dopaminergic cells were seen. Thus the present report does not provide biochemical support for the observation that glucose is able to decrease the functional activity of nigrostriatal dopaminergic neurons in (non-anaesthetized) rats.

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The absence of sodium ions does not explain the failure of sulpiride to inhibit, in vitro, rat striatal dopamine-sensitive adenylate cyclase

N. M. J. RUPNIAK, P. JENNER, C. D. MARSDEN*, *University Department of Neurology, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.*

Sulpiride acts as a dopamine receptor antagonist in animals and man (see Jenner & Marsden 1979 for review). This drug is thought, however, to act selectively at dopamine receptors not linked to the enzyme adenylate cyclase since it does not inhibit the dopamine stimulation of striatal adenylate cyclase activity in vitro (Trabucchi et al 1975; Elliott et al 1977; Jenner et al 1978). The classification of sulpiride as acting at adenylate cyclase independent receptors forms a substantial part of the evidence for the division of cerebral dopamine receptors into adenylate cyclase-linked and adenylate cyclase-independent systems (Kebabian & Calne 1979).

However, we have recently demonstrated that the specific binding of [^{3}H]sulpiride, but not [^{3}H]spiperone, to rat striatal membranes in vitro is critically dependent on the presence of sodium ions (Theodorou et al 1980). Thus, the specific binding of [^{3}H]sulpiride was almost completely prevented by incubation of tissue in a sodium-free buffer system, an effect reversed by the incorporation of sodium chloride (25–200 mM). One interpretation of these data is that sodium ions may be required for the association of sulpiride and other substituted benzamide drugs with the dopamine receptor. This is confirmed by the fact that displacement of [^{3}H]spiperone by substituted benzamide drugs is sodium-dependent, while that for other neuroleptics is sodium-independent (Stefanini et al 1980).

Since sodium ions are not a normal constituent of the incubation buffer employed in assays of dopamine-sensitive adenylate cyclase in brain homogenates, the failure of sulpiride to inhibit the dopamine stimulation of cyclic (c)AMP formation may reflect a failure to meet the cation requirements for sulpiride to interact with dopamine receptors linked to adenylate cyclase. We have therefore compared the effects of the isomers of sulpiride on basal and dopamine-stimulated rat striatal adenylate cyclase activity in vitro in the presence and absence of 120 mM sodium chloride.

* Correspondence.

Male Wistar rats (150 ± 10 g; Olac Southern Ltd) were killed by cervical dislocation and decapitation and the brain rapidly removed and placed on ice. Striatal tissue from 10 rats was pooled and basal and dopamine-stimulated cAMP formation assayed according to the method of Miller et al (1974). Aliquots of striatal homogenate (50 μl) were added to assay tubes containing 250 μl of buffer consisting of 80 mM Tris-maleate, 2 mM magnesium sulphate, 10 mM theophylline and 0·2 mM EGTA (brought to pH 7·4 with Trizma base; Sigma Chemical Co), or the same buffer to which was added individually or in combination: sodium chloride (10–200 mM), dopamine (1–1,000 μM) and (+)- or (−)-sulpiride (*N*-[1'-ethyl-2'-pyrrolidinylmethyl]-2-methoxysulphamoyl benzamide) (10^{-7} – 10^{-4} M as final concentrations; SESIF, France). The effects of all additions to the incubation buffer were assessed in quadruplicate on tissue from the same homogenate pool and on at least three separate occasions. The cAMP content of the incubates was determined by the saturation assay of Brown et al (1972) which was linear over the range 0·5–50 pmol cAMP.

In the standard sodium-free incubation buffer, cAMP formation was increased by incorporation of dopamine (1–1000 μM) (ED50 20 μM dopamine). A sub-maximal concentration (100 μM) was used to produce an approximate doubling of cAMP formation (Table 1). In agreement with previous findings the isomers of sulpiride (10^{-7} – 10^{-4} M) had no effect on dopamine-stimulated adenylate cyclase activity (Table 1). This contrasts with the concentration-dependent inhibition of dopamine (100 μM)-stimulated cAMP formation by *cis*-flupenthixol (IC50 $2·4 \times 10^{-8}$ M). Basal adenylate cyclase activity also was not affected by the incorporation of sulpiride (10^{-7} – 10^{-4} M) (mean (± 1 s.e.m.) basal cAMP formation was 52·5 ± 5·5 pmol/2·5 min in 2 mg tissue; in the presence of (−)-sulpiride 10^{-4} M, it was 49·0 ± 8·4 pmol/2·5 min in 2 mg tissue; in the presence of (+)-sulpiride 10^{-4} M, it was 49·5 ± 10·7 pmol/2·5 min in 2 mg tissue).

The incorporation of sodium chloride (10–200 mM) alone

Table 1. The effect of (+)- and (−)-sulpiride on dopamine (100 μM) and sodium chloride (120 mM)-stimulated striatal adenylate cyclase activity and on cAMP formation stimulated by sodium chloride (120 mM) plus dopamine (100 μM). The results are the mean (± 1 s.e.m.) of determinations carried out in quadruplicate at each drug concentration using at least three separate tissue pools. Data obtained in the presence and absence of the isomers of sulpiride was compared using a two-tailed Student's *t*-test. None of the values obtained in the presence of the isomers of sulpiride were significantly different from control values at the $P < 0.05$ level.

	pmol cAMP formed in 2·5 min per 2 mg striatal tissue		
Addition	100 μM DA	120 mM NaCl	120 mM NaCl + 100 μM DA
None	106·3 ± 12·0	87·7 ± 10·3	102·8 ± 12·3
(−)-Sulpiride			
10^{-7} M	102·4 ± 17·5	82·7 ± 11·6	114·1 ± 14·3
10^{-6} M	99·4 ± 15·5	74·9 ± 11·5	122·2 ± 23·9
10^{-5} M	98·3 ± 17·2	77·1 ± 12·0	112·6 ± 3·0
10^{-4} M	93·6 ± 15·2	86·9 ± 19·9	121 1 ± 14·0
(+)-Sulpiride			
10^{-7} M	100·8 ± 11·4	92·7 ± 23·5	153·0 ± 24·0
10^{-6} M	103·7 ± 14·1	95·5 ± 24·0	158·8 ± 36·9
10^{-5} M	125·6 ± 15·1	121·4 ± 10·8	158·2 ± 29·1
10^{-4} M	122·6 ± 13·2	98·9 ± 7·1	149·0 ± 26·8

caused a concentration-dependent increase in cAMP formation (ED50 80 mM NaCl). A physiological concentration of sodium chloride (120 mM) increased cAMP formation by approximately 60% (Table 1). The inclusion of either (−)- or (+)-sulpiride (10^{-7} – 10^{-4} M) did not inhibit sodium chloride-activated cAMP formation. Indeed, (+)-sulpiride tended to increase cAMP formation but this was not a concentration-dependent phenomenon.

Activation of adenylate cyclase by sodium chloride has previously been reported for both rat liver and fat cell preparations (Katz et al 1980a,b). The mechanism for adenylate cyclase activation by sodium chloride is not understood, but an increase in extracellular sodium might cause membrane hyperpolarization and thus mimic some post-synaptic effects of dopamine. However, the activation induced by sodium chloride appears not be be specific for either the anion or the cation (unpublished data) and may be attributable to a general phenomenon of activation by high concentrations of salts.

A combination of dopamine (100 μM) and sodium chloride (120 mM) caused no further increase in cAMP formation compared with dopamine (100 μM) or sodium chloride (120 mM) alone (Table 1). Again, the incorporation of sulpiride (10^{-7} – 10^{-4} M), whether as the active (−)- or inactive (+)-isomer, did not antagonize the stimulation of cAMP formation due to dopamine (100 μM) plus sodium chloride (120 mM) (Table 1). There was a tendency again for (+)-sulpiride to further increase cAMP formation but this was not concentration-dependent.

The failure of sulpiride to antagonize the action of dopamine in stimulating adenylate cyclase cannot be attributed to the absence of sodium ions in this system. It is more likely, therefore, that the failure of sulpiride to inhibit dopamine-sensitive adenylate cyclase reflects the inability of the drug to interact with the adenylate cyclase linked dopamine receptor. Indeed, sulpiride and some other substituted benzamide compounds are unable in the presence of sodium ions to displace [^{3}H]cis-flupenthixol from its specific binding site on rat striatal preparations, although this is closely linked to the adenylate cyclase receptor (Hyttel 1980). In addition, dopamine-stimulated cAMP formation in striatal slices is not antagonized by the isomers of sulpiride in the presence of 120 mM chloride in vitro (Spano et al 1979) and the in vivo administration of sulpiride does not result in decreased cAMP formation despite presumably optimal ionic conditions for interacting with its receptor site (Trabucchi et al 1975).

Another explanation for the failure of substituted benzamide drugs to inhibit adenylate cyclase may lie in the poor lipid membrane penetration of such molecules (Woodruff et al 1980), since newer substituted benzamide drugs have been synthesized that are claimed to be potent and selective cyclase-linked receptor antagonists (Usuda et al 1979).

In conclusion, sulpiride does not inhibit the dopamine stimulation of adenylate cyclase in the presence or absence of sodium ions, a finding consistent with the proposed specific effect of this substituted benzamide drug on adenylate cyclase independent dopamine receptors.

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