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Prevention by (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin of both catalepsy and the rises in rat striatal dopamine metabolism caused by haloperidol

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1 The influence of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on haloperidol-induced increases in the dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA), was measured in three microdissected brain regions of the rat following a quantitative assessment of catalepsy.

2 Haloperidol alone (2.66 μmol kg^{-1}, i.p.) caused a robust cataleptic response. Given 30 min after haloperidol, 8-OH-DPAT (76 or 760 nmol kg^{-1}, s.c.) prevented catalepsy in 30% and 100% of rats, respectively.

3 Haloperidol significantly increased the DOPAC (by 2 to 4 fold) and HVA (by 3 to 7 fold) contents of the caudate-putamen, nucleus accumbens and medial prefrontal cortex. Given alone, only the lower dose of 8-OH-DPAT caused a significant biochemical change, a doubling of cortical DOPAC.

4 In the cases where catalepsy was prevented by either dose of 8-OH–DPAT, the haloperidol-induced increases in DOPAC and HVA were consistently lower in the caudate-putamen. This pattern was true for the rise in cortical HVA but only in response to the lower dose of 8-OH-DPAT. In contrast, neither dose of 8-OH-DPAT was able to influence the haloperidol-induced rises in cortical DOPAC. In the nucleus accumbens, 8-OH-DPAT did not affect the haloperidol-induced increases in the dopamine metabolites, irrespective of the dose employed or the resulting behaviour. When catalepsy was not prevented, 8-OH-DPAT did not alter the neurochemical responses to haloperidol in any region.

5 These results suggest that part of the mechanism by which 8-OH-DPAT prevents haloperidol-induced catalepsy is reflected by a reversal of the compensatory increase in meso-striatal and/or meso-cortical dopamine neuronal activity that normally accompanies postsynaptic dopamine receptor blockade with haloperidol.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT; catalepsy; caudate-putamen; DOPAC; dopamine; haloperidol; HVA; medial prefrontal cortex; metabolism; nucleus accumbens

Introduction

Motor-related side effects are commonly encountered in the treatment of schizophreniform psychoses with so-called 'classical' antipsychotic drugs (APDs) such as haloperidol, that are known to block central dopamine receptors (Baldessarini & Tarsy, 1980; Casey, 1991). Animal models of the parkinsonian symptoms induced in man by APDs often employ an APD-induced behavioural state known as catalepsy in which animals display long periods of immobility and fail to correct externally imposed postures. It is not fully understood how catalepsy is produced although agents acting at a wide variety of pharmacological receptors can induce it. These include dopamine receptor antagonists, cholinoceptor agonists and opioid stimulants (see Klemm, 1989 for review).

However, it is also known that there are interactions between dopaminergic and 5-hydroxytryptaminergic neurones in the CNS which may be of relevance to the catalepsy syndrome. Indeed, substantial evidence has long supported a role for 5-HT in the modulation of haloperidol-induced catalepsy although its precise role is unclear and even disputed. For example, Kostowski *et al.* (1972) reported a reduction of haloperidol-induced catalepsy by destruction of 5-hydroxytryptaminergic and other neurones in the midbrain raphe or by depletion of central 5-hydroxytryptamine (5-HT) stores with *p*-chlorophenylalanine. Equally, Carter & Pycock (1977) and Balsara *et al.* (1979) observed a potentiation of haloperidol-induced catalepsy by the use of a 5-HT agonist (quipazine) or a 5-HT-uptake inhibitor (clomipramine) and its reduction by pretreatment with the 5-HT antagonist, methysergide. Conversely, other studies have reported that catalepsy was unaffected by the 5-HT antagonists, metergoline and methysergide, or by depletion of 5-HT with *p*-chlorophenylalanine (see, for example, Sarnek & Baran, 1975; Fregnan & Vidali, 1982).

Following the discovery of multiple 5-HT receptor subtypes, it is now apparent that selective agonists of the 5-HT_{1A} receptor subtype, *e.g.* (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and the partial 5-HT_{1A} agonist, buspirone, can prevent the catalepsy induced by different APDs (Invernizzi *et al.*, 1988; Hicks, 1990; Wadenberg & Ahlenius, 1991).

As far as dopamine transmission is concerned, previous studies have indicated that APD-induced catalepsy is mediated by blockade of postsynaptic dopamine receptors in the basal ganglia (Sanberg, 1980; Calderon *et al.*, 1988), but the precise site(s) of cataleptogenic action remains uncertain. For example, some microinjection studies indicate that catalepsy can be elicited by bilateral infusions of haloperidol made into the caudate-putamen or medial prefrontal cortex but not into the nucleus accumbens (Klockgether *et al.*, 1988). Conversely, others report that the nucleus accumbens is a pivotal region for induction of haloperidol-induced catalepsy (Hartgraves & Kelly, 1984; Ossowska *et al.*, 1990). In any event, each of these brain regions receives a 5-HT input from the midbrain raphe nuclei (Azmitia & Segal, 1978) and so it is possible that 8-OH-DPAT may interact with dopaminergic neurotransmission in one or more of these dopamine-rich brain regions to influence the cataleptic response to haloperidol and other APDs.

Since one consequence of dopamine receptor blockade with an APD is an increase in dopamine neuronal activity leading to

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an elevation of dopamine release and dopamine metabolism (Commissiong, 1985), we tested the above hypothesis by examining whether the anti-cataleptic action of 8-OH-DPAT was mediated or at least reflected by an influence on dopamine metabolism in one or more of the caudate-putamen, nucleus accumbens, and medial prefrontal cortex. Some of these data have been presented in preliminary form to the British Pharmacological Society (Andersen & Kilpatrick, 1995).

Methods

Animals

Male Porton rats (250–300 g, Wistar-derived) were used in this study. The animals were brought to the laboratory on the day of the experiment at least 30 min before the first injection in order to allow the animals to adjust to the new environment.

Drug treatment

All injections were made in a volume of 1.0 ml kg^{-1} body weight. 8-OH-DPAT·HBr was dissolved in 154 mM (0.9%) saline, while haloperidol was first dissolved in a minimum volume of glacial acetic acid and then made up to volume in 154 mM saline (final pH = 2.9). All drugs were freshly prepared on the day of the experiment and protected from light throughout their use.

All injection protocols followed an identical pattern such that the second, subcutaneous (s.c.) injection was given 30 min after the first, intraperitoneal (i.p.) injection. The doses of 8-OH-DPAT were chosen to reflect (1) reports that a low dose of 8-OH-DPAT (76 nmol kg^{-1}; equivalent to 25 μg kg^{-1}) selectively activates meso-cortical dopaminergic neurones (see Arborelius *et al.*, 1993a,b) without having a marked anti-cataleptic effect (Neal-Beliveau *et al.*, 1993) and (2) a higher dose (760 nmol kg^{-1}; equivalent to 250 μg kg^{-1}) that has been reliably demonstrated to antagonize haloperidol-induced catalepsy (Invernizzi *et al.*, 1988; Neal-Beliveau *et al.*, 1993). The time courses were chosen so that haloperidol (2.66 μmol kg^{-1}; equivalent to 1 mg kg^{-1}) was able to express its maximum behavioural effect at the same time as 8-OH-DPAT has its pharmacodynamic maximum (see Zetterström *et al.*, 1984; Perry & Fuller, 1989). The following drug treatment groups were established: (a) Vehicle control (haloperidol vehicle i.p. + saline s.c.); (b) haloperidol control (2.66 μmol kg^{-1} haloperidol i.p. + saline s.c.); (c) 8-OH-DPAT control (haloperidol vehicle i.p. + 76 or 760 nmol kg^{-1} 8-OH-DPAT s.c.); (d) haloperidol + 8-OH-DPAT (2.66 μmol kg^{-1} haloperidol i.p. + 76 or 760 nmol kg^{-1} 8-OH-DPAT s.c.).

Catalepsy

Catalepsy was assessed 45 min following the second injection by a vertical grid test, adapted from the method of Fuenmayor & Vogt (1979). Briefly, the rat was placed on a vertical wire grid (mesh size = 50 × 25 mm; wire diameter = 2 mm; overall dimensions 27 × 41 cm) with the nose pointing upwards and so that both hind- and forepaws were able to grip separate cross-members on the grid. The latency (s) to move and replace any paw from and onto the grid was recorded with a maximum immobility period set at 180 s. In order to avoid induction of 'learned' catalepsy (see Klemm, 1989), catalepsy was assessed only once.

Sample preparation

The tissue was prepared and analysed for dopamine and metabolites by h.p.l.c. as described in detail by Kilpatrick *et al.* (1986). Briefly, rats were killed immediately after the catalepsy assessment (48 min after the last injection) by cervical dislocation after initial stunning and their brains rapidly (< 1 min) removed onto an ice-chilled Petri dish. The caudate-putamen, nucleus accumbens and medial prefrontal cortex of both sides were rapidly dissected using a chilled razor blade and immediately frozen on a dry ice-cooled aluminium plate. Frozen tissue samples were individually thawed, blotted, weighed (accuracy 0.1 mg) and placed in an ice-cooled 1.8 ml polypropylene flip-top vial. Each sample was then homogenized for 1 min in a tapered PTFE motorized pestle in a ratio of 40 μl ice-cooled mobile phase per mg wet tissue. After centrifugation (17,000 *g*, 30 min, 2°C) in a Centra-3RS refrigerated centrifuge (Damon/IEC, Dunstable, England), the supernatant layer was removed into a 1-ml syringe and filtered through a 0.2 μm Acro LC3 filter unit (Gelman Sciences, Northampton, England). The samples were frozen at −70°C until the time of assay, which did not exceed five days.

H.p.l.c. assay of dopamine and its metabolites

A 40 μl filtrate was injected via a Rheodyne 7125 valve onto an Ultratechsphere 5 μm ODS 150 × 4.6 mm column (HPLC Technology Ltd, Macclesfield, England). The column was perfused with a mobile phase containing 100 mM sodium acetate, 83 mM citric acid, 0.45 mM 1-octanesulphonic acid (OSA), 0.27 mM disodium EDTA and 10.5% v/v methanol at pH 3.85. The pH was adjusted with citric acid. Before use, the mobile phase was filtered through a 0.45 μm hydrophobic membrane and degassed by sparging with helium for 15 min. Occasional alteration to the mobile phase (e.g. addition of extra OSA) was required due to alterations in the column retention profile (see Kilpatrick, 1991). The flow rate was maintained at 1.0 ml min^{-1} using a model 303 pump and a model 802 manometric module (Gilson Medical Electronics, Villiers-le-Bel, France). The detection system consisted of an analytical cell (model 5011, ESA, Bedford, U.S.A.) and the potentials across the electrodes (detector 1: −0.00 V and detector 2: +0.40 V) were applied from an ESA Coulochem model 5100A controller. The output from detector 2 was connected to a flatbed chart recorder. Under these conditions, it was possible to separate the compounds of interest with baseline resolution within 10 min and an on-column detection limit of 3 pg per compound.

Chemicals

Haloperidol and 8-OH-DPAT·HBr were purchased from Research Biochemicals International (Natick, MA, U.S.A.). Dopamine·HCl, DOPAC and HVA were obtained from Sigma (St Louis, MO, U.S.A.). Methanol for h.p.l.c. was purchased from BDH (Poole, England). All other chemicals were obtained from Fluka (Buchs, Switzerland).

Statistics

Data are presented as the mean tissue concentration of analyte (in pmol mg^{-1} wet tissue) ± standard error of the mean (s.e.mean). Where applicable, a Student's two-tailed, unpaired *t* test was used to assess differences between group values. It should be noted that the groups treated with the combined low dose of 8-OH-DPAT and haloperidol were also divided into separate groups: those that exhibited catalepsy and those that did not.

A minimum significance level was set at $P < 0.05$ and this is indicated by single markers on the histograms. Duplicate symbols represent a significance of $P < 0.01$ and triplicate symbols a significance of $P < 0.001$.

Results

Behaviour

No overt behavioural effect was seen following 76 nmol kg^{-1} 8-OH-DPAT alone. However, 760 nmol kg^{-1} 8-OH-DPAT induced a behavioural syndrome that is characteristic of many

5-HT agonists. This included forepaw treading, hind limb abduction, a flattened body posture and head weaving which started approximately 10 min after the injection (see Hjorth *et al.*, 1982; Tricklebank *et al.*, 1984). The forepaw treading and flat body posture components are thought to be due to 5-HT_{1A} receptor activation (Tricklebank *et al.*, 1984).

Haloperidol alone (2.66 μmol kg^{-1}) induced a robust cataleptic response such that every animal remained immobile on the grid for at least 35 s (111 ± 31 s; mean ± s.e.mean; $n=6$). Vehicle controls did not display any catalepsy or any other overtly unusual behaviours.

When 760 nmol kg^{-1} 8-OH-DPAT was administered 30 min after haloperidol, catalepsy was completely prevented in all 6 rats tested. A dose of 76 nmol kg^{-1} 8-OH-DPAT given 30 min after haloperidol prevented catalepsy in 3 out of the 10 rats tested. The remaining 70% stayed immobile for an average of 44 ± 20 s on the grid.

The '8-OH-DPAT syndrome' was also seen when 760 nmol kg^{-1} 8-OH-DPAT and haloperidol were co-administered. In the 3 animals given the combination of haloperidol and the lower dose of 8-OH-DPAT and in which catalepsy did not appear, no abnormal behaviours could be discerned.

Neurochemistry

Dopamine concentrations In controls ($n=6$), the dopamine concentrations (in pmol mg^{-1} wet tissue) in the three microdissected brain regions were as follows: caudate-putamen (77.3 ± 1.7), nucleus accumbens (44.9 ± 2.8) and medial prefrontal cortex (0.9 ± 0.1). None of the treatments employed in this study had a significant effect on the dopamine content of any of the brain regions studied.

DOPAC concentrations (Figures 1 and 2) Haloperidol (2.66 μmol kg^{-1}) significantly increased the DOPAC content of the caudate-putamen relative to the vehicle control (+217%; $P<0.001$; $d.f.=11$; Figure 1). When the haloperidol-induced catalepsy was prevented by either 76 or 760 nmol kg^{-1} 8-OH-DPAT, the increases were respectively restricted to +138% and +59%. These increases were significantly less than those generated by haloperidol treatment alone ($P<0.05$; $d.f.=8$ and $P<0.001$; $d.f.=11$, Figure 1) but were still significantly elevated above the vehicle control values ($P<0.001$ in both cases).

In the 7 cases where catalepsy was not prevented by 76 nmol kg^{-1} 8-OH-DPAT, the magnitude of the haloperidol-induced increase in the DOPAC concentration (+230%; Figure 1) was similar to that observed for haloperidol alone.

In the nucleus accumbens and the medial prefrontal cortex, haloperidol treatment increased DOPAC concentrations relative to those of the vehicle control by +197% ($P<0.001$; $d.f.=11$; Figure 2) and +435% ($P<0.001$; $d.f.=11$; Figure 2), respectively. In contrast to the effect seen in the caudate-putamen, these increases were not prevented by either of the 8-OH-DPAT doses studied.

Given alone, neither dose of 8-OH-DPAT had any significant influence on the DOPAC content of the caudate-putamen or of the nucleus accumbens. In the medial prefrontal cortex, however, the lower dose of 8-OH-DPAT alone significantly increased the DOPAC content relative to the vehicle control by +106% ($P<0.01$; $d.f.=10$; Figure 2). The higher dose of 8-OH-DPAT caused a mean rise of +71% in cortical DOPAC which did not reach statistical significance ($P=0.091$; $d.f.=9$; Figure 2).

HVA concentrations (Figures 3 and 4) Essentially the same pattern recorded for changes in DOPAC content in the caudate-putamen and in the nucleus accumbens was seen for the

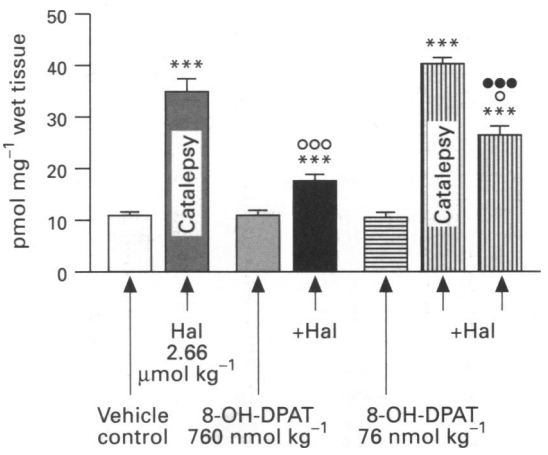

Figure 1 Influence of haloperidol and/or 8-OH-DPAT on DOPAC concentrations in the caudate-putamen. Each column represents the mean ± s.e.mean in pmol mg^{-1} wet tissue of 5–7 separate experiments, except for the group which failed to show catalepsy after receiving haloperidol and 76 nmol kg^{-1} 8-OH-DPAT (extreme right-hand column; this group consists of 3 experiments). Rats received 2.66 μmol kg^{-1} haloperidol (Hal) or the vehicle 30 min before either 76 or 760 nmol kg^{-1} 8-OH-DPAT or its vehicle. Groups displaying catalepsy when tested 45 min after the second injection are indicated as such within that group column. Treatment groups are identified by the arrows. Rats were killed 3 min after catalepsy testing. (*)Significant differences from the vehicle control group; (○) significant differences between Hal + vehicle and Hal + 8-OH-DPAT treatment; (●) significant differences between the groups of cataleptic and non-cataleptic rats after combined Hal and 76 μmol kg^{-1} 8-OH-DPAT treatment.

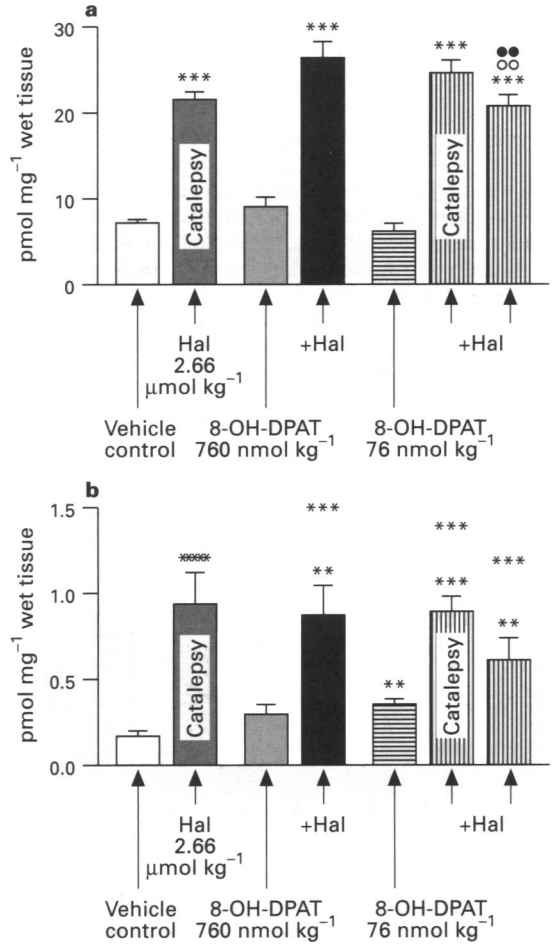

Figure 2 Influence of haloperidol (Hal) and/or 8-OH-DPAT on DOPAC concentrations in the nucleus accumbens (a) and medial prefrontal cortex (b). Other details can be found in the legend to Figure 1.

HVA content in these regions. Thus, haloperidol caused a significant increase in the HVA content of the caudate-putamen (+319%; $P<0.001$; $d.f.=11$; Figure 3) and the nucleus accumbens (+451%; $P<0.001$; $d.f.=11$; Figure 4) relative to the vehicle control. Similarly, neither dose of 8-OH-DPAT significantly altered the HVA content of these two regions when administered alone. In those animals treated with both drugs and in which the cataleptic response did not appear, the HVA increase in the caudate-putamen was restricted to +195% by the lower dose of 8-OH-DPAT and to +104% by the higher dose (Figure 3). Again, these changes were significantly less than those evoked by haloperidol alone ($P<0.01$; $d.f.=8$ and $P<0.001$; $d.f.=11$, respectively) but the resulting values were still greater than those of the vehicle control ($P<0.001$ in both cases). In the nucleus accumbens of animals treated with haloperidol and either dose of 8-OH-DPAT, the haloperidol-induced rises in HVA were preserved, regardless of whether the animals were cataleptic (Figure 4).

In the medial prefrontal cortex, haloperidol increased the HVA content by +712% ($P<0.001$; $d.f.=11$; Figure 4) relative to the vehicle control. This increase was slightly but nonetheless significantly ($P<0.05$; $d.f.=8$) prevented by 76 nmol kg^{-1} 8-OH-DPAT in the 'non-cataleptic' group. A significant interaction between haloperidol and the higher dose of 8-OH-DPAT on cortical HVA levels could not be discerned. On its own, 8-OH-DPAT did not significantly influence the HVA content in this particular region (Figure 4).

Discussion

These results show that in addition to preventing haloperidol-induced catalepsy, 8-OH-DPAT prevents the rise in DOPAC and HVA content of the caudate-putamen that normally accompanies haloperidol treatment. This neurochemical phenomenon also occurs to a lesser extent in the medial prefrontal cortex, but is completely absent from the nucleus accumbens.

The regional action of haloperidol

At least three different mechanisms are thought to underlie the increase in DOPAC and HVA contents of the caudate-putamen and nucleus accumbens after acute haloperidol treatment, although the contribution made by any single mechanism is difficult to establish.

These include (i) postsynaptic blockade of dopamine D_2-receptors and activation (or interruption) of a neuronal loop reflex (see for example, Essig & Kilpatrick, 1991a,b), (ii) a direct antagonist action of haloperidol on impulse-regulating autoreceptors localized on dopaminergic cell bodies and/or dendrites (Hand *et al.*, 1987) and (iii) a blockade of D_2-receptors that are negatively coupled to both dopamine synthesis and dopamine release and are located on dopamine terminals (Garcia-Munoz *et al.*, 1977; Wuerthele & Moore, 1979; 1980; Westerink & De Vries, 1989). In the medial prefrontal cortex, it is thought that blockade of release-modulating autoreceptors on meso-cortical dopamine terminals is largely responsible for the increase in dopamine utilisation seen after acute haloperidol treatment (Chiodo *et al.*, 1984; Talmaciu *et al.*, 1986; Kilpatrick & Rogers, 1987; Wolf & Roth, 1987).

The interaction of 8-OH-DPAT with haloperidol

How does 8-OH-DPAT counteract haloperidol-induced dopamine metabolism increases in one area but not in others? In the caudate-putamen, this may ostensibly be explained by the report of Johnson *et al.* (1993), in which 8-OH-DPAT could apparently activate 5-HT$_{1A}$ receptors present on dopamine terminals in this area to inhibit tyrosine hydroxylase. *In vivo* then, both dopamine and DOPAC formation should be reduced yet, when 8-OH-DPAT was administered alone in the current study, no effect on dopamine or its metabolites was seen in either the caudate-putamen or the nucleus accumbens. Given that immobility is typically linked to a breakdown of dopamine transmission, it is also difficult to envisage how a *reduction* in dopamine synthesis (and arguably, an eventual decline in dopamine release) could lead to the prevention of an immobilising response.

In the only other reported study to examine the influence of systemic 8-OH-DPAT on dopamine metabolism, Ahlenius *et*

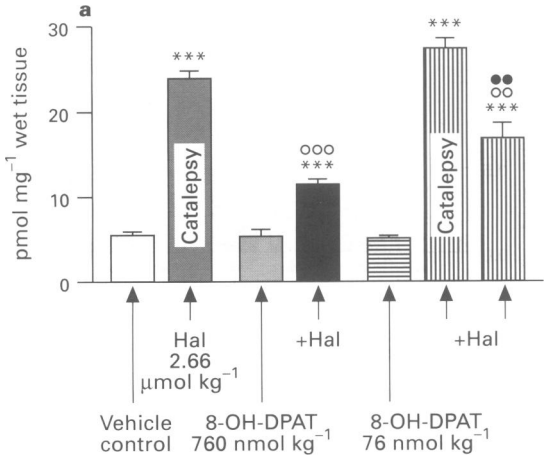

Figure 3 Influence of haloperidol (Hal) and/or 8-OH-DPAT on HVA concentrations in the caudate-putamen. Other details can be found in the legend to Figure 1.

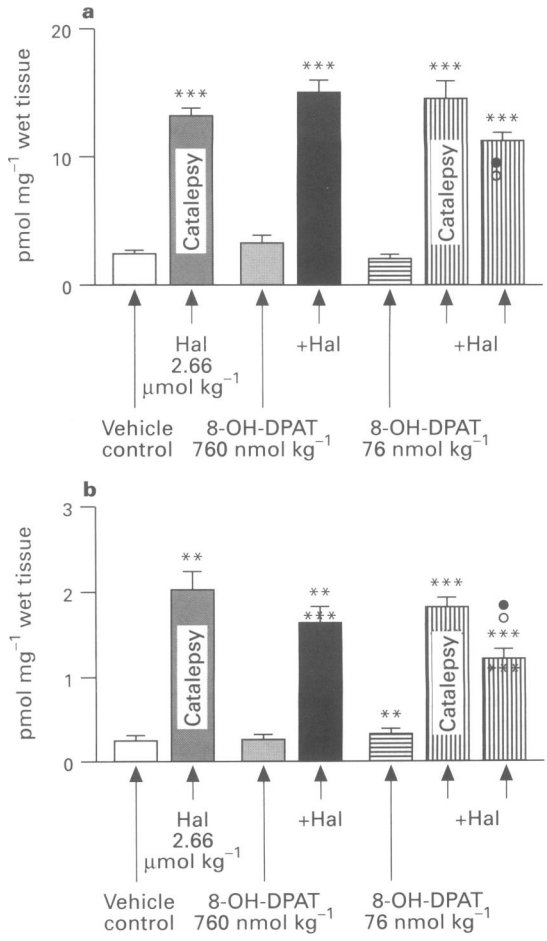

Figure 4 Influence of haloperidol (Hal) and/or 8-OH-DPAT on HVA concentrations in the nucleus accumbens (a) and medial prefrontal cortex (b). Other details can be found in the legend to Figure 1.

al. (1990) found that 8-OH-DPAT (800 nmol kg^{-1}, s.c.) increased dopamine turnover in samples of the ventral striatum but (as reported here) not in the dorsal striatum (caudate-putamen) 40 min after injection. Although those results for the ventral striatum cannot be directly compared with the results from our study (since their tissue samples comprised the nucleus accumbens, the olfactory tubercle, the diagonal band of Broca and the bed nucleus of the stria terminalis), we also detected a small, albeit non-significant, increase in dopamine metabolism in the nucleus accumbens after a similar dose of 8-OH-DPAT. However, *in vivo* studies rarely find such actions of 8-OH-DPAT (Jiang *et al.*, 1990).

8-OH-DPAT has been reported to act as a dopamine D_2 agonist in peripheral tissue (Smith & Cutts, 1990) and the CNS (Ahlenius *et al.*, 1989) and 8-OH-DPAT could therefore simply compete with haloperidol at D_2 receptors. However, if this were the case, 8-OH-DPAT treatment alone would have been expected to generate some or all of the effects that are associated with postsynaptic D_2 receptor stimulation. These include marked *decreases* in the tissue content of DOPAC and/or HVA (Boyar & Altar, 1987) and the appearance of stereotyped behaviours including sniffing, licking and gnawing (Arnt & Hyttel, 1990); none of these was seen.

Data from *in vivo* microdialysis studies using 8-OH-DPAT are equally conflicting. Thus, 8-OH-DPAT delivered directly into the caudate-putamen is reported to stimulate dopamine release at that site (Benloucif & Galloway, 1991; Benloucif *et al.*, 1993; Golembiowska *et al.*, 1993). However, Arborelius *et al.* (1993b) found that systemic (***R***)-8-OH-DPAT had no effect on dopamine release in this structure. Indeed, these latter authors reported that this low dose of (***R***)-8-OH-DPAT (76 nmol kg^{-1}, s.c.) selectively increased dopamine release in the medial prefrontal cortex. This agrees with data from their electrophysiological study, in which Arborelius *et al.* (1993a) found that this same low dose of the 8-OH-DPAT enantiomer increased the firing of neurones in the parabrachial pigmentosus nucleus (a subregion of the ventral tegmental area containing meso-cortical dopaminergic neurones). It also accords with our observation that the low dose of the 8-OH-DPAT racemate caused both a significant increase in the DOPAC content of the medial prefrontal cortex and a non-significant rise in the HVA content of this region. It is somewhat surprising then, that the *separate* increases in cortical DOPAC caused by 8-OH-DPAT and haloperidol alone, were not in any way additive when the drugs were given in combination, even when catalepsy was not prevented. If anything, both doses of 8-OH-DPAT tended to *reduce* the haloperidol-evoked increases in cortical DOPAC and HVA, especially when catalepsy was prevented. These reductions were greatest with the lower dose of 8-OH-DPAT which, on its own, promoted the largest *rise* in cortical DOPAC. Taken in combination with the report that moderate to high doses of 8-OH-DPAT actually *inhibit* the activity of all types of presumed dopaminergic neurone in the midbrain (Arborelius *et al.*, 1993a), the reasons for these phenomena are not immediately apparent. Nevertheless, it is clear that extracellular dopamine concentrations in both the *in vivo* caudate-putamen (Benloucif & Galloway, 1991; Benloucif *et al.*, 1993; Bonhomme *et al.*, 1995) and the nucleus accumbens (Jiang *et al.*, 1990; Parsons & Justice, 1993) are consistently elevated by infusions of 5-HT itself into those structures although a variety of 5-HT receptors appear to be responsible, including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 and 5-HT_4 subtypes.

Whilst the data of Arborelius and colleagues (1993a,b) indicate that the medial prefrontal cortex is the most sensitive area to changes in dopamine release caused by (***R***)-8-OH-DPAT, it must be borne in mind that their dose of (***R***)-8-OH-DPAT is likely to be only weakly anti-cataleptic (Neal-Beliveau *et al.*, 1993). In the present study, the lower dose of the 8-OH-DPAT racemate was also only marginally effective in preventing the haloperidol-evoked catalepsy and the associated rises in dopamine metabolism. This may simply reflect the potency of 8-OH-DPAT or the fact that the (***S***)-enantiomer is only a partial 5-HT_{1A} agonist (Cornfield *et al.*, 1991), although it should be noted that partial 5-HT_{1A} agonists are potent anti-cataleptic agents in their own right (Neal-Beliveau *et al.*, 1993).

It is feasible that when given in combination with haloperidol, higher doses of 8-OH-DPAT actually *promote* an elevation of dopamine release in the caudate-putamen over and above that caused by haloperidol. In this scheme, the additional dopamine overflow would displace haloperidol and have a dual action. Postsynaptically, catalepsy would be alleviated. Presynaptically, as well as postsynaptically, dopamine synthesis and metabolism would be reduced leading to the observed falls in tissue metabolites. For whatever reason, this mechanism would not apply to the nucleus accumbens and be only marginally active in the prefrontal cortex.

Of course, the anti-cataleptic effect of 8-OH-DPAT could be mediated independently of dopamine transmission and/or in a brain region that was not investigated in this study. Yet it follows that since catalepsy is a postsynaptically-derived response (see Introduction) and 8-OH-DPAT prevented *both* catalepsy *and* increased dopamine metabolism in the caudate-putamen, then the latter action of 8-OH-DPAT is likely to be a consequence of the former *i.e* an interaction with the reflex result of *postsynaptic* dopamine receptor blockade rather than an action on haloperidol's blockade of terminal or somatodendritic autoreceptors. Such a route is currently obscure.

Dopamine metabolism increases do not consistently reflect catalepsy

In the present study, there is a clear correlation of the neurochemical findings with the behaviour. Thus, haloperidol increases dopamine metabolism and induces catalepsy, whereas 8-OH-DPAT prevents catalepsy and concurrently reduces the rises in striatal dopamine metabolism caused by haloperidol. Equally, in a preliminary investigation, Hinds & Kilpatrick (1994) observed a return of haloperidol-induced catalepsy 120 min after 8-OH-DPAT was administered at a time when no influence of 8-OH-DPAT on the enhanced dopamine metabolism could be shown. Collectively, however, these results do not necessarily mean that there is a *functional* correlation between dopamine metabolism and behaviour. Indeed, similar increases in regional brain dopamine metabolism can be induced by an atypical APD, remoxipride, at doses which do not even begin to elicit a cataleptic response (see Ögren *et al.*, 1984; Hinds & Kilpatrick, 1994).

Conclusions

Explanations of the reported behavioural and neurochemical actions may invoke the known reciprocity between 5-HT and dopamine release in which 8-OH-DPAT, acting via 5-HT_{1A} autoreceptors on raphe-striatal somata (Sharp *et al.*, 1989), would suppress 5-HT release in the caudate-putamen and consequently enhance dopamine release. Yet this would not account for the lack of neurochemical change seen with 8-OH-DPAT alone. Whilst 5-HT_{1A} receptors do seem to be involved in the anti-cataleptic action of 8-OH-DPAT against the atypical APD, raclopride (Wadenberg *et al.*, 1994), this may not be a universal mechanism.

It is plain that the behavioural actions of 8-OH-DPAT do not simply need to be superimposed upon those of haloperidol at the expense of catalepsy, since the low dose of 8-OH-DPAT was able to prevent the appearance of catalepsy without invoking any overt behaviours itself. Nevertheless, assuming that the anti-cataleptic mechanism of 8-OH-DPAT involves stimulation of 5-HT_{1A} receptors that affect skeletal muscle activity, a number of targets exist. Thus, 5-HT_{1A} receptors are relatively dense in brain regions such as the raphe nuclei, amygdala, cerebral cortex and hippocampus but are notably less concentrated in areas known to influence movement directly, *viz.* the globus pallidus, substantia nigra and especially, the caudate-putamen from which they are virtually absent yet

in which the strongest neurochemical interaction between 8-OH-DPAT and haloperidol was observed in the present study (Marcinkiewicz *et al.*, 1984; Chalmers & Watson, 1991; Radja *et al.*, 1993). 5-HT_{1A} receptors are also present in the spinal cord, although Wadenberg *et al.* (1993) suggest that this, too, is not the site from which 8-OH-DPAT prevents haloperidol-induced catalepsy. Whatever the 5-HT receptor(s) involved, the target is likely to be a postsynaptic one (Needham *et al.*, 1994).

Haloperidol is unlikely to be unique in displaying an apparent correlation between dopamine metabolism and behaviour in a variety of conditions. Further investigation is therefore warranted to aid our understanding of how 8-OH-DPAT prevents catalepsy produced not only by haloperidol but by a range of APDs.

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