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Stimulation of brain dopamine autoreceptors by remoxipride administration in reserpine-treated male rats

SVEN AHLENIUS, EVALENA ERICSON, AGNETA WIJKSTRÖM*, *Departments of Behavioral Pharmacology and Bioanalysis*, Astra Arcus AB, S-151 85 Södertälje, Sweden*

Abstract—Male Sprague-Dawley rats were treated subcutaneously with reserpine (5 mg kg^{-1}, −18 h) and with the aromatic amino acid decarboxylase inhibitor, NSD-1015 (3-hydroxybenzylhydrazine) (100 mg kg^{-1} −30 min). Remoxipride 0·8, 3·2 or 12·8 mg kg^{-1} was administered subcutaneously at −50 min. Immediately following decapitation (0 h), the ventral striatum and the anterior neocortex were dissected. Dopa and 5-hydroxytryptophan accumulation in these brain areas were analysed by HPLC with electrochemical detection. Reserpine produced a marked increase in striatal and neocortical dopa accumulation, in comparison with glucose vehicle + NSD-1015-treated controls, and this increase was dose-dependently antagonized by remoxipride treatment. Thus, together with demonstrated dopamine receptor antagonist actions in intact animals, remoxipride behaves as a mixed dopamine receptor agonist-antagonist. Such properties could contribute to the favourable endocrine and extrapyramidal side effect profile of remoxipride as an antipsychotic agent.

Remoxipride, [*S*(−)-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl) methyl] benzamide HCl, is a new dopamine D_2 receptor blocking agent (Florvall & Ögren 1982; Ögren et al 1984; Magnusson et al 1986) with antipsychotic properties (Chouinard 1987; McCreadie et al 1990). Well tolerated in healthy volunteers (Grind et al 1989), and with few or mild signs of extrapyramidal motor side-effects in clinical and laboratory studies (Ögren et al 1984), remoxipride shows potential as a new valuable antipsychotic agent (see Sedvall 1990).

The systemic administration of remoxipride produces increased neostriatal dopamine synthesis and turnover in the rat. Furthermore, remoxipride antagonizes dopamine-receptor-agonist-induced suppression of neostriatal dopamine synthesis in the γ-butyrolactone (GBL) model, a model where the dopamine synthesis is increased due to cessation of impulse flow and subsequent lifting of an autoreceptor-mediated tonic inhibition of dopamine synthesis and release (see Roth 1984). In both these preparations, however, the maximal effect of remoxipride appears less than that produced by haloperidol (Magnusson et al 1986, 1987, 1988). A less than maximal response is an effect expected by a partial dopamine agonist. Should this indeed be the case for remoxipride, the above observations, as well as the low propensity of remoxipride to produce extrapyramidal motor effects, could be explained by weak dopamine receptor stimulation intrinsic to the remoxipride treatment. In the present experiments, this possibility was investigated by the administration of remoxipride to rats pretreated with reserpine. The antagonism of reserpine-induced increase in brain dopamine synthesis, presumably mediated via presynaptic dopaminergic autoreceptors, has proven a sensitive index of dopamine agonist properties of dopaminergic agents (see Carlsson 1975; Roth 1984). Brain dopamine synthesis was therefore estimated by measuring the accumulation of dopa, following 3-hydroxybenzylhydrazine (NSD-1015), in reserpine-treated rats (Carlsson et al 1972).

Correspondence: S. Ahlenius, Department of Behavioral Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden.

Materials and methods

Animals. Adult male Sprague-Dawley rats, 280–320 g, were supplied by ALAB Laboratorietjänst AB (Sollentuna, Sweden), and maintained under controlled conditions of temperature (20–21°C), relative humidity (55–65%) and a 12:12 h light-dark cycle (lights off 0600 h). The animals were acclimatized for at least 10 days before being used in experiments.

Drugs. The following drugs were used: reserpine (Fluka, Buchs, Switzerland), 3-hydroxybenzylhydrazine 2HCl (NSD-1015) (Sigma, St Louis, MO), haloperidol (Janssen, Beerse, Belgium), (−)-sulpiride (ICFI, Milan, Italy) and remoxipride HCl (batch F14) (Astra, Södertälje, Sweden). Reserpine, haloperidol and sulpiride were dissolved in a minimal quantitiy of glacial acetic acid, and the final volume was made up with 5·5% glucose. The other compounds were dissolved in physiological saline. All compounds were injected subcutaneously in a volume of 2 mL kg^{-1}. Controls received the corresponding amount of the vehicle. The doses refer to the drug forms indicated above.

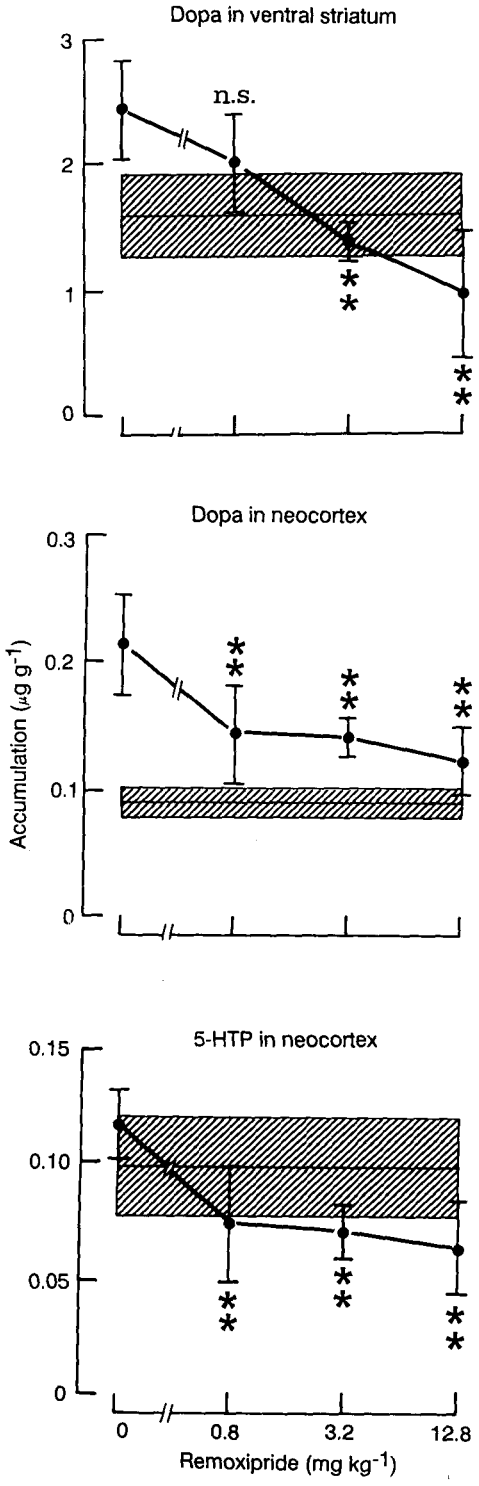

FIG. 1. Effects of remoxipride on brain dopa and 5-HTP accumulation following decarboxylase inhibition in reserpine-treated rats. The animals were treated with reserpine (or glucose vehicle) and NSD-1015. Remoxipride (0·8–12·8 mg kg^{-1} subcutaneously) or the saline vehicle, was administered at −50 min. The figure shows means ± s.d., based on 4–8 values per group. The dopa and 5-HTP accumulation in glucose-treated controls is indicated by the shaded area in the figure. Statistical evaluation was performed by means of a one-way analysis of variance, followed by Dunnett's *t*-test for comparison with reserpine-treated controls, as indicated in the figure. Dopa: $F_{4,24}=12{\cdot}79$, $P<0{\cdot}001$ (ventral striatum); $F_{4,25}=19{\cdot}25$, $P<0{\cdot}001$ (neocortex); 5-HTP: $F_{4,25}=7{\cdot}82$, $P<0{\cdot}001$ (neocortex). $^{n.s.}P>0{\cdot}05$, $^{**}P<0{\cdot}01$.

Table 1. Effects of haloperidol and (−)-sulpiride on brain dopa and 5-HTP accumulation in reserpine-treated rats.

	Dopa (μg g^{-1}) Ventral striatum	Dopa (μg g^{-1}) Neocortex	5-HTP (μg g^{-1}) Neocortex
Glucose controls[1]	1·52 ± 0·29**	0·09 ± 0·01**	0·10 ± 0·02
Haloperidol (mg kg^{-1})			
0·0	2·74 ± 0·24	0·21 ± 0·02	0·09 ± 0·02
0·1	2·44 ± 0·69^{ns}	0·19 ± 0·02^{ns}	0·09 ± 0·02^{ns}
0·6	2·60 ± 0·44^{ns}	0·23 ± 0·03^{ns}	0·08 ± 0·02^{ns}
(−)-Sulpiride (mg kg^{-1})			
0·0	2·38 ± 0·39	0·21 ± 0·04	0·12 ± 0·02
0·6	2·21 ± 0·32^{ns}	0·17 ± 0·01^{ns}	0·09 ± 0·01^{ns}
2·5	2·19 ± 0·71^{ns}	0·17 ± 0·05^{ns}	0·19 ± 0·02^{ns}
10·0	2·07 ± 0·85^{ns}	0·17 ± 0·04^{ns}	0·10 ± 0·03^{ns}

Haloperidol or (−)-sulpiride was administered subcutaneously 50 min before decapitation. The results are presented as means ± s.d., based on 4 observations per group. [1]Pooled controls from the two experiments with haloperidol and sulpiride. $^{**}P<0{\cdot}01$, (Student's *t*-test for comparisons with the respective reserpine treated controls). $^{ns}P>0{\cdot}05$ (Dunnett's *t*-test for comparisons with reserpine-treated controls).

Brain dissections. The animals were decapitated with a guillotine. 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 (0·07 mm diameter) stainless steel wire. Dissections were made from these slices, placed on an ice-chilled petri dish. The rostral edge of the slices 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 orientation of the horizontal plane in the atlas of Paxinos & Watson (1986). The ventral striatum (including the nucleus accumbens, the bed nucleus of the stria terminalis, part of the olfactory tubercle ventrally, and the diagonal band of Broca medially) and neocortex were dissected from the most rostral of these slices. The brain samples were immediately frozen on dry ice and stored at −70°C for later analysis.

Dopa and 5-HTP determinations. Dopa and 5-hydroxytryptophan (5-HTP) were extracted from the brain tissue by sonication in perchloric acid, containing internal standard (*N*-ethylnoradrenaline). The supernatants were analysed for their content of dopa and 5-HTP by coupled column liquid chromatography with electro-chemical detection. Dopa and 5-HTP levels were quantified with an intra-assay precision of 1–2%. The limit of detection was about 0·2 ng per sample (2–4 ng g^{-1}). For further details see Magnusson et al (1980).

Experimental procedures and statistics. The animals were pretreated with reserpine, 5 mg kg^{-1}, or the glucose vehicle at −18 h (in relation to decapitation). Remoxipride, haloperidol or sulpiride were administered at −50 min. All animals received the aromatic L-amino acid decarboxylase inhibitor NSD-1015 (100 mg kg^{-1}) at −30 min. For further details see the legends to Fig. 1 and Table 1. Statistical analysis was performed by means of a one-way analysis of variance, followed by Dunnett's *t*-test for individual comparisons.

Results

There was a statistically significant increase in dopa accumulation following reserpine treatment in the ventral striatum as well as in the neocortex ($P<0{\cdot}01$). This increase in dopa

accumulation was antagonized by treatment with remoxipride (0·8–12·8 mg kg^{-1}, 1·9–30·0 μmol kg^{-1}) in both brain areas (Fig. 1). In addition, remoxipride produced a statistically significant, dose-dependent, decrease in the dopa and 5-HTP accumulation in the neocortex (Fig. 1). There were no statistically significant effects on dopa or 5-HTP accumulation in any brain area in reserpine-treated animals given haloperidol, 0·1–0·6 mg kg^{-1} (0·3–1·6 μmol kg^{-1}) or (−)-sulpiride, 0·6–10·0 mg kg^{-1} (1·9–30·0 μmol kg^{-1}) (Table 1).

Discussion

Our results demonstrate that remoxipride produces the effects of an agonist at brain dopamine autoreceptors. The effects of remoxipride are in this respect similar to the effects displayed by partial dopamine agonists, such as (−)3-3(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP), 2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo[5,4-*d*] (B-HT 920) or terguride in reserpine-treated rats (Clark et al 1985). The efficacy of remoxipride as a dopamine receptor agonist, however, appears to be considerably less than for these compounds, as also evidenced by the predominant antagonist profile of remoxipride in intact animals (Magnusson et al 1986). In contrast to remoxipride, no statistically significant effects were obtained by haloperidol or (−) sulpiride in the present test model for dopamine agonist properties. As a dopamine receptor antagonist, this implies that the dopamine receptor blockade produced by remoxipride, although primarily responsible for its pharmacological effects, will be less complete than for the other two compounds, due to concomitant weak dopamine receptor stimulation. The possibility that the atypical neuroleptic profile of remoxipride may be explained by such dopamine receptor agonist properties merits further investigation.

It should also be noted, however, that in the present test model, there was evidence for both noradrenaline and 5-hydroxytryptamine (5-HT) agonist properties of remoxipride, as estimated by the dopa and the 5-HTP accumulation in the neocortex, respectively. In fact, remoxipride was more potent in this respect than with regard to stimulation of dopamine receptors. In the present dose range, however, no clear dose-related effects were obtained although the effects, particularly on 5-HTP accumulation, were prominent. It also remains to be clarified to what extent these properties contribute to the pharmacodynamic profile of remoxipride.

It is concluded that remoxipride behaves as a mixed agonist/antagonist at brain dopamine receptors. Such properties of remoxipride may explain its favourable preclinical profile as regards extrapyramidal side-effects. Furthermore, this profile could be of importance for the treatment of negative symptomatology in schizophrenia, in addition to its proven effect on positive symptoms (Lewander et al 1990).

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