesis was blocked with AMPT was also decreased (Table 2). Endogenous catecholamine measured without drug pretreatment were not significantly altered (data not shown). To determine sympathetic nervous system activity at this point,

Table 2. *Brain and adrenal catecholamines 1 h after bromocriptine (0·5 mg kg^{-1}).* Rats received α-methyl-*p*-tyrosine, 250 mg kg^{-1}, i.p. 1 h before death.

	Catecholamines	
	Control, nmol g^{-1} or pair	Bc % control
Brain		
Dopamine	3·47 ± 0·15	131 ± 15%*
Noradrenaline	1·61 ± 0·05	126 ± 6%*
Adrenal		
Dopamine	0·73 ± 0·08	102 ± 8%
Noradrenaline + adrenaline	1·64 ± 0·24 ($\times 10^2$)	110 ± 9%

Mean ± s.e. of 4 values in nmol g^{-1} (control brain), nmol per pair (control adrenals) or % of respective control value (Bc treated rats).
* $P < 0·05$ *vs* control (statistics by matched pairs).

the disappearance of adrenal noradrenaline and adrenaline after AMPT was measured. There was a slight but not significant decrease (Table 2).

Catecholamine turnover during motor excitation

Two h after 5 mg kg^{-1} bromocriptine, brain catecholamine synthesis was reduced as shown by a decrease in dopa accumulation. The brain dopa of rats receiving 5 mg kg^{-1} bromocriptine was 68 ± 9% of control (n = 4, control = 4·02 ± 0·54 nmol g^{-1}, $P < 0.05$).

Dopamine release was also reduced (Table 3) and there was a slight net elevation in endogenous dopamine at all time intervals after bromocriptine, 5 mg kg^{-1}. Dopamine values at 1, 2 and 4 h were 112 ± 5% (n = 7), 106 ± 3% (12) and 108 ± 4% (7), respectively ($P < 0.05$). In light of the interaction of GABA and dopamine in response to drugs (Andén, Magnusson & Stock, 1973), it is of interest that the decreased dopamine turnover was accompanied by a small increase in brain GABA to 116 ± 3% of control (n = 5, control = 2·44 ± 0·31 μmol g^{-1}, $P < 0.01$).

Brain concentrations of noradrenaline were unchanged after 5·0 mg kg^{-1} bromocriptine but, in contrast to 0·5 mg kg^{-1}, increased disappearance of noradrenaline after AMPT occurred (Table 3).

Table 3. *Brain and adrenal catecholamines 2 h after bromocriptine, 5·0 mg kg^{-1}.*

		Catecholamines	
		Control, nmol	Bc
Brain	n	g^{-1} or pair	% control
Dopamine	12	4·51 ± 0·43	106 ± 3%*
Noradrenaline	14	1·83 ± 0·12	100 ± 7%
Dopamine	8†	2·79 ± 0·42	122 ± 9%**
Noradrenaline	7†	1·33 ± 0·18	79 ± 6%*
Adrenal			
Dopamine	8	1·32 ± 0·14	125 ± 8%**
Noradrenaline + adrenaline	12	1·84 ± 0·25 (× 10^2)	95 ± 5%
Dopamine	7†	0·40 ± 0·09	99 ± 16%
Noradrenaline + adrenaline	7†	1·68 ± 0·10 (× 10^2)	77 ± 6%**

Mean ± s.e. in nmol g^{-1} brain, nmol per pair adrenals or % of respective control value (Bc treated rats). † rats received α-methyl-*p*-tyrosine, 250 mg kg^{-1}, i.p. 2 h before death.

* $P < 0.05$ *vs* respective control value.

** $P < 0.01$ *vs* respective control value (statistics by matched pairs).

Sympathetic nervous system activity was also increased as shown by increases in adrenal noradrenaline and adrenaline release and endogenous dopamine concentration (Table 3). Elevation in adrenal dopamine is associated with sympathetic stimulation (Carlsson, Snider & others, 1973).

DISCUSSION

The results show that (1) both inhibition and stimulation of motor behaviour after bromocriptine are accompanied by reduced release of brain dopamine, (2) release of noradrenaline in brain is reduced during motor inhibition but increased during motor excitation, and (3) whole brain catecholamine synthesis is reduced under both conditions.

The biphasic response of motor activity to bromocriptine is similar to that reported for apomorphine (Carlsson, 1975a). This pattern was not seen in measurements of spontaneous motility in bromocriptine-treated mice (Puech & others, 1975). But, the exploratory phase of motor behaviour may be a more sensitive indicator of dopaminergic function than spontaneous motility (Carlsson, 1975b), and other ergot drugs such as agroclavine (Stone, 1973) do cause a biphasic motor response.

The alterations of catecholamine synthesis and turnover in our experiments are similar to those obtained in experimental animals by Fuxe & others (1974) for bromocriptine and by Carlsson, Kehr & Lindqvist (1976) for apomorphine. There was an inhibition of brain dopamine and noradrenaline synthesis at both low and high doses whilst endogenous dopamine increased and noradrenaline decreased at high doses. It is probable that bromocriptine inhibits dopamine metabolism in man as well since homovanillic acid is reduced in the cerebrospinal fluid (csf) during bromocriptine treatment (Curzon, 1975).

Carlsson & others (1976) as well as Fuxe (1974) postulate that the low dose inhibition of motor activity and dopamine metabolism caused by dopamine agonists is the result of preferential activation of inhibitory receptors located on dopamine neurons. Carlsson & others (1976) have coined the term, autoreceptor, for these. Large doses of the dopamine agonist compound may activate both the autoreceptor and the postsynaptic receptor, resulting in inhibition of dopamine metabolism and increased rather than decreased motor activity. The effects of apomorphine or bromocriptine on noradrenaline metabolism appear to be caused by change in the activity of dopamine neurons (Fuxe & others, 1974; Carlsson & others, 1976).

Our results are consistent with the hypothesis that bromocriptine activates dopamine autoreceptors and postsynaptic receptors in rat brain in a manner similar to that of apomorphine, differing only in the longer time course (Fuxe & others, 1974). This conclusion does not explain the qualitatively different behavioural effects of bromocriptine. It is possible that the latter are due not to a different mode of action on dopamine receptors but to an effect of bromocriptine on other neurotransmitter systems, e.g. 5-HT (Corrodi, Farnebo & others, 1975; Snider, Hutt & others 1975). Our recent data indicate that bromocriptine may reduce 5-HT release in rats since brain 5-hydroxyindoleacetic acid (5-HIAA) is decreased 30–40% by bromocriptine treatment (Snider & others, 1975). 5-HIAA concentrations are also decreased in the csf of bromocriptine-treated patients (Curzon, 1975).

The potential clinical value of high-dose bromocriptine in the treatment of diseases such as parkinsonism has been demonstrated (Calne, Teychenne & others, 1974). A possible application for low-dose bromocriptine therapy could be considered in hyperkinetic movement disorders such as tardive dyskinesia and Huntington's chorea since low doses of bromocriptine appear to act preferentially on the inhibitory presynaptic dopamine receptor. It is pertinent that during the build-up phase of bromocriptine treatment in Parkinson patients, a transient worsening of the motor disorder is sometimes seen (Carlsson, 1975b). This may be the clinical equivalent of autoreceptor activation.

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