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Effect of glucose on dopamine metabolism in the rat striatum

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In a recent report Saller & Chiodo (1980) described that glucose administration suppressed the firing of central dopaminergic neurons within the zona compacta of the substantia nigra. This effect was observed even with doses of glucose sufficient to raise glucose concentrations in the blood to levels equivalent to those produced by a meal or stress. In addition, it was found that glucose prevented the increase in discharge rate of dopaminergic cells normally elicited by the antipsychotic agent haloperidol. These observations may have important implications for our understanding of the functions in which dopamine (DA) is involved as a neurotransmitter.

It is well known that pharmacological treatments which influence the firing of nigral dopaminergic neurons induce characteristic changes in DA metabolism (Roth et al 1976). We therefore studied the effect of glucose administration on the concentration of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) in the striatum of the rat.

Female Wistar rats (180 200 g) were treated with D(+)-glucose (Merck, 250 mg kg^{-1} i.v. as well as i.p.) and/or haloperidol (Serenase, Janssen, 0·2 mg kg^{-1} i.v.) or 0·9% NaCl (saline). In the first experiment rats were decapitated 12 or 20 min after administration of glucose or 15 min after saline. Brains were rapidly removed, dissected within 3 min and frozen on dry ice. DA, DOPAC and HVA were determined by a concurrent automatic (continuous flow) fluorimetric method, after manual isolation on small Sephadex G 10 columns (Westerink & Korf 1977). Table 1 shows that glucose administration did not influence the concentration of DA, DOPAC and HVA. In a second experiment rats were treated with saline, glucose, haloperidol or glucose + haloperidol and killed 20 min later by focused microwave radiation (Litton Systems 70/50; 6 s). Striatal concentrations of 3-MT were determined by h.p.l.c. with electrochemical detection (Westerink & Spaan 1981). Glucose administration did not influence the concentrations of DA, DOPAC, HVA and 3-MT, nor could it prevent the increase in DOPAC, HVA and 3-MT which was observed after haloperidol treatment (Table 1).

Saller & Chiodo (1980) have shown that glucose (250 mg kg^{-1} i.v. or s.c.) inhibited the activity of all dopaminergic neurons sampled. The cell discharge rate ceased completely 3 to 7 min after injection and last for at least 30 min. This inhibition could not be reversed by additional administration of haloperidol (0·2 to 0·5 mg kg^{-1}. i.v.). Such pronounced changes in firing of dopaminergic neurons would have been expected to influence the concentration of DA or its metabolities. Treatments such as with apomorphine, amphetamine, γ-hydroxybutyric acid or mechanical lesioning of the nigro-striatal pathway are known to decrease, within minutes, both the activity of dopaminergic neurons as well as the levels of DOPAC in the striatum (Roth et al 1976). γ-Hydroxybutyric acid or a lesion of the nigro-striatal pathway causes a characteristic increase in DA concentrations within 20 min (Gianutsos et al 1976). In addition 3-MT concentrations appear to be a sensitive index of the decreased activity of nigro-striatal dopaminergic neurons (Di Giulio et al 1978; Westerink, unpublished).

Although the dose of glucose used in the present study was twice as high as the highest dose used by Saller & Chiodo, no effects characteristic for a decreased activity of

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Table 1. Effects of glucose and haloperidol and haloperidol + glucose on DA, DOPAC, HVA and 3-MT concentrations (% of controls ± s.e.m.) in the striatum of the rat.[a] For each experimental group 5 rates were used.

Drug	Dose (mg kg^{-1})	Killed after injection (min)	DA	DOPAC	HVA	3-MT
Experiment 1						
Glucose	250 i.v. + 250 i.p.	12	123 ± 11	104 ± 5	89 ± 5	N.D.[b]
Glucose	250 i.v. + 250 i.p.	20	114 ± 6	111 ± 13	97 ± 4	N.D.[b]
Experiment 2						
Glucose	250 i.v. + 250 i.p.	20	99 ± 8	99 ± 17	86 ± 6	107 ± 13
Haloperidol	0·2 i.v.	20	85 ± 6	337 ± 55[c]	194 ± 18[c]	177 ± 13[c]
Haloperidol + Glucose	0·2i.v. 250i.v. + 250i.p.	20	93 ± 11	466 ± 75[c]	218 ± 42[c]	160 ± 5[c]

[a]: Control values (μg g^{-1} ± s.e.m.) were, experiment 1, DA: 7·59 ± 0·44; DOPAC: 1·28 ± 0·06; HVA: 0·99 ± 0·08, and for experiment 2: DA: 6·29 ± 0·56; DOPAC: 0·79 ± 0·17; HVA: 0·50 ± 0·07; 3-MT: 0·022 ± 0·002. These values were not corrected for the recoveries, which varied between 85 and 95%. [b]: N.D. = not determined. [c]: significantly increased over controls, $P<0·01$ (Student's *t*-test).

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

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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.

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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