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Differential Effects of Dopamine Antagonists on Evoked Dopamine Release from Slices of Striatum and Nucleus Accumbens in Rats

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Abstract

The effects of dopamine-receptor antagonists on electrically-evoked dopamine release were compared in the nucleus accumbens and striatal slices of rats.

(−)-Sulpiride induced a concentration-dependent increase in the evoked dopamine release from both regions, the increase in the nucleus accumbens being significantly greater than that in the striatum. Clozapine also increased evoked dopamine release from the nucleus accumbens, but not from the striatum. The haloperidol-induced increase in evoked dopamine release from the nucleus accumbens was less than that from the striatum.

These findings indicate that, in terms of dopamine transmission, (−)-sulpiride and clozapine, but not haloperidol, predominantly affect the nucleus accumbens rather than the striatum. We have previously reported that the contribution of D_3 receptors to the regulation of dopamine release from dopamine nerve terminals is much greater in the nucleus accumbens than that in the striatum. (−)-Sulpiride and clozapine have relatively higher affinity for D_3 receptors than does haloperidol. The regional differences in responsiveness of dopamine release to dopamine antagonists could be due to the different affinities to D_2 or D_3 receptors of the dopamine antagonists.

Dopamine-receptor antagonists block both pre- and post-synaptic dopamine receptors. As a consequence of this blockade, compensatory increases occur in the firing rates of dopaminergic neurons (Bunney & Aghajanian 1974) and in the concentrations of dopamine and its metabolites in intra-cerebral dialysates (Zetterstrom et al 1985). It has been reported that there is a regional difference between the striatum and nucleus accumbens in regard to the changes induced in dopamine metabolism by dopamine-receptor antagonists (Meltzer 1991). The atypical neuroleptics, clozapine and thioridazine, have little effect on dopamine turnover in the striatum, but increase it in the nucleus accumbens, which action may account for the low incidence of extrapyramidal side-effects with these drugs (Andén & Stock 1973; Weisel & Sedvall 1975; Zivkovic et al 1975). Moreover, haloperidol-induced increases in dopamine metabolites in the striatum were tolerated within one week of treatment with the drug, whereas it took more than five weeks to induce this tolerance in the nucleus accumbens (Tsutsumi et al 1982). However, the mechanisms underlying the regional differences in drug-induced changes in dopamine turnover remains unclear. Evoked dopamine release is mediated by dopamine autoreceptors in dopamine nerve terminals. D_3 receptors have now been cloned and the binding properties of these receptors to dopamine agonists and antagonists have been determined (Sokoloff et al 1990); the D_3 receptors seem to act as dopamine autoreceptors mainly in the nucleus accumbens and not in the striatum (Yamada et al 1994). We conducted this study to observe the effects of haloperidol, clozapine and (−)-sulpiride on electrically-evoked dopamine release from the striatum and nucleus accumbens in rats, to determine whether the blockade of D_3 receptors by these drugs contributes to the regional differences in the drug-induced changes in dopamine release.

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Materials and Methods

Male Wistar rats, 200–250 g, were housed in a light-, humidity- and temperature-controlled environment for at least eight days before the experiments were conducted. The animals were decapitated and the brains quickly removed. Coronal sections (0·3 mm thick) were made with a Micro Slicer (Dosaka E.M. Co.) at A 9726 to A 7630, according to the atlas of König & Klippel (1963), the slices were punched out in ice-cold Krebs solution with a stainless-steel tube (i.d. 2 mm). Each section was placed in a chamber made from a Teflon tube, with platinum electrodes at the top and bottom. The preparation was superfused with Krebs solution at a flow rate of 0·7 mL min^{-1} at 37°C and equilibrated with 95% O_2–5% CO_2. The composition of the Krebs solution was (mM): NaCl 118·0, $MgCl_2$ 1·18; KCl 4·9; $NaHCO_3$ 25·0; $NaHPO_4$ 1·25; $CaCl_2$ 1·25, and glucose 11·9. Nomifensine (3 μM) was added to prevent dopamine uptake into the dopamine nerve terminals. The stimuli were applied as trains (1 Hz, 2 ms, 20 mA). Following superfusion with control Krebs solution for 57 min, each slice was stimulated for 2 min (S1), and then superfused for 30 min either with control Krebs solution or with Krebs solution containing various concentrations of haloperidol, (−)-sulpiride or

clozapine. A second stimulation (S2), also 2 min in duration, was performed 21 min after the first stimulation. The superfusate was collected in tubes during the 7-min periods preceding and following each stimulation. Dopamine released into the superfusate was adsorbed on alumina, eluted with 300 μL of 0·5 M acetic acid, and quantified by high performance liquid chromatography and electrochemical detection according to the method of Kissinger et al (1972), with slight modifications. Details of the extraction procedure have been described in a previous report (Yamada et al 1988). Evoked release during the S1 or S2 period was calculated as total release minus spontaneous release. The spontaneous release during each stimulation was estimated from the sample collected during the 7-min period preceding stimulation. Statistical comparisons were formed by analysis of variance, followed by Scheffe's test.

Results

The evoked dopamine release from the striatal slices was 2·15 ± 0·16 ng (mg protein)$^{-1}$/7 min fraction (mean ± s.e.m., n = 18). This was higher than the amount released from the nucleus accumbens slices (1·07 ± 0·09 ng (mg protein)$^{-1}$/7 min fraction (n = 21). The control S2/S1 value for the striatum was similar to that of the nucleus accumbens (1·23 ± 0·08 for the striatum and 1·27 ± 0·09 for the accumbens). Fig. 1 shows the dose-response curve of the evoked dopamine release from the striatum and nucleus accumbens for haloperidol, clozapine, and (−)-sulpiride 15 min after drug superfusion. Clozapine (10 μM) induced a significant increase in evoked dopamine release from the nucleus accumbens (149 ± 15% of control S2/S1 ratio, mean ± s.e.m., n = 10), this was greater than that for the striatum (108 ± 8% of control S2/S1 ratio, mean ± s.e.m., n = 10). Low concentrations of clozapine (1 and 0·1 μM) induced a slight increase in evoked dopamine release from both regions. On the other hand, a low concentration of haloperidol (0·1 μM) increased the evoked dopamine release from both regions (143 ± 11% from striatum and 140 ± 10% from nucleus accumbens). Haloperidol (1 μM) induced a greater increase in evoked dopamine release from the striatal slices (157 ± 19%) than from the nucleus accumbens (119 ± 16%, $P < 0·05$). The (−)-sulpiride (10 μM)-induced increase in evoked dopamine release from the nucleus accumbens (189 ± 21%) was greater than from the striatum (150 ± 16%, $P < 0·05$).

The time courses of changes in evoked dopamine release induced by low concentrations of clozapine (0·1 μM) and haloperidol (0·1 μM) are shown in Table 1. Both drugs induced a time-dependent increase in evoked dopamine release from the nucleus accumbens. Clozapine (0·1 μM) induced a greater increase in evoked dopamine release from the nucleus accumbens compared with the striatum 45 min after superfusion with the drug. However, there was no difference between the haloperidol-induced increase in evoked dopamine release in the two regions throughout the experimental periods.

Discussion

Clozapine increased the evoked dopamine release from the nucleus accumbens, but not from the striatum, this being consistent with previous reports that clozapine had a greater effect on dopamine turnover in the limbic system (Andén & Stock 1973; Weisel & Sedvall 1975; Zivkovic et al 1975) and that acute administration of clozapine increased the firing

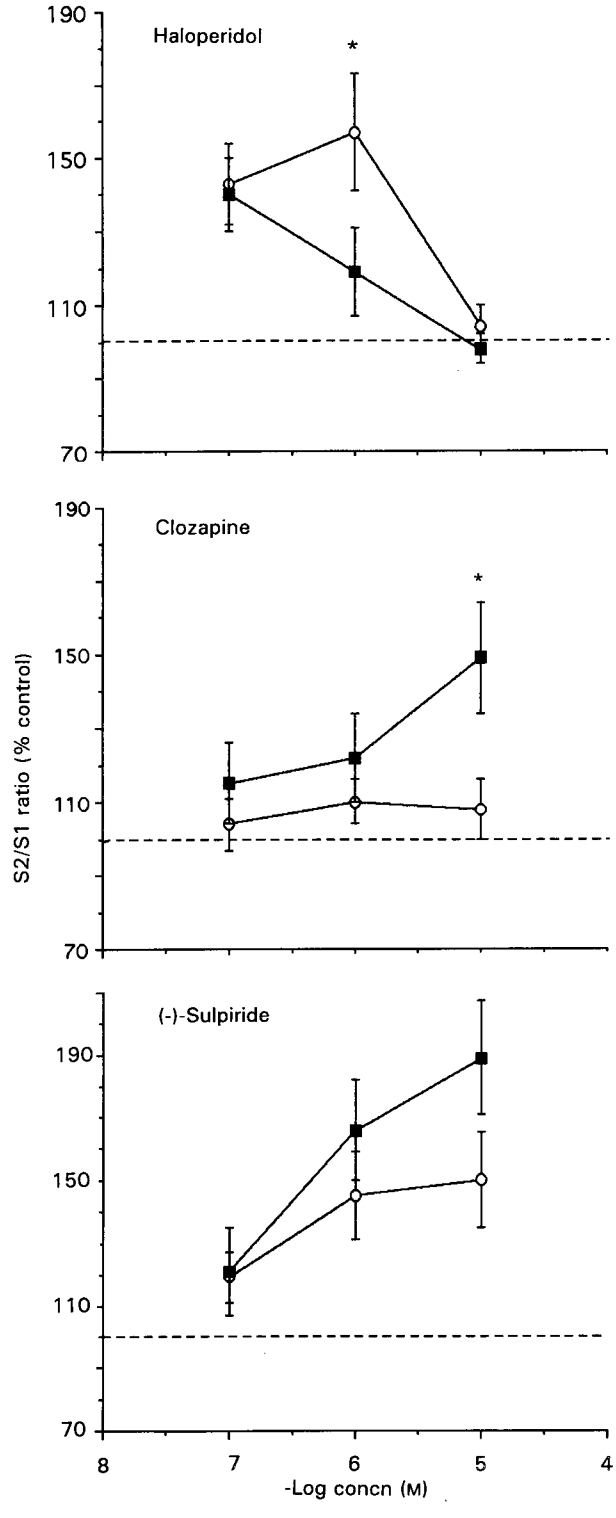

FIG. 1. Effects of haloperidol, clozapine, and (−)-sulpiride on evoked dopamine release from striatal (○) and nucleus accumbens (■) slices. Values are mean % ± s.e.m. of the control S2/S1 ratio. *$P < 0·05$, compared with the striatum (n = 8–10 at each point).

Table 1. Time-course of changes in evoked dopamine release from the striatum and nucleus accumbens in the presence of clozapine and haloperidol.

Drug	Concn (μM)	Region	Dopamine release (% of control)		
			15 min	30 min	45 min
Haloperidol	0·1	Striatum	143 ± 11**	157 ± 11**	173 ± 15**
		Nucleus accumbens	140 ± 10**	172 ± 12**	192 ± 15**
Clozapine	0·1	Striatum	102 ± 11	112 ± 13	118 ± 9
		Nucleus accumbens	115 ± 10	126 ± 11*	145 ± 22*[a]

Values are mean % ± s.e.m. of the control S2/S1 ratio. [a] $P < 0·01$ compared with striatum, **$P < 0·01$, *$P < 0·05$ compared with control S2/S1 ratio.

rate of A10, but not A9, neurons (Hand et al 1987). (−)-Sulpiride increased the evoked dopamine release from both striatal and nucleus accumbens slices. This finding is consistent with previous reports that electrically-stimulated [^{3}H]dopamine release from rabbit caudate nucleus (Starke et al 1978; Nowak et al 1983) and from rat striatal slices (Dwoskin & Zahniger 1986; Yamada et al 1993) was enhanced by (−)-sulpiride, which antagonizes dopamine agonists. The stimulating effect of (−)-sulpiride on the nucleus accumbens was greater than that on the striatum. On the other hand, haloperidol induced a greater increase in evoked dopamine release from the striatum than from the nucleus accumbens (Fig. 1). In agreement with the present results, Moghaddam & Bunney (1990) reported that haloperidol (0·1 mg kg^{-1}, i.v.) elevated extracellular dopamine levels in the striatum 20–60 min after injection, but that a significant increase in dopamine levels in the nucleus accumbens was observed only 60 min after drug injection. We found here that the stimulatory effects of (−)-sulpiride and clozapine on evoked dopamine release were greater in the nucleus accumbens than in the striatum. In contrast, the haloperidol-induced increase in evoked dopamine release from the striatum was greater than that from the nucleus accumbens. These findings indicate that, in terms of dopamine transmission, (−)-sulpiride and clozapine, but not haloperidol, predominantly affected the nucleus accumbens. However, the mechanism underlying these regional differences in the responsiveness of dopamine release to dopamine-receptor antagonists remains unclear. The order of potency for these drugs in increasing the evoked dopamine release from the striatum was haloperidol>(−)-sulpiride> clozapine; this was correlated with the order of binding affinity for D_2 receptors in the striatum and with the order of inducing extrapyramidal side-effects. As stated above, the D_3-dopamine receptor has been cloned and its pharmacological properties characterized; it appears to act as a dopamine autoreceptor, like D_2 receptors (Sokoloff et al 1990; Levesque et al 1992). We have previously reported that the D_3-selective agonists, quinpirole and 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin, predominantly inhibit evoked dopamine release from the nucleus accumbens compared with the striatum, which findings indicate that the contribution of D_3 receptors in the nucleus accumbens to the regulation of dopamine nerve terminals is much greater than that in the striatum (Yamada et al 1994). Sokoloff et al (1990) reported that the ratio of the k_i value for D_3-receptor binding affinity to that for D_2-receptor binding affinity was 0·36 for (−)-sulpiride and 0·31 for clozapine; these values were six times higher than that for haloperidol (0·05). The higher values of the ratio for (−)-sulpiride and clozapine than for haloperidol may explain the greater response induced in evoked dopamine release from the nucleus accumbens than from the striatum by the blockade of D_3 receptors. Haloperidol, which has a lower value for this ratio (0·05), may induce a greater increase in evoked dopamine release from the striatum than from the nucleus accumbens by the blockade of D_2 receptors. However, it is well known that clozapine blocks not only dopamine receptors but also histaminergic, α_1-adrenergic, 5-HT_2-ergic, and cholinergic receptors. Thus, the broad spectrum of clozapine's receptor-blocking action may account for its minor effect on evoked dopamine release from the striatum. In fact, it has been reported that co-administration of the α_1-adrenoceptor antagonist, prazosin, or an anticholinergic agent, trihexyphenydyl, with haloperidol has a similar region-specific electro-physiological effect to that of clozapine (Chiodo & Bunney 1985). However, since (−)-sulpiride is a D_2- or D_3- receptor antagonist, only its binding properties to receptors other than D_2 or D_3 being negligible, it is possible that differences in the regional distribution of D_3 receptors in dopamine terminals in the striatum and nucleus accumbens may account for the regional differences of responsiveness in evoked dopamine release to (−)-sulpiride. D_3 mRNA has been detected in the islands of Calleja, olfactory bulb, olfactory tubercles and nucleus accumbens (Levesque et al 1992); these findings indicate that postsynaptic D_3 receptors are predominantly located in limbic areas and somewhat less in the striatum. Thus, another possible explanation of the regional differences in responsiveness to D_3-dopamine antagonists is that the blocking of postsynaptic D_3 receptors by dopamine antagonists may increase evoked dopamine release via intrinsic neurons in the nucleus accumbens.

References

Andén, N.-E., Stock, G. (1973) Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. J. Pharm. Pharmacol. 25: 346–348

Bunney, B. A., Aghajanian, G. K. (1974) The effect of antipsychotic drugs on the firing of dopaminergic neurons. A reappraisal. In: Sadvall, G., Uvnas, B., Zotterman, Y. (eds) Antipsychotic Drugs: Pharmacologics and Pharmacokinetics. Pergamon, New York, pp 305–318

Chiodo, L. A., Bunney, B. S. (1985) Possible mechanisms by which repeated clozapine administration differentially affects the

activity of two subpopulations of midbrain dopamine neurons. J. Neurosci. 5: 2539–2544

Dwoskin, L., Zahniger, N. R. (1986) Robust modulation of ^{3}H-dopamine release from striatal slices by D-2 dopamine receptors. J. Pharmacol. Exp. Ther. 239: 442–446

Hand, T. H., Hu, X.-T., Wang, R. Y. (1987) Differential effects of acute clozapine and haloperidol on the activity of ventral tegmental (A10) and nigrostriatal (A9) dopamine neurons. Brain Res. 415: 257–269

Kissinger, P. T., Refshauge, C., Drering, R., Adams, R. N. (1972) An electrochemical detector for liquid chromatography with picogram sensitivity. Anal. Lett. 6: 465–477

König, J. F. R., Klippel, R. A. (1963) The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore

Levesque, D., Diaz, J., Pilon, C., Martres, M. P., Giros, B., Souil, E., Schott, D., Morgat, J. L., Schwartz, J. C., Sokoloff, P. (1992) Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-3H-hydroxy-*N*,*N*-di-n-propyl-2-aminotetralin. Proc. Natl. Acad. Sci. USA 89: 8155–8159

Meltzer, H. Y. (1991) The mechanism of action of novel antipsychotic drugs. Schizophr. Bull. 17: 263–288

Moghaddam, B., Bunney, S. (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. J. Neurochem. 54: 1755–1760

Nowak, J. Z., Arbilla, A., Galzin, A. M., Langer, S. Z. (1983) Changes in sensitivity of release modulating dopamine autoreceptors after chronic treatment with haloperidol. J. Pharmacol. Exp. Ther. 226: 558–564

Sokoloff, P., Giros, B., Martres, M. P., Bouthenet, M. L., Schwartz, J. C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347: 146–151

Starke, K., Reiman, W., Zumstein, A., Herttung, G. (1978) Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. Naunyn Schmiedebergs Arch. Pharmacol. 323: 298–306

Tsutsumi, T., Kojima, H., Anraku, S., Inanaga, K. (1982) The short- and long-term effects of haloperidol on rat central dopamine turnover. Brain Res. 232: 485–488

Weisel, F. A., Sedvall, G. (1975) Effects of antipsychotic drugs on homovanillic acid levels in striatum and olfactory tubercle of the rat. Eur. J. Pharmacol. 30: 364–367

Yamada, S., Kojima, H., Yokoo, H., Tsutsumi, T., Takamuki, K., Anraku, S., Nishi, S., Inanaga, K. (1988) Enhancement of dopamine release from striatal slices of rats that were subchronically treated with methamphetamine. Biol. Psychiatry 24: 399–408

Yamada, S., Yokoo, H., Nishi, S. (1993) Modulation of (−)-sulpiride-induced increase in electrically-evoked release of dopamine from rat striatal slices. J. Pharm. Pharmacol. 45: 479–481

Yamada, S., Yokoo, H., Nishi, S. (1994) Differential effects of dopamine agonists on evoked dopamine release from slices of striatum and nucleus accumbens in rats. Brain Res. 648: 176–179

Zetterstrom, T., Sharp, T., Ungerstedt, U. (1985) Effect of neuroleptic drugs on striatal dopamine release and metabolism in awake rat studied by intracerebral dialysis. Eur. J. Pharmacol. 106: 27–37

Zivkovic, G., Guidotti, A., Revuelta, A., Costa, E. (1975) Effect of thioridazine, clozapine and other antipsychotics in the kinetic state of tyrosine hydroxylase and on the turnover state of dopamine in striatum and nucleus accumbens. J. Pharmacol. Exp. Ther. 194: 37–46