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Increased dopamine turnover in the ventral striatum by 8-OH-DPAT administration in the rat

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Abstract—The administration of the 5-HT_{1A} agonist 8-OH-DPAT (0·8 μmol kg^{-1} s.c. −40 min) produced an increase in dopamine (DA) turnover, estimated by the quotient (DOPAC + HVA) DA^{-1}, in the ventral striatum of the rat. No statistically significant effects were obtained in the dorsal striatum. The accumulation of 3-MT in pargyline-treated animals (375 μmol kg^{-1} s.c. −60 min) was not affected by 8-OH-DPAT treatment (0·15–2·4 μmol kg^{-1} s.c. −30 min). These findings indicate that 8-OH-DPAT has weak antagonist properties at striatal DA receptors in normal rats. Both the 5-HT_{1A} agonist flesinoxan (0·06–17·8 μmol kg^{-1} s.c. −50 min) and the mixed 5-HT_1 and 5-HT_2 agonist 5-MeODMT (1·6–26·0 μmol kg^{-1} s.c. −50 min) produced a decrease in forebrain 5-HTP accumulation (striatum and neocortex), following decarboxylase inhibition by means of NSD-1015 in reserpine treated rats, indicating stimulation of central 5-HT receptors by these two compounds. At the same time, the DOPA accumulation in the ventral striatum was decreased by flesinoxan and increased by 5-MeODMT treatment. These observations show that, under these conditions, the decrease in DA synthesis is not directly coupled to the decreased 5-HT synthesis produced by flesinoxan, as previously demonstrated for 8-OH-DPAT. Taken together with previous observations, the present results suggest that 8-OH-DPAT, depending on the experimental conditions, is an agonist or antagonist at striatal DA receptors, in all probability due to partial DA receptor agonist properties of the compound.

In a recent report it was shown that brain dopamine (DA) synthesis was decreased by the administration of the 5-hydroxytryptamine (5-HT) agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) in reserpine-treated rats (Ahlenius et al 1989). Although 8-OH-DPAT has been characterized as a direct acting agonist at brain 5-HT_{1A} receptors (Hjorth et al 1982; Middlemiss & Fozard 1983), the effects of 8-OH-DPAT on brain DA synthesis are probably not mediated via 5-HT_{1A} receptors, since the effect was completely antagonized by treatment with the DA D_2 receptor antagonist raclopride (Köhler et al 1985), but not by the 5-HT_{1A} receptor antagonist (−)-pindolol (see Palacios et al 1987). Reserpine treatment, to a large extent, excludes indirect effects via other monoaminergic neurons, but it does not exclude indirect effects locally at the synaptic level, as in the case of (+)-amphetamine (see Carlsson 1970). Thus, the effects on striatal DA synthesis, produced by 8-OH-DPAT administration, could be due to direct agonist actions at brain DA receptors and/or indirect receptor stimulation due to release of striatal DA.

In the present experiments, we have investigated the effects of 8-OH-DPAT on striatal DA turnover and release by measuring the deaminated products DOPAC and HVA, and by measurements of the extraneuronally formed *O*-methylated product 3-MT. We also further studied the relation between changes in striatal 5-HT and DA synthesis by use of the new 5-HT_{1A} receptor agonist flesinoxan (Ramage et al 1988), and by use of the 5-HT receptor agonist 5-methoxy-*N*,*N*-dimethyl-tryptamine (5-MeODMT), the latter of which also has some affinity for the 5-HT_2 receptor site (see Peroutka 1987). Brain monoamine synthesis was estimated in reserpine treated animals by measuring the accumulation of DOPA and 5-HTP, following inhibition of cerebral decarboxylase (see Carlsson et al 1972).

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Materials and methods

Animals. Adult male Sprague–Dawley rats (280–320 g) (ALAB Laboratorietjänst AB, Sollentuna, Sweden) arrived in the laboratory at least one week before use and were housed under controlled conditions of circadian rhythm (12:12 h, lights off 06.00 h), temperature (20–21°C) and relative humidity (55–65%). Food (R3, Ewos, Södertälje, Sweden) and tap water were freely available in the home cage. Experiments were performed between 09.00 and 16.00 h.

Drugs. 8-Hydroxy-2-(di-*n*-propylamino) tetralin HBr (8-OH-DPAT) (RBI, Natick, MA), flesinoxan HCl (Duphar, Weesp, The Netherlands), 5-methoxy-*N*,*N*-dimethyltryptamine oxalate (5-MeODMT) (RBI), pargyline HCl (Sigma, St Louis, MO), reserpine (Fluka, Buchs, Switzerland), 3-hydroxy-benzylhydrazine 2HCl (NSD-1015) (Sigma). Reserpine was dissolved in a minimal quantity of glacial acetic acid and the final solution was made up to volume with 5·5% glucose. The other compounds were dissolved in 0·9% NaCl (saline). The injected volume was 2 mL kg^{-1} and the route of administration, subcutaneously (s.c.) or intraperitoneally (i.p.), is indicated in figure legends. Controls received the corresponding volume of the appropriate vehicle.

Biochemistry. The animals were decapitated and the brain, including the olfactory bulb rostrally and the medulla oblongata caudally, was quickly removed, and placed in a mould where it could be sliced in 2·5 mm sections by a thin stainless steel wire (diam = 70 μm). The ventral striatum (including the nucleus accumbens, the olfactory tubercle, the diagonal band of Broca and the bed nucleus of the stria terminalis) and the dorso-lateral neostriatum were dissected on ice from one of these slices. The rostral edge of the slice was approximately +2·1 mm in relation to bregma. The brain was cut at an inclination of approximately 7°, such that ventrally the sections extended slightly rostrally, according to the horizontal plane in the atlas of Paxinos & Watson (1986). The mean weight (grand means ± s.d. from the different experiments) of the ventral and the dorsal striatum, as defined here, were 37·3 ± 1·8 and 23·1 ± 0·1 mg, respectively. The brain samples were immediately frozen on dry ice and stored at −70°C until processing. Di-hydroxy-phenylalanine (DOPA), 5-hydroxy-tryptophan (5-HTP), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT) and dopamine (DA) were determined in the brain samples by means of coupled column liquid chromatography with electrochemical detection. The preparation of the samples and further details are given in Magnusson et al (1980) and in Mohringe et al (1986).

Results

Effects of 8-OH-DPAT on striatal dopamine turnover and release in the rat. The administration of 8-OH-DPAT, 0·8 μmol kg^{-1}, produced a statistically significant increase in DA turnover in the ventral, but not in the dorsal, striatum (Fig. 1). The DA turnover was estimated by the quotient (DOPAC + HVA) DA^{-1}. There were no changes in DA levels by the 8-OH-DPAT treatment in the ventral striatum, as compared with saline-

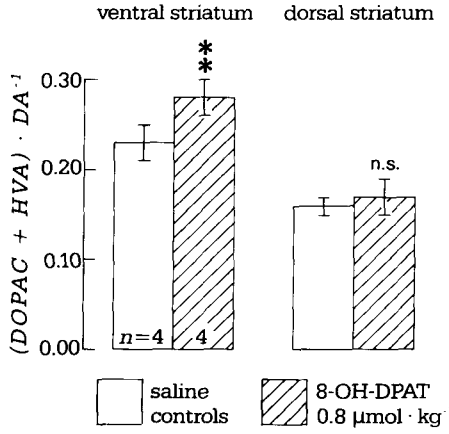

FIG. 1. Effects of 8-OH-DPAT on brain dopamine turnover in rats. 8-OH-DPAT was administered s.c. 40 min before decapitation. The figure shows means ± s.d., based on 4 observations per group. Statistical analysis was performed by means of Student's *t*-test, as indicated in the figure. $^{n.s.}P > 0.05$ $^{**}P < 0.01$.

treated controls (44·0 ± 6·2 and 42·5 ± 3·7 nmol g^{-1}, respectively $t_6 = 0.41$, $P > 0.05$). The corresponding figures in the dorsal striatum were 82·0 ± 7·1 and 80·2 ± 5·9 nmol g^{-1}, $t_6 = 0.37$, $P > 0.05$.

The determination of 3-MT in pargyline pretreated rats (375 μmol kg^{-1}), following the administration of 8-OH-DPAT (0·15–2·4 μmol kg^{-1}) did not indicate that DA metabolism via *O*-methylation was increased (Table 1).

Table 1. Effects of 8-OH-DPAT on brain 3-MT accumulation in pargyline treated rats. 8-OH-DPAT was administered s.c. 30 min before decapitation. All animals were pretreated with pargyline, 375 μmol kg^{-1} s.c. 60 min before decapitation. The Table shows mean values ± s.d. (nmol g^{-1}) based on 4 determinations per group. Statistical evaluation was performed by means of a one-way ANOVA (see Winer 1971). Ventral striatum: $F_{3,12} = 0.42$, $P > 0.05$; Dorsal striatum: $F_{3,12} = 0.52$, $P > 0.05$.

	8-OH-DPAT (μmol kg^{-1} s.c.)			
	0	0·15	0·60	2·40
Ventral striatum	3·33 ± 0·64	2·89 ± 0·71	2·98 ± 0·60	3·12 ± 0·48
Dorsal striatum	6·43 ± 0·76	6·23 ± 0·33	5·89 ± 0·60	6·58 ± 1·31

Effects of flesinoxan and 5-MeODMT on DOPA and 5-HTP accumulation in the ventral striatum in reserpine and NSD-1015 treated rats. The DOPA accumulation was, in both experiments, increased by the reserpine treatment, compared with NSD-1015 treated controls: $t_{25} = 4.41$, $P < 0.01$ (flesinoxan experiment) and $t_{14} = 4.12$, $P < 0.01$ (5-MeODMT). The 5-HTP accumulation, however, was not significantly changed by the reserpine treatment. In comparison with reserpine-treated controls, flesinoxan produced a dose-dependent decrease in the DOPA as well as the 5-HTP accumulation. The administration of 5-MeODMT also produced a decrease in 5-HTP accumulation, which was not clearly dose-dependent, however. In contrast to the effects produced by flesinoxan, 5-MeODMT administration resulted in a dose-dependent increase in the DOPA accumulation. With the notable exception of the decrease in DOPA accumulation, which was selective for the ventral striatum in flesinoxan treated animals, similar effects, as shown for the ventral striatum in Fig. 2, were obtained in the dorsal striatum and in the neocortex with both compounds. Furthermore, in the case of flesinoxan the

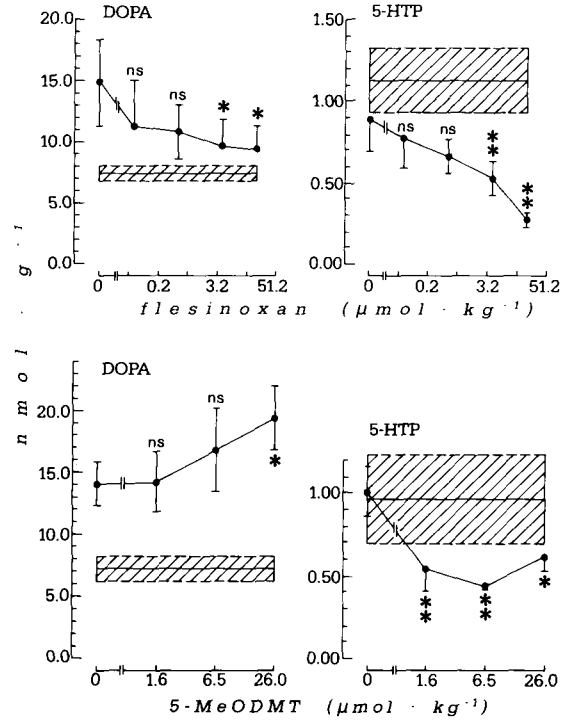

FIG. 2. Effects of flesinoxan and 5-MeODMT on the accumulation of DOPA and 5-HTP in the ventral striatum following inhibition of cerebral aromatic amino acid decarboxylase in reserpine-treated rats. The animals were pretreated with reserpine, 8·2 μmol kg^{-1}, or the glucose vehicle, s.c. 18 h before decapitation. The corresponding time for the administration of flesinoxan or 5-MeODMT in doses (s.c.) as indicated in the figure, or the saline vehicle, was 50 min. All animals received NSD-1015, 475 μmol kg^{-1} i.p. 30 min before decapitation. The results are presented as means ± s.d., based on 3–8 determinations per group. The glucose/NDS-1015 control values are indicated by the hatched areas in the figure. Statistical analysis was performed by means of a one-way ANOVA, followed by the Dunett's t-test for comparisons with reserpine-treated controls, as indicated in the figure (see Winer 1971). Flesinoxan: $F_{5,25} = 6.81$, $P < 0.001$ (DOPA); $F_{5,25} = 18.38$, $P < 0.001$ (5-HTP). 5-MeODMT: $F_{4,14} = 16.19$, $P < 0.001$ (DOPA); $F_{4,14} = 9.13$, $P < 0.001$ (5-HTP). $^{n.s.}P > 0.05$ $^{*}P < 0.05$ $^{**}P < 0.01$.

neocortical decrease in 5-HTP accumulation was statistically significant already at the 0·5 μmol kg^{-1} dose (data not shown).

Discussion

The present results show that DA turnover was increased in the ventral striatum following treatment with 8-OH-DPAT. The relatively small effect (about 130% of controls) was only marginally further increased by higher doses of 8-OH-DPAT, up to 19·4 μmol kg^{-1} (data not shown). The DA receptor blocking agents raclopride or haloperidol, under comparable conditions, produce an increase of at least 400% (e.g. Hillegaart et al 1988). In a previous report it was shown that 8-OH-DPAT produced a decrease in striatal DOPA accumulation, following decarboxylase inhibition, in reserpine-treated rats, and that this effect was antagonized by treatment with the DA D_2 receptor antagonist raclopride, but not the 5-HTA_{1A} antagonist (−)-pindolol (Ahlenius et al 1989). These observations can be reconciled on the assumption that 8-OH-DPAT is a partial DA agonist. In normal animals, as in the present experiments, antagonist properties are disclosed, as indicated by the increased DA turnover, whereas in reserpine-treated animals agonist properties at dopaminergic autoreceptors are seen. This is in general agreement with observations on effects of partial DA agonists

like (−)3-PPP, terguride and OPC-4392 on brain DA synthesis and turnover (Hjorth et al 1983; Kehr 1984; Yasuda et al 1988). The characterization of 8-OH-DPAT as a partial DA agonist is further supported by the observation that firing of DA neurons in the substantia nigra is inhibited by the intravenous administration of 8-OH-DPAT and that this effect is antagonized by haloperidol treatment (Sinton & Fallon 1988). Finally, 8-OH-DPAT produces contralateral turning in rats with a unilateral 6-OH-DA lesion of the ascending nigro-striatal pathway (Gerber et al 1988).

The interesting possibility that at least some of the effects of 8-OH-DPAT on striatal DA turnover are mediated via 5-HT receptors is not supported by our observations. As mentioned above, the decrease in DOPA accumulation produced by 8-OH-DPAT in reserpine-treated animals can be antagonized by raclopride, but not (−)-pindolol, administration. Furthermore, (−)-pindolol with affinity for the 5-HT_{1A} receptor site (see Palacios et al 1987) by itself suppressed the 5-HTP accumulation in reserpine-treated rats. This decrease, however, was not accompanied by a decrease in the DOPA accumulation (Ahlenius et al 1989). In the present experiments we studied the effects of two additional 5-HT agonists, flesinoxan and 5-MeODMT on striatal DOPA and 5-HTP accumulation in reserpine-treated rats. Flesinoxan, like 8-OH-DPAT, produced a decrease in both DOPA and 5-HTP accumulation, whereas 5-MeODMT produced a different pattern of effects: decrease in 5-HTP and an increased DOPA accumulation. Although flesinoxan and 5-MeODMT differ in their affinity for various proposed 5-HT receptor sites, as defined in receptor ligand studies, our previous observations on 8-OH-DPAT-(−)-pindolol interactions make a role for the proposed 5-HT_{1A} receptor site less likely and, as shown by the 5-MeODMT experiments, possibly unrelated to 5-HT receptor stimulation. Needless to say, a clear definition of the functional role of 5-HT receptor subtypes may disclose interactions not possible to demonstrate with the chemical tools available today. The intrinsic activity of the various compounds discussed here also appear to be different, with potentially important consequences for their pharmacodynamic profile. It appears for instance that the efficacy of flesinoxan as a 5-HT agonist is greater than for 5-MeODMT in the present experiments, and this may apply also in comparison with 8-OH-DPAT, as shown by others and ourselves under similar experimental conditions (Hjorth et al 1982; Ahlenius et al 1989). Finally, it is worth noting that in a recent study on the biochemical effects of four possible C3-methylated derivatives of 8-OH-DPAT, the effects on 5-HT synthesis were dissociated from effects on DA turnover (Björk et al 1987). Thus, of the two compounds consistently decreasing 5-HTP accumulation after decarboxylase inhibition, only one produced an increase in striatal HVA levels. Furthermore, the two compounds with no or less effects on the 5-HTP accumulation, both produced an increased striatal HVA level.

In the present experiments, as in our previous report (Ahlenius et al 1989), DA receptors in the limbic striatal areas appear to be selectively affected by the 8-OH-DPAT treatment. Curiously, this was true also for flesinoxan, where the DOPA accumulation in the posterior striatum was 16·9, 16·8, 16·1 and 17·4 nmol g^{-1} at the doses of 0, 0·06, 0·5 and 3·75 μmol kg^{-1}, respectively, in reserpine treated animals. The cause of this selectivity remains to be clarified.

Conclusion. The present finding that 8-OH-DPAT can produce an increased DA turnover in the striatum of the rat indicates that the compound can act as an antagonist at DA receptors. Together with previous observations on the effects of 8-OH-DPAT on striatal DA synthesis in reserpine-treated rats, this suggests that 8-OH-DPAT is a mixed agonist/antagonist at striatal DA receptors, probably due to partial agonist properties of the compound.

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Smoking, eicosanoids and ulcerative colitis

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Abstract—In this study, which is the first of its kind using normal tissue samples that are very difficult to obtain, we have investigated the hypothesis that smoking protects against ulcerative colitis by altering the colonic mucosal formation of prostaglandins and related substances. Colonic mucosa biopsied from healthy young men produced prostaglandin E, 6-keto-$PGF_{1\alpha}$ (formed from PGI_2), leukotriene B_4 and leukotriene $C_4/D_4/E_4$ as determined by radioimmunoassay. With each substance, the median yield was lower in the group of smokers who smoked 3 cigarettes in the 2 h before biopsy, than in the non-smokers. However, with each eicosanoid the statistical probability approached only the 10% level, but the fact that the trend was the same for all eicosanoids somewhat strengthens the possibility of a real difference between the groups.

Prostaglandins and leukotrienes are important mediators of inflammation whose synthesis in human gastric mucosa is influenced by smoking (Quimby et al 1986). A similar effect in the colon might be pertinent to the aetiology of ulcerative colitis, and its inverse relationship to smoking (Harries et al 1982). We have investigated this hypothesis by measuring, for the first time, prostaglandin, thromboxane and leukotriene synthesis in biopsies of rectal mucosa from smokers and non-smokers.

Subjects and methods

There were 25 healthy adult males who were either non-smokers (n = 13, mean age 44·5 years, range 22–74) or smokers (n = 12, mean age 44·8, range 23– 67). They were friends or relatives of patients with colitis, and they volunteered for the study which had the approval of the Ethical Committee of the University Hospital of Wales. None of the subjects were taking medication. Those in the test group were accustomed to smoking 10 to 30 cigarettes daily; they smoked three cigarettes in the 2 h before biopsies were taken.

All of the volunteers appeared to have normal rectal and colonic mucosa on sigmoidoscopy performed without enema preparation. Two biopsies were taken from the anterior rectal wall 8 cm from the anal margin, washed immediately in 154 mM NaCl, dropped into liquid nitrogen, and then stored at −80°C for nine months before extraction and analysis at King's College School of Medicine and Dentistry, London. Although the period of storage was long, it is well known that cyclo-oxygenase in stored tissue retains activity over many years, and lipoxygenase activity occurs in tissues that have been stored for several months (J A Salmon, personal communication). If any enzymic deterioration had occurred during storage, we would have expected it to be similar in the test and control samples.

The samples were thawed, carefully weighed (100 ± 5 mg) and pre-incubated in 1 mL phosphate buffer pH 7·4 (PBS) at 0°C for 30 min. The pre-incubation fluid was then discarded and replaced by 1 mL of fresh fluid which, after further incubation at 37°C for 30 min, was removed and stored at −20°C until radioimmunoassay in duplicate.

The antisera, sources and % immunological specificities for radioimmunoassays were:

PGE (ICN Biomedicals Ltd); cross reactions: PGE_2 100; PGE_1 240; $PGF_{1\alpha}$ 0·35; $PGF_{2\alpha}$ 0·5; 15-keto-PGE_2 0·1; PGD_2 0.04; 6,15-diketo-$PGF_{1\alpha}$ < 0·04; 15-keto-$PGF_{1\alpha}$ < 0·04; 13, 14-dihydro-6-keto$PGF_{1\alpha}$ < 0·04; 6-keto-$PGF_{1\alpha}$ 0·6; TXB_2 < 0·04. Since the antibody does not distinguish between PGE_1 and PGE_2, the assay results are expressed as PGE. *6-Keto-$PGF_{1\alpha}$* (Wellcome); cross-reactions: 6-keto-$PGF_{1\alpha}$, 100; PGE_2, 0·01; $PGF_{2\alpha}$, 3·0; TXB_2, 0·02. *TXB_2* (Wellcome); cross-reactions: TXB_2, 100; $PGF_{2\alpha}$, 0·1; PGE_2, 0·1; 6-keto-$PGF_{1\alpha}$, 0·1.

The immunological specificities of the RIA kits used were: *$LTC_4/LTD_4/LTE_4$* (Amersham); cross-reactions: LTC_4, 100; LTD_4, 64; LTE_4, 64; LTB_4, 0·001; prostaglandins and TXB_2 < 0·001. *LTB_4* (Amersham); cross reactions: LTB_4 100; 20-OH-LTB_4 0·4; other arachidonate metabolites < 0·05.

The assays were completed without knowledge of the groups from which the biopsies were taken. Intra-assay coefficients of variation at 50% binding were (%): LTB_4 2·8, $LTC_4/D_4/E_4$ 1·9, TXB_2 3·7, 6-keto-$PGF_{1\alpha}$ 2·2, PGE 6·8. The results were analysed by the Mann–Whitney U-test (2-tailed).

Results and discussion

Synthesis of eicosanoids by the rectal mucosa varied greatly between biopsies (Fig. 1). The measured products formed (overall range pg mg^{-1}/30 min) were: PGE 16·3–175; thromboxane B_2 29·4–548; 6-keto-$PGF_{1\alpha}$ 29·4–124; $LTC_4/D_4/E_4$ 7·1–97·2; LTB_4 0·8–4·5.

The median yield of each eicosanoid was lower in smokers, compared with non-smokers, but in each case the statistical probabilities approached only the 10% level. However, the fact that the median was lower in every case, and each set of results is an independent measurement of eicosanoid synthesis, somewhat strengthens the possibility of a real difference. Since both the cyclo-oxygenase and lipoxygenase products showed a tendency for reduction, smoking might affect both these enzyme pathways and/or arachidonate release. Studies on more subjects might

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