Differential actions of substituted benzamides on pre- and postsynaptic dopamine receptor mechanisms in the nucleus accumbens

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The action of neuroleptic agents to reduce psychomotor activity may relate to an ability to inhibit postsynaptic dopamine receptor mechanisms in the mesolimbic and striatal systems (Pijnenburg et al 1973; Jackson et al 1975; Costall & Naylor 1976). However, it has become apparent that dopamine receptor mechanisms may also be located presynaptically, and that stimulation of such mechanisms by dopamine or a dopamine agonist such as apomorphine may effectively switch off dopamine synthesis and/or release (Kehr et al 1972; Carlsson 1975; Strömbom 1976; Walters & Roth 1976; Di Chiara et al 1978). Thus, an additional action for neuroleptic agents may be to inhibit presynaptic dopamine receptor mechanisms and thereby enhance dopamine neurotransmission. These two actions of neuroleptics have been demonstrated via an experimental model using amphetamine to 'drive' the accumbens dopamine system and apomorphine to 'switch' the system off: larger doses of neuroleptic can inhibit the amphetamine response per se (postsynaptic action) whilst lower doses specifically prevent the effect of apomorphine (presynaptic action) (Costall et al 1979, 1980). From a wide range of neuroleptics evaluated, a benzamide derivative, sulpiride, was shown to most effectively differentiate between pre- and postsynaptic action. The present studies were therefore designed to analyse the pre-/postsynaptic action of a number of benzamide derivatives and, where appropriate, to determine the active conformation.

The studies used male Sprague-Dawley (CFE) rats weighing 300–325 g at the time of operation. The rats were prepared for intra-accumbens injections as previously described (Costall & Naylor 1976). Briefly, guide cannulae were stereotaxically implanted for the subsequent insertion of injection units to the centre of the nucleus accumbens (Ant. 9·4, Vert. 0·0, Lat. ± 1·6; De Groot 1959). Injections were made into the nucleus accumbens 14 days after surgery; volumes of 1 μl were administered bilaterally over 1 min. Animals were used on a maximum of three occasions with intervening 14 day recovery periods. At the end of the experiment the brain of every 5th rat was examined histologically to determine the precise points of injection; these were all confined to the area of the nucleus accumbens (data indistinguishable from that previously reported: Costall & Naylor 1976). The behavioural index in all studies was hyperactivity which was measured by placing rats immediately after intracerebral injection in individual cages each fitted with one photocell unit. Interruptions of the light beams were measured electromechanically and hyperactivity expressed in counts per 5 min. All hyperactivity experiments were carried out between 08·00 and 18·00 h in a sound-proofed, diffusely illuminated room maintained at 21 ± 2 °C. Hyperactivity was induced by 10 μg bilateral intra-accumbens (+)-amphetamine (SO_4, Sigma, prepared in distilled water; dose established as the minimum to cause a maximal hyperactivity response) and inhibited by a concomitant intra-accumbens injection of 6·5 μg apomorphine (HCl, Macfarlan Smith, prepared in distilled water containing 0·1% sodium metabisulphite; optimal dose for maximum inhibition, see Costall et al 1980).

A classical neuroleptic, haloperidol (Janssen, prepared in 1% lactic acid) and the benzamide derivatives (±)-sulpiride, (+)-sulpiride, (—)-sulpiride, (±)-sultopride, (+)-sultopride, (—)-sultopride, tiapride and metoclopramide (SESIF, France, prepared in distilled water or in a minimum quantity of hydrochloric acid neutralized with sodium bicarbonate) were given i.p. either 30 min (haloperidol) or 1 h (benzamide derivatives) before the intracerebral injections to determine potential to antagonize at postsynaptic dopamine mechanisms (antagonism of amphetamine response per se) and at presynaptic dopamine mechanisms (ability to reverse the antagonism of amphetamine by apomorphine, doses not affecting the amphetamine response per se). The dependence of these responses on dopamine was assessed using 1 h pretreatments with yohimbine (.HCl, Sigma, prepared in distilled water, 1·25–10 mg kg^{-1} i.p.), propranolol (.HCl, ICI, prepared in distilled water, 5 mg kg^{-1} i.p.), aceperone (Janssen, prepared in minimum quantity of *NN*-dimethylformamide, 2·5 mg kg^{-1} i.p.) and cyproheptadine (.HCl, Merck, Sharp and Dohme, prepared in minimum quantity of *NN*-dimethylformamide, 2·5–5 mg kg^{-1} i.p.), and a 30 min pretreatment with atropine (.SO_4, Sigma, prepared in distilled water, 2·5–5 mg kg^{-1} i.p.). The indicated doses of these agents, which failed to modify the amphetamine response per se, did not affect the antagonism of amphetamine by apomorphine.

Haloperidol, 0·0125–0·025 mg kg^{-1} i.p., caused a reduction/abolition of the amphetamine response, 0·00625 mg kg^{-1} i.p. haloperidol failed to modify the amphetamine response per se but did reverse the apomorphine inhibition of amphetamine (amphetamine alone 59–71 counts/5 min, amphetamine + apomorphine 10–26 counts/5 min, amphetamine + apomorphine + 0·00625 mg kg^{-1} i.p. haloperidol 50–69

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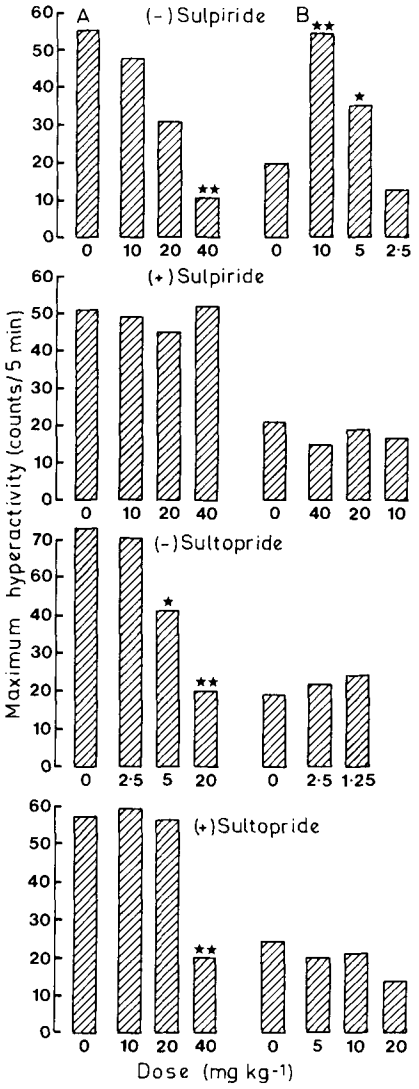

FIG. 1. Effect of the (—)- and (+)-isomers of sulpiride and sultopride on A. The hyperactivity induced by 10 μg bilateral intra-accumbens (+)-amphetamine and B. The activity following combined bilateral intra-accumbens amphetamine, 10 μg, and apomorphine, 6·5 μg. Doses of the benzamides, given 1 h before the intracerebral injections, are indicated in mg kg^{-1} i.p. Control, solvent injections are indicated as 0. Hyperactivity was measured in counts/5 min. n = 6–10. s.e.m.s < 20%. Effects significantly different from solvent effects to $^{*}P < 0{\cdot}01$, $^{**}P < 0{\cdot}001$ (Student's t-test).

counts/5 min). A dose of 0·00313 mg kg^{-1} i.p. haloperidol did not affect the apomorphine inhibition of amphetamine. Similarly to haloperidol 40–80 mg kg^{-1} i.p., (±)-sulpiride caused a reduction of the amphetamine responses; 20 mg kg^{-1} i.p. (±)-sulpiride, which failed to modify amphetamine alone, did reverse the apomorphine inhibition (amphetamine alone 49–62 counts/5 min, amphetamine + apomorphine 10–19 counts/5 min, amphetamine + apomorphine + 20 mg kg^{-1} i.p. (±)-sulpiride 50–59 counts/5 min). (—)-Sulpiride was approximately twice as potent as (±)-sulpiride whilst (+)-sulpiride was inactive both against amphetamine and apomorphine (Fig. 1). (±)-Sultopride reduced the amphetamine response dose-dependently at 10–40 mg kg^{-1} i.p. although 5 mg kg^{-1} i.p. failed to modify the apomorphine inhibition of amphetamine. (—)-Sultopride was approximately twice as potent as (±)-sultopride in reducing the amphetamine effect and again failed to modify the action of apomorphine. Only a large dose of (+)-sultopride reduced the amphetamine response and lower doses failed to modify the inhibitory action of apomorphine (Fig. 1). Similarly to sultopride, tiapride (20–40 mg kg^{-1} i.p.) and metoclopramide (0·25–2 mg kg^{-1} i.p.) were shown to reduce the response to amphetamine in a dose-dependent manner but not to reverse the action of apomorphine (2·5–10 mg kg^{-1} i.p. tiapride, 0·0625–0·25 mg kg^{-1} i.p. metoclopramide).

Thus, from a series of benzamide derivatives examined, only sulpiride could effectively inhibit a presynaptic, apomorphine sensitive receptor site: the (—)-isomer of sulpiride was the active form. It is suggested that the apomorphine-sensitive site affected by sulpiride is also a neuroleptic-sensitive site since it was blocked by haloperidol but not by the classical α- and β-adrenergic blocking agents, aceperone and propranolol, the presynaptic α antagonist, yohimbine, or by the acetycholine and 5-HT antagonists, atropine, and cyproheptadine. That sulpiride was found to be 4 to 8 fold more effective on pre- as compared with postsynaptic mechanisms, would distinguish it from classical neuroleptic agents which are no more than twice as effective on presynaptic mechanisms. Thus, it is easier to separate the two effects of sulpiride and to use this agent as a tool for analysis of presynaptic mechanisms. The pharmacological significance of this presynaptic action must remain speculative, although it may be related to the known alerting action of sulpiride both in animals and in the clinic.

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Caffeine disposition and effects in young and one-year-old rats

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Age often influences both the kinetics and toxicity of foreign compounds (Triggs & Nation 1975; Crooks et al 1976). One-year-old rats are more sensitive than younger ones to the lethal effects of caffeine (Peters & Boyd 1967). We have therefore studied the disposition of caffeine in 40-day and one-year-old rats after a single dose of 10 mg kg^{-1} to see whether there is a pharmacokinetic basis for the difference in toxicity.

We have also looked for biological determinants of the difference; this was done by using the liver perfusion technique (Bartošek et al 1973). We also wanted to establish whether any of the drug's biochemical effects, such as the increase in plasma free fatty acids (FFA) and corticosterone, were influenced by age. Two groups of male Sprague Dawley rats (Bio-Breeding Lab of Canada, Ottawa) 40-day and one-year-old, received 10 mg kg^{-1} of caffeine in water (1% w/v solution, NSDA†, Blended source) by gavage. Five animals were killed at each time (5, 15, 30, 60, 120, 240, 480 min) after administration. Blood was collected in tubes containing 0·1 ml EDTA (4%), centrifuged, and plasma was immediately frozen. Tissues were removed, rinsed in chilled 0·9% NaCl wiped and frozen. All tissues were homogenized in twice-distilled water (1 : 10 w/v).

The livers from four young and four old rats were isolated and perfused with recycling at 1 ml g^{-1} min^{-1} of flow following the technique of Bartošek et al (1973). Two doses of caffeine were used, a 10 mg kg^{-1} dose and ten times less in order to calculate kinetic parameters without any saturation of the eliminating systems that occur at higher concentrations (Aldridge et al 1977; Latini et al 1978). Microsamples (100 μl) were obtained from the circulating medium every 10 min for 180 min.

Caffeine was assayed by h.p.l.c. (Bonati et al 1979) in plasma and in some tissues at the time of plasma peak and 480 min after administration.

The concentration of caffeine vs time curves were analysed following a one-compartment open model system after extravascular administration. Experimental points were fitted by linear regression, after transformation by the peeling method. Apparent volume of distribution (Vd) and plasma clearance (Cl_p) were calculated from the following formulae:

$$Vd = \frac{F\,D}{AUC\,\beta}_{0\to\infty} \qquad Cl_p = \frac{F\,D}{AUC}_{0\to\infty}$$

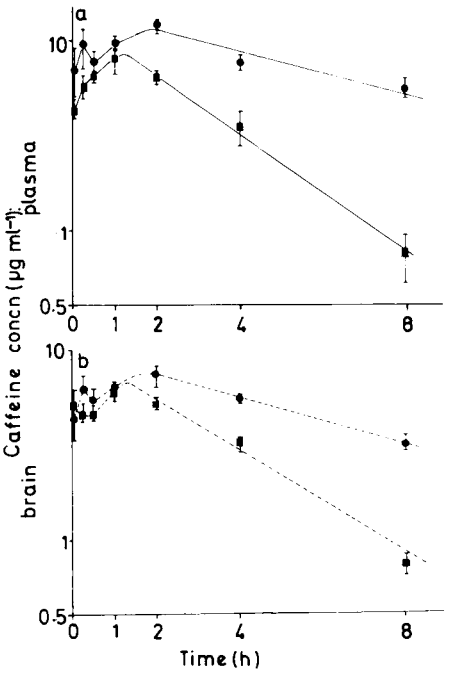

FIG. 1. a. Plasma caffeine concentrations in 40-day (■—■) and one-year-old rats (●—●) after 10 mg kg^{-1} by gavage. b. Brain caffeine concentrations in 40-day (■—■) and one-year-old rats (●—●) after 10 mg kg^{-1} by gavage. Vertical bars represent standard errors.

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