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Effects of bromocriptine on adenylate cyclase and phosphodiesterase activities of rat striatum

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The activity of 2-bromo-α-ergokryptine (bromocriptine) (5 mg kg^{-1}, i.p.) on adenylate cyclase and on phosphodiesterase (PDE I–PDE II) of rat striatum, has been examined both *in vitro* and *in vivo*. *In vitro* and *in vivo* bromocriptine stimulated adenylate cyclase activity, but reduced the stimulating effect of dopamine on adenylate cyclase activity. Bromocriptine showed a dose-dependent biphasic action on phosphodiesterases *in vitro* while *in vivo* it stimulated them. The results obtained proved bromocriptine to have an agonist-antagonist action at striatal dopamine receptor level, with a relevant effect on the cAMP system.

Various ergot alkaloids and their derivatives are active on adenylate cyclase or phosphodiesterases of different tissues and consequently on cAMP concentrations (Murad, Chi & others, 1962; Iwangoff & Enz, 1971, 1972; Markstein & Wagner, 1975; Meier-Ruge & Iwangoff, 1976; Schorderet, 1976; Govoni, Iuliano & others, 1977).

Some derivatives of ergot alkaloids have dopaminergic properties *in vivo* (Johnson, Vigouret & Loew, 1973; Pagnini, Camanni & others, 1976). Among them 2-bromo-α-ergokryptine (bromocriptine) has proved to have effects on the dopamine system (Corrodi, Fuxe & others, 1973; Calne, Kartzinel & Shoulson, 1976; Loew, Vigouret & Jaton, 1976). On the basis of its action both at the level of dopamine presynaptic autoreceptors and at the level of dopamine postsynaptic receptors bromocriptine would seem to behave as a partial agonist (Fuxe, Fredholm & others, 1978).

Dopamine is known to be a highly specific stimulant of adenylate cyclase of the striatum as well as other central areas (Kebabian & Greengard, 1971; Horn, Cuello & Miller, 1974; Spano, Di Chiara & others, 1976). Consequently, adenylate cyclase activation has been considered to be representative of dopaminergic receptor response (Kebabian, Petzold & Greengard, 1972; Iversen, 1975).

In our studies on the actions of ergot alkaloids on the cns, we have investigated the effects of bromocriptine on adenylate cyclase and phosphodiesterase (PDE I, high Km; PDE II, low Km) activities in rat striatum.

MATERIALS AND METHODS

Materials: (^{3}H)-cAMP (30 000 mCi $mmol^{-1}$) and (^{3}H)-ATP (30 000 mCi $mmol^{-1}$) (Radiochemical Centre, Amersham, U.K.); cAMP and ATP (Sigma Chem. Co.); 2-bromo-α-ergokryptine (bromocriptine) (Sandoz, Basel); dopamine (BDH, Milan); Dowex 50-H^+ resin (Fluka) (Dowex 50 W × 4, 200–400 mesh).

Preparation of enzymes. Adenylate cyclase and phosphoidesterases were obtained from Sprague Dawley male rats, 220–250 g, from which the striatum had been taken according to Glowinski & Iversen (1966). Adenylate cyclase was obtained according to Krishna, Weiss & Brodie (1968). Phosphodiesterases were obtained using a modified method of Schönhöfer, Skidmore & others (1972) as described by (Pagnini & others, 1976). The protein concentration was measured by Lowry, Rosebrough & others' technique (1951), using bovine serum albumin as standard.

Determination of the adenylate cyclase activity. The method of Krishna & others (1968) was used. Incubation was carried out at 37° for 30 min; the final volume was 1·2 ml in tris HCl (4×10^{-2} M, pH 7·4) $MgSO_4$ ($3{\cdot}3 \times 10^{-3}$ M), and contained 3 mg of the enzyme, 0·2 ml of theophylline (1×10^{-2} M); the ATP concentration was 2×10^{-3} M (^{3}H-ATP spec. act. 30 mCi $mmol^{-1}$).

Dopamine was used at concentrations ranging from 1×10^{-4} to 2×10^{-7} M, bromocriptine from 1×10^{-4} to 1×10^{-7} M.

When used together dopamine was at 1×10^{-5} M and bromocriptine was at concentrations ranging from 1×10^{-4} to 1×10^{-7} M.

Determination of the phosphodiesterase activity. The method of Schönhöfer & others (1972) was used. Incubation was at 37° for 30 min; the final volume of the solution was 1 ml and contained tris HCl

* Correspondence.

(4×10^{-2} M, pH 7·4) $MgSO_4$ ($3{\cdot}3 \times 10^{-3}$ M), cAMP at 2×10^{-4} M (high Km) and 4×10^{-6} M (low Km). Bromocriptine concentrations ranged from 2×10^{-5} to 1×10^{-8} M.

In vivo experiments. Male Sprague-Dawley rats, 220–250 g, were treated intraperitoneally with saline or bromocriptine (5 mg kg^{-1}) and killed 10, 20, 30, 60 min later. The brains were removed, and the striatum rapidly separated according to Glowinski & Iversen (1966), and the adenylate cyclase and phosphodiesterase (PDE I and PDE II) activities evaluated.

RESULTS

Dopamine at doses ranging between 1×10^{-7} and 2×10^{-5} M induced a dose-dependent increase of adenylate cyclase activity. At doses higher than 2×10^{-5} M no further increase was obtained (Fig. 1). Bromocriptine at the lower concentrations used stimulated adenylate cyclase activity higher than that induced by dopamine, but above $1{-}5 \times 10^{-7}$ M there is either a slight increase, or no variation at all (Fig. 1).

Bromocriptine, 1×10^{-6} to 1×10^{-4} M, inhibited the stimulation of adenylate cyclase induced by dopamine 1×10^{-5} M. This inhibiting effect was proportional to the concentration of bromocriptine (Fig. 2 a, b).

Bromocriptine showed a biphasic action on phosphodiesterase activity (PDE I, PDE II): it activated the enzyme at concentrations between 2×10^{-7} and 1×10^{-6} M, while a dose dependent inhibition occurred for concentrations higher than 2×10^{-6} M (Fig. 3). Furthermore, maximal stimulation for both PDE I and PDE II was reached at 1×10^{-6} M.

Bromocriptine (5 mg kg^{-1}, i.p.) induced a stimulating effect on striatal adenylate cyclase that

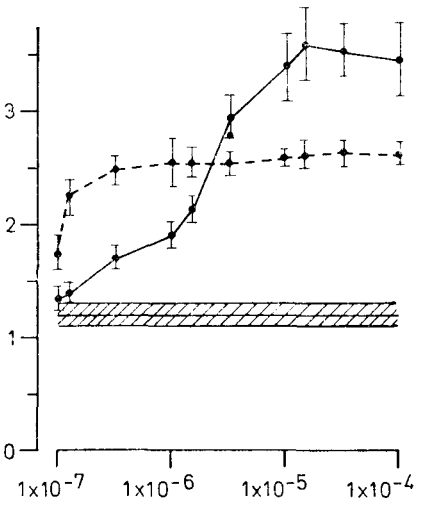

FIG. 1. Effect of *in vitro* addition of dopamine (●—●) or bromocriptine (● – – ●) on striatal adenylate cyclase activity of rat brain. Each point represents a mean of 8 determinations ± s.e. Ordinate: nmol cAMP mg^{-1} per 30 min. Abscissa: Concentration (mol $litre^{-1}$).

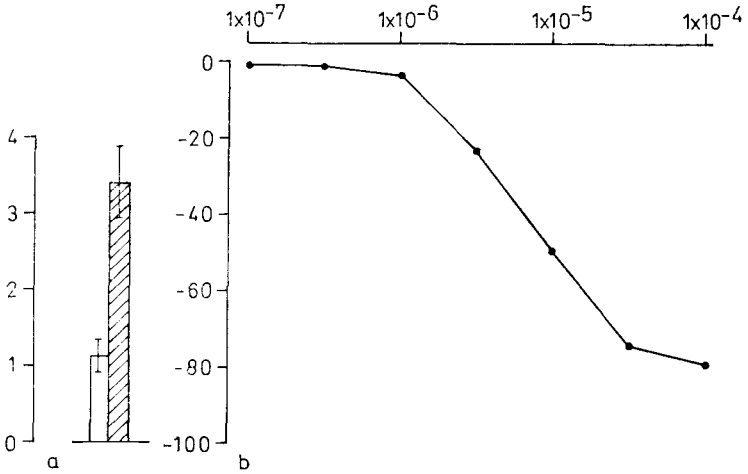

FIG. 2. (a) *In vitro* action of dopamine (1×10^{-5} M) on striatal adenylate cyclase activity. Results are expressed as mean of 4 determinations ± s.e. Basal (open column). Basal + dopamine (1×10^{-5} M) (hatched column). Ordinate: nmol cAMP mg^{-1} per 30 min. (b) *In vitro* bromocriptine % inhibition of stimulating action of dopamine (1×10^{-5} M) on adenylate cyclase activity. Each point represents a mean of 4 determinations. Ordinate: % variation. Abscissa: Concentration of bromocriptene (M).

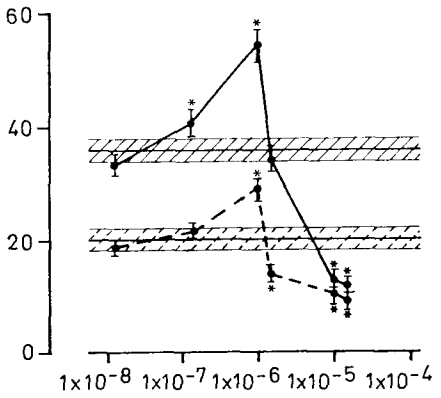

FIG. 3. Effect of *in vitro* addition of bromocriptine on striatal phosphodiesterase activity (●—● PDE I; ● - - ● PDE II). Each point represents a mean of 4 determinations ± s.e. Hatched areas = basal values ± s.e. Ordinate: nmol cAMP mg^{-1} per 30 min. Abscissa: Drug concentration (mol $litre^{-1}$).* $P<0.01$ in respect to control values.

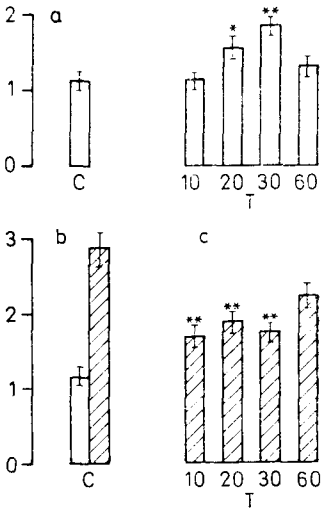

FIG. 4. (a) Striatal adenylate cyclase activity of rats untreated (C) or treated (T) with bromocriptine (5 mg kg^{-1}, i.p.) and killed after 10, 20, 30 and 60 min. * $P < 0.05$, * * $P < 0.02$ in respect to control values. (b) Adenylate cyclase activity in rat striatum without (open column) or after addition of dopamine (1×10^{-5} M) (hatched column). (c) Effect of the *in vitro* addition of dopamine (1×10^{-5} M) on the striatal adenylate cyclase of rats previously treated with bromocriptine (5 mg kg^{-1}, i.p.) and killed after 10, 20, 30 and 60 min. * * $P < 0.02$ in respect to control value (hatched column) (b). Results are a mean of 4 determinations ± s.e. Ordinate: nmol cAMP mg^{-1} per 30 min.

reaches a maximum after 30 min (Fig. 4a). When dopamine (1×10^{-5} M) was added *in vitro* to the striatal adenylate cyclase of rats it had a stimulating effect on the control animals (Fig. 4b), but its effect was noticeably reduced in the striatum of rats treated with bromocriptine (Fig. 4c). The inhibiting activity of bromocriptine on the dopamine stimulation tended to disappear after 60 min.

Phosphodiesterase PDE I and PDE II striatal activities were stimulated by a treatment with bromocriptine, reaching their maximum 20–30 min after its administration (Fig. 5).

DISCUSSION

Bromocriptine proved able to stimulate striatal adenylate cyclase, but it also depressed dopamine's sbility to stimulate this enzyme. Our results are in complete agreement with those of Fuxe & others (1976), but they do not agree with results obtained with a different method by Trabucchi, Spano & others (1976). However, bromocriptine can be said to be both an agonist and an antagonist at the level of the dopamine receptors. This is also true for other ergot alkaloids (Goldstein, Lieberman & others, 1978). However, other results have shown the existence of different functional dopamine receptors (Cools & van Rossum, 1976) for which bromocriptine could have a different affinity.

Phosphodiesterase activities are inhibited by some ergot alkaloids, such as dihydroergotoxine (Meier-Ruge & Iwangoff, 1976), but they are stimulated by bromocriptine at low concentrations.

As intracellular cAMP concentrations are related both to adenylate cyclase and phosphodiesterase activities, bromocriptine is likely to induce variations of cAMP concentrations that may differ according to the dose used.

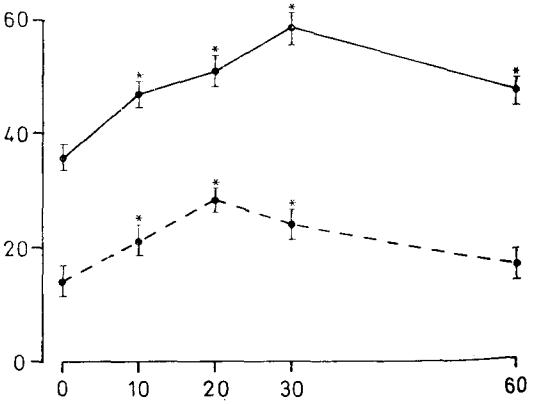

FIG. 5. Striatal phosphodiesterase activity (●—● PDE I; ● - - ● PDE II) of rats treated with saline or bromocriptine (5 mg kg^{-1}, i.p.) and killed after 10, 20, 30 and 60 min. Each point represents a mean of 4 determinations ± s.e. * $P < 0.01$ in respect to basal values. Ordinate: nmol cAMP mg^{-1} per 30 min. Abscissa: Time (min).

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