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Bromocriptine-induced decrease in blood pressure in conscious spontaneously hypertensive rats: evidence for a peripheral site of action

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Abstract—The aim of the study was to discover whether the dopamine agonist bromocriptine has a central or peripheral site of action on blood pressure. An intraperitoneal injection of bromocriptine (0·5 mg kg^{-1}) induced a long-lasting decrease in blood pressure in conscious spontaneously hypertensive rats (SHR). This effect was blocked by peripheral pretreatment with haloperidol or domperidone, but not by central treatment with haloperidol. A central injection of bromocriptine had only minor effects on blood pressure. These results suggest that primarily peripheral, rather than central, mechanisms are involved in the hypotensive effects of bromocriptine.

Bromocriptine is a derivative of ergot alkaloids and has been shown to be agonistic to dopaminergic receptors (Corrodi et al 1973; Fuxe et al 1978; Lokhandwala 1979). The drug has been used as a centrally acting treatment in Parkinson's disease (see Greenacre et al 1976; Quinn et al 1981; Montastruc et al 1985). Several reports have also described an antihypertensive action of bromocriptine in man and experimental animals (for references, see van den Buuse et al 1986). Despite the consensus about its blood pressure effects, the mechanism and site of action of bromocriptine is as yet still unclear. For instance, it may activate presynaptic dopamine receptors on the sympathetic nervous system and thereby decrease noradrenaline release (Ziegler et al 1979; Montastruc & Montastruc 1981; Tadepalli & Novak 1983). Alternatively, intrarenal effects and changes in cardiac output may be involved in the action of bromocriptine (Struyker-Boudier et al 1984). Other authors have suggested a principal central site of action of the drug (Nagahama et al 1984). Central dopamine systems play an important role in the regulation of cardiovascular homeostasis and the development of hypertension (van den Buuse et al 1986).

We have investigated the cardiovascular effects and principal site of action of bromocriptine in conscious spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). A preliminary account of this study has been published previously (De Jong et al 1986).

Materials and methods

Male rats were used (200–300 g), either SHR or WKY, derived from a breeding colony at our institute. The animals were kept 4–5 per cage at a constant light-dark rhythm and temperature (20°C) with standard pellet food and free access to tap water.

For the direct measurement of blood pressure, rats were anaesthetized with ether and the femoral artery cannulated with a 6 cm fine Teflon part, which was brought into the femoral artery until approximately the aorta, a short connecting piece of polyethylene-50 and a longer piece of polyethylene-100 tubing, which was tunnelled under the skin and exteriorized in the neck. After the incisions were closed, the rat was allowed to recover from the anaesthesia and individually housed.

At least 24 h after the operation, 6–8 rats were brought to a quiet experimentation-room and connected to a Statham P23AC transducer and a Grass model 7C polygraph. After a rest-period of 1 h, basal blood presure was recorded. Either haloperidol (0·5 mg kg^{-1}) or domperidone (0·5 mg kg^{-1}) was injected 30 min before the bromocriptine (0·5 mg kg^{-1}); all drugs were injected intraperitoneally (i.p.) in a volume of 1 mL kg^{-1}. Mean arterial pressure (MAP) was then recorded for 4 h. Neither haloperidol nor domperidone induced significant changes in blood pressure on their own. In one experiment, heart rate readings were taken regularly by increasing the speed of the chart-recorder, thus allowing the number of heart-beats to be counted during an interval of about 1 min.

One week before the cardiovascular experiments, two groups of SHR were provided with a small cannula in the lateral cerebral ventricle (Brakkee et al 1979) to allow intracerebroventricular (i.c.v.) injection of drugs. The further protocol was identical as described above.

The drugs we used were: bromocriptine mesylate (a gift from Sandoz, Arnhem, The Netherlands), haloperidol and domperidone (Haldol, Motilium, Janssen Pharmaceutica, Beersse, Belgium). Bromocriptine was dissolved in polyethyleneglycol which was then diluted to a 20% solution with 0·9% saline. In pilot experiments we observed that this vehicle had little or no effect on blood pressure. For haloperidol and domperidone we diluted the commercially available solutions with saline.

Data were analysed with analysis of variance (ANOVA) followed by Student-Newman-Keuls test (SNK). Data are mean ± standard error of the mean (s.e.m.) and expressed as change in mm Hg (or beats min^{-1}) from the resting value. Differences between groups were considered significant when $P < 0.05$.

Results

Fig. 1 shows the effect of an i.p. injection of 0·5 mg kg^{-1} bromocriptine on blood pressure of conscious SHR (resting MAP 185 ± 6 mm Hg). A rapid fall in pressure occurred after bromocriptine administration, the maximal effect reached between t = 15 and t = 30 min. The fall in blood pressure was greater than in WKY (not shown, resting MAP 124 ± 2 mm Hg). For instance, after 30 min the decrease was −26 ± 5 mm Hg in SHR and −8 ± 4 mm Hg in WKY ($P < 0.05$). The hypotensive

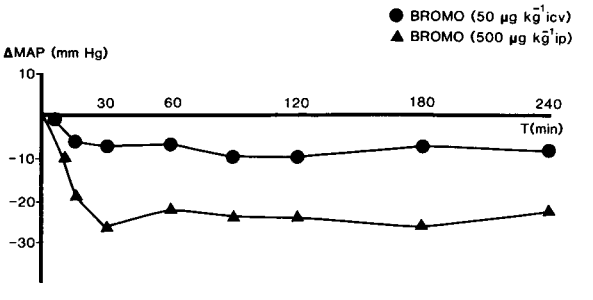

FIG. 1. The effect of bromocriptine (0·5 mg kg^{-1} i.p. or 0·05 mg kg^{-1} i.c.v.) on blood pressure in conscious SHR, expressed as change in MAP (mm Hg). I.p., but not i.c.v. administration induced a marked fall in blood pressure, which lasted for at least 4 h. Average s.e.m. for the group treated with 0·5 mg kg^{-1} was 3·9 (n = 10); average s.e.m. for the group treated with 0·05 mg kg^{-1} was 2·9 (n = 15).

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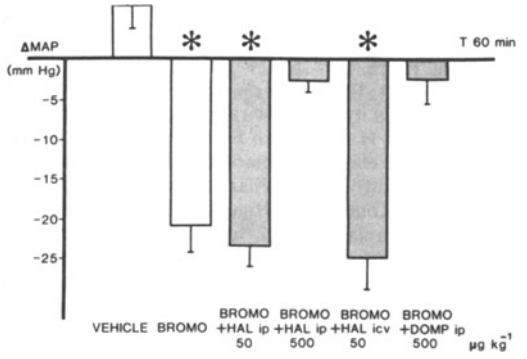

FIG. 2. The effect of pretreatment (30 min before) with haloperidol or domperidone on the change in blood pressure induced by bromocriptine (0·5 mg kg^{-1} i.p.) in conscious SHR. A low dose of i.p. haloperidol and i.c.v. haloperidol did not influence the effect of bromocriptine. A higher dose of i.p. haloperidol and domperidone completely blocked the effect of bromocriptine. Data are expressed as change in MAP (mm Hg) ± s.e.m.

effect of bromocriptine in SHR lasted for at least 4 h. In contrast to the i.p. administration, i.c.v. injection of bromocriptine (10 or 50 $\mu g\ kg^{-1}$) had little effect on blood pressure (see Fig. 1 for the 50 μg dose). At 60 min after 10 $\mu g\ kg^{-1}$ injection: 2 ± 3 mm Hg; at 60 min after 50 $\mu g\ kg^{-1}$: −7 ± 4 mm Hg.

Bromocriptine induced a decrease in heart rate. In SHR this decrease was maximum at 30 min after administration (−40 B min^{-1}) and lasted for at least 3 h (data not shown).

Fig. 2 shows the effect of haloperidol or domperidone on the hypotensive action of bromocriptine in SHR. Pretreatment i.p. with 0·05 mg kg^{-1} haloperidol did not affect the fall in blood pressure, whereas the 0·5 mg kg^{-1} dose almost completely blocked it. In contrast, i.c.v. injection of 50 $\mu g\ kg^{-1}$ haloperidol did not influence the effect of bromocriptine. Pretreatment of the SHR with 0·5 mg kg^{-1} domperidone blocked the effect of bromocriptine on blood pressure.

Discussion

The results show a marked hypotensive effect of bromocriptine on blood pressure in SHR. This confirms previous results in these rats, as well as in other species (for references, see Nagahama et al 1984; van den Buuse et al 1986). However, the site of action of bromocriptine has been unclear (see Introduction). In our experiments, a higher dose of haloperidol or domperidone blocked the action of bromocriptine, confirming the dopaminergic nature of the hypotensive response. Previous reports have suggested a possible α-adrenergic or β-adrenergic component in the effect of bromocriptine (Lew et al 1977; Gibson & Samini 1978; Hamilton 1981), but this may well be related to the higher doses used in those studies. At the present dose of bromocriptine, its effect could be completely blocked by the two i.p. administered dopamine antagonists. Haloperidol thus given is an antagonist on central and peripheral dopamine receptors. However, domperidone does not cross the blood-brain-barrier when injected peripherally (Laduron & Leysen 1979). The finding of almost complete inhibition of the hypotensive action of bromocriptine by domperidone therefore suggests that bromocriptine exerts its action through dopamine receptors located in the periphery. This is strengthened by the fact that central administration of haloperidol does not change the action of i.p. bromocriptine on blood pressure, indicating that central blockade of dopamine receptors does not influence the effect of bromocriptine on blood pressure. Moreover, central injection of bromocriptine had only minor effects on blood pressure when compared with systemic treatment. It is unlikely that α-blocking properties of haloperidol or domperidone could have contributed to their peripheral effect on the action of bromocriptine, since no change in basal blood pressure was found after administration of these antagonists.

Nagahama and co-workers have suggested a central site of action of bromocriptine on blood pressure (Nagahama et al 1984, 1985). In their experiments, domperidone did not block the hypotensive effect of bromocriptine in SHR, whereas the centrally-acting antagonist metoclopramide did. It is as yet unclear why the results of those authors differ so fundamentally from ours. The dose of bromocriptine and of domperidone was the same, although in their study Nagahama et al administered drugs intravenously. Interestingly, plasma prolactin levels were stimulated by both metoclopramide and domperidone. Plasma hormone levels have been used as an indication of the central effects of bromocriptine (Stumpe et al 1977; McMurtry et al 1979; Hutchinson et al 1981) but are obviously not a reliable index for the site of action of the drug. Plasma noradrenaline was not changed in the study of Nagahama et al but plasma adrenaline was increased. Other studies have suggested a decrease in plasma noradrenaline levels or sympathetic activity induced by bromocriptine treatment (Lokhandwala 1979; Lokhandwala et al 1979; Ziegler et al 1979; Kolloch et al 1980; Sowers 1981). Obviously plasma catecholamine levels have to be interpreted with care. Local injections of bromocriptine, or the use of particular antagonists with distinct and well-described mechanisms of action, may provide the answer. From such an approach in the present study we conclude that (i) bromocriptine may induce a marked and long-lasting decrease in blood pressure in the conscious SHR; (ii) this effect is most probably mediated by an action of this drug on dopamine receptors located in the periphery.

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Absence of [^{3}H]SCH 23390 binding sites in the rat adrenal gland

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Abstract—The binding of D_2-dopamine receptor ligand [^{3}H]spiperone and selective D_1-ligand [^{3}H]SCH 23390 to the rat adrenal gland and striatum has been compared. [^{3}H]Spiperone showed specific binding in both tissues revealing a B_{max} of 887 fmol mg^{-1} protein and K_D of 0·38 nM, and B of 34 fmol mg^{-1} protein and K_D of 0·66 nM in the striatum and adrenal gland, respectively. On the other hand, [^{3}H]SCH 23390 showed a specific binding to the striatal tissue with B_{max} of 747 fmol mg^{-1} protein and K_D of 0·70 nM, while in the adrenal tissue no specific binding was observed. These results apparently indicated only D_2-dopamine receptor binding sites being present in the rat adrenal gland.

Dopamine-sensitive adenylate cyclase was demonstrated in the rat adrenal gland (Relja & Lacković 1984; Missale et al 1985a, 1986) indicating the presence of D_2-dopamine receptors. Binding studies, on the other hand, indicated the existence of D_2-receptor sites (Dunn & Bosmann 1981; Relja & Lacković 1984; Missale et al 1985b; Lyon et al 1987; Quik et al 1987). However, in these studies non-selective or even D_2-ligands as [^{3}H]haloperidol, [^{3}H]spiperone and [^{3}H]sulpiride were used. Recently, [^{3}H]SCH 23390 has been reported to be a selective ligand of central D_1-receptors (Billard et al 1984; Schulz et al 1985). To evaluate the type of dopamine receptor binding sites in the rat adrenal gland, in the present study the binding of [^{3}H]SCH 23390 and D_1-ligand [^{3}H]spiperone has been compared.

Materials and methods

The following radiolabelled drugs were used: [^{3}H]SCH 23390, (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol, 80 Ci mmol^{-1} and [^{3}H]spiperone 21·3 Ci mmol^{-1} (Amersham Laboratories, UK). All other compounds used were purchased from Sigma Co (St Louis, MO, USA).

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The fresh adrenal and striatal tissue of male, adult Wistar rats was homogenized (Ultra Turrax) in 100 vol (w/v) of ice-cold 50 mM Tris buffer. The homogenate was centrifuged at 1000*g* and the supernatant was recentrifuged at 20000*g* for 10 min at 4°C. The resulting pellet was washed twice and finally resuspended in 50 mM Tris buffer containing (mM) NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$1, pargyline 10μM and 0·1% ascorbic acid.

For [^{3}H]spiperone binding, 1 mL aliquots of tissue suspensions were incubated in the presence of [^{3}H]spiperone of various concentrations (0·01–3 nM) for 15 min at 37°C. The specific binding was defined in the presence of 10 μM haloperidol. The final protein concentration was between 200–300 μg mL^{-1}.

To provide [^{3}H]SCH 23390 binding to the striatal tissue, 1 mL aliquots of the tissue suspensions were incubated in the presence of various concentrations of [^{3}H]SCH 23390 (0·01–6 nM) for 15 min at 37°C. The specific binding was defined in the presence of 1 μM SCH 23390. The protein concentration was 200 μg mL^{-1}.

For [^{3}H]SCH 23390 binding to the adrenal tissue, 250 μL aliquots of the tissue suspensions were incubated in the presence of [^{3}H]SCH 23390 of various concentrations (0·5–25 nM) for 15 min at 37°C. The specific binding was defined in the presence of 10 μM SCH 23390. The protein concentration was 1000 μg mL^{-1}.

Incubations were terminated by filtration through Whatman GF/B filters. Radioactivity trapped on filters was determined in a Beckman LC counter (LC 1701) after a 5 mL of Aquasol (NEN, Mass.) had been added.

Results

The saturable specific binding of [^{3}H]spiperone was observed in striatal and adrenal membrane suspensions (Fig. 1). Scatchard