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Stimulation of rat caudate nucleus adenylate cyclase activity by BW 245 C, a prostaglandin analogue with prostacyclin-like activity

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We have previously reported that in chicks BW 245 C, an hydantoin prostaglandin analogue with prostacyclin-like activity (Caldwell et al 1979, 1980), given into the IIIrd cerebral ventricle produces dose-dependent behavioural stimulation, vocalization, increase in locomotor activity, circling and escape responses accompanied by electrocortical desynchronization and marked decrease in total as well as in 0–4, 4–8 Hz electrocortical voltage power (Rotiroti et al 1981). This symptomatology resembles that evoked by intraventricular injection, into the same species, of apomorphine and cholera toxin (Nisticò et al 1978). The central effects of cholera toxin both in chicks (Nisticò et al 1978) and in mammalian species (Miller & Kelly 1975; Iversen et al 1975), are likely to be due to an activation of a dopamine-sensitive adenylate cyclase. The purpose of the present experiments was to extend the previous study by following behavioural changes in a mammalian species after infusion of BW 245 C into the rat caudate nucleus and more specifically to ascertain whether the drug's effects were mediated by an increase in the intracellular concentration of 3′,5′-cAMP.

Methods

Adult male Wistar-Morini rats, 250–280 g had stereotactic implantation of cannulae performed under chloral-hydrate anaesthesia according to the coordinates of De Groot (1959). BW 245 C was dissolved in aqueous phosphate buffer (pH 7) and prepared freshly each time before injection. The same volumes (0·5–1 μl) of the vehicle lacked effects on gross behaviour and motor activity. All drugs were given, at least 4 days after implantation of the cannulae, to unrestrained freely moving animals.

For the in vitro determination of adenylate cyclase activity, succinyl-cyclic (c)AMP tyrosine-methyl-ester (^{125}I) and specific cAMP antiserum complex were purchased from New England Nuclear; Tris maleate and EGTA were from Fluka and ATP was from Boerhinger Mannheim Gmbh, Germany.

The caudate nuclei of rats after decapitation were rapidly placed on ice, then gently homogenized with a Teflon/glass homogeniser in 25 vol (w/v) of 2 mM Tris-maleate buffer pH 7·4 containing 2 mM EGTA. Adenylate cyclase activity was measured in an assay system containing 50 μl of homogenate, 250 μl of 80 mM Tris maleate buffer, 2 mM $MgSO_4$, 0·2 mM EGTA, 10 mM theophylline and various drugs as indicated. The incubation tubes were kept on ice/salt bath, while ATP was added to a final concentration of 0·5 mM. The incubation tubes were then placed at 30 °C for 3 min. At the end of this time 1 ml of ethanol was added and tubes centrifuged at 2500 *g* for 15 min; 100 μl samples of supernatant were then transferred to assay tubes and dried in a vacuum desiccator for radioimmunoassay of cAMP according to Steiner et al (1972).

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Results

Data are expressed as mean values ± s.e.m. In order to evaluate the relative potency of dopamine and BW 245 C in stimulating adenylate cyclase, the ratio between the ED50 was calculated by evaluating the correlation coefficient and applying Student's *t*-test.

The infusion of BW 245 C (0·1, 1 and 10 μg) into the caudate nucleus of freely moving rats (at least 6 for each dose) produced an intense pattern of stereotyped movements (sniffing, licking, grooming, rearing and wet-dog syndrome) and increased exploratory behaviour and locomotor activity (specific data are not

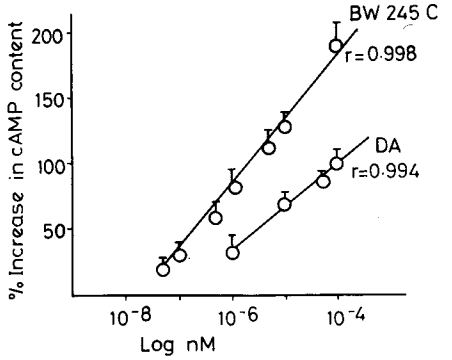

FIG. 1. Dose-dependent stimulation by dopamine (DA) and BW 245 C of adenylate cyclase activity, as shown by the increase in cAMP content in rat caudate homogenates. Each point represents the mean value ± s.e.m. of five to six experiments each assayed in duplicate. Basal levels of cAMP in control rat caudate homogenates was 34·5 ± 4·1 pmol per assay tube (approximately 2 mg wet weight tissue). Maximal stimulating concentrations both for dopamine and BW 245 C were 10^{-4} M since no further increase was obtained with higher concentrations. BW 245 C dose-response curve was significantly ($P < 0.001$) different from that for DA. Sodium fluoride (10^{-4} M) produced an increase in cAMP content of 140% from basal values (6 experiments, each assayed in duplicate).

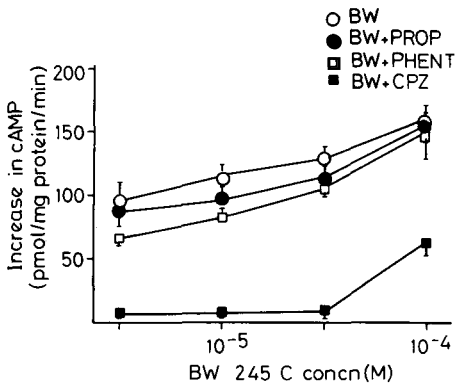

FIG. 2. Effects of 10^{-4} M concentration of chlorpromazine (CPZ), propranolol (PROP) and phentolamine (PHENT) on cAMP increase in rat caudate nucleus produced by several concentrations of BW 245 C. Basal values of cAMP content in control rat caudate homogenates (4–8 experiments in duplicate) was 32·7 ± 3·9 pmol per assay tube (approximately 2 mg wet weight tissue). Each point represents the mean value ± s.e.m. of at least six experiments each assayed in duplicate. Dose-response curve by BW 245 C + chlorpromazine was significantly different ($P < 0.01$) from that of BW 245 C alone, whereas curves regarding BW 245 C + propranolol or phentolamine were not different ($P > 0.05$) from that of BW 245 C alone.

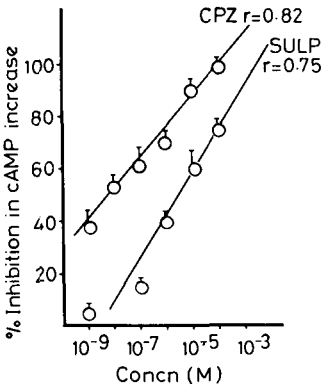

FIG. 3. Percent inhibition by chlorpromazine (CPZ) or (-)-sulpiride (SULP) at various concentrations of cAMP rise produced by 5×10^{-5} M BW 245 C in rat striatal homogenates. Basal values of cAMP production were 28·7 ± 2·46 pmol per assay tube, and in presence of BW 245 C (5×10^{-5} M) were 63·71 ± 4·63 pmol.

reported here). These effects lasted 40–60 min according to the dose used. Haloperidol (0·5 mg kg^{-1} i.m. 15 min before) prevented stereotyped behaviour and the increase locomotor activity elicited by subsequent administration of BW 245 C (1 μg, n = 6).

BW 245 C produced a dose-dependent stimulation in rat caudate nucleus adenylate cyclase activity (Fig. 1). In comparison with dopamine, BW 245 C produced a higher increase in cAMP and its relative dose-response curve was significantly ($P < 0.001$) shifted to the left (Fig. 1). The ED50 for dopamine was 5×10^{-6} M, whereas for BW 245 C was 2×10^{-7} M thus making the latter 25 times more potent than dopamine. In addition, the maximal increase of adenylate cyclase activity was 100 with a dopamine concentration of 10^{-4} M and 190% with the same concentration of BW 245 C (Fig. 1). Higher doses of BW 245 C (10^{-3} M) did not produce further increase in cAMP formation. The effects of BW 245 C were significantly ($P < 0.01$) antagonized by chlorpromazine whereas antagonists at α- and β-adrenoceptors were almost ineffective ($P > 0.05$) (Fig. 2). Chlorpromazine IC50 was 9×10^{-9} M. In addition, (-)-sulpiride (IC50 5×10^{-6} M), a selective antagonist at dopamine D_2 receptors, was significantly less potent in inhibiting the rise in cAMP induced by BW 245 C (Fig. 3).

Discussion

The present experiments show that BW 245 C given into the rat caudate nucleus induces stereotyped behaviour and increases locomotor activity. These effects suggest that BW 245 C may act as a dopamine receptor agonist or through dopamine release, an idea supported also by the antagonism obtained with haloperidol. In addition, the in vitro experiments show that this prostaglandin analogue stimulates dopamine-sensitive adenylate cyclase and its effects were much more potent than those exhibited by dopamine itself. Dopamine receptor antagonists such as chlorpromazine and (-)-sulpiride were able to prevent BW 245 C-induced increase in cAMP whereas no effects were obtained with α- and β-adrenoceptor antagonists. However, chlorpromazine was significantly more effective than (-)-sulpiride in antagonizing cAMP increase induced by BW 245 C suggesting the compound acts specifically on dopamine receptors linked to adenylate cyclase (Seeman 1981). The possibility that behavioural and motor effects of BW 245 C are due to dopamine release seems to be unlikely from in vitro experiments since the drug was more powerful than dopamine and since in caudate homogenates the drug is able to produce a marked increase in cAMP which suggests a direct stimulatory effect on dopamine receptors linked to adenylate cyclase. Data exist in the literature relating the behavioural and motor effect of dopamine and dopamine receptor agonists to the increase in cAMP (Miller & Kelly 1975; Iversen et al 1976). On the other hand, BW 245 C has biochemical effects that are not surprising since in cultured endothelial cells (Dembinska-Kiec et al 1979), in bovine coronary arteries (Dembinska-Kiec et al 1979) and in platelets (Moncada & Vane 1978) prostacyclin exerts its powerful antiaggregatory and vasodilatating effects through an increase in intracellular cAMP content. In addition, dopamine induced vasodilatation in the renal vascular bed (Goldberg 1972) seems to be due to an increase in renal artery adenylate cyclase activity (Murthy et al 1976). Recently the

occurrence of receptors for prostacyclin in the neuronal tissue has been suggested by the presence of a prostacyclin-dependent activation of adenylate cyclase in a neuronal somatic cell hybrid (Blair et al 1980).

In conclusion, the present experiments show that the prostaglandin analogue BW245 C possesses powerful dopamine-like effects in rat brain which are very likely mediated by an increase in intracellular content of cAMP and this could be of importance also in the interpretation of results with this compound in other test systems.

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