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Rat brain monoamine oxidase activity is not affected by repeated administration of haloperidol

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Compared with a single injection, repeated administration of neuroleptic drugs induces tolerance to the increase in the striatal levels of the dopamine (DA) metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Scatton 1977). This phenomenon is generally ascribed to development of supersensitivity of striatal DA receptors following repeated blockade of these receptors by the neuroleptics. In conformity with this idea increased binding of DA receptor ligands has been observed under these conditions (for review see Muller & Seeman 1978).

Recently Leelavathi & Smith (1980) reported that after prolonged administration of haloperidol and fluphenazine the activity of monoamine oxidase (MAO) in rat cortex and hippocampus is substantially decreased. If the same should also hold for striatal tissue, this MAO inhibitory effect might be, at least partly, responsible for the decreased rise in DA metabolites found after repeated administration of neuroleptics, making metabolite levels a less suitable parameter for such studies.

However, Leelavathi & Smith (1980) did not measure MAO activity in striatum (or in other DA-rich brain areas). In addition, in contrast with what might be expected on account of their findings, data of Schwartz et al (1974) and of Mann et al (1980) suggest that neuroleptics do not affect MAO activity in human brain. Therefore we began investigations into the effect of repeated administration of haloperidol on rat striatal MAO activity.

Materials and methods

Male Wistar rats were used. For the chronic haloperidol experiments rats (ca 175 g) were injected i.p. daily for 3 weeks (exp. I) or 4 weeks (exp. II) with 5 mg kg^{-1} (exp. I) or 2 mg kg^{-1} (exp. II) haloperidol or with the vehicle. Seven days (exp. I) or 9 days (exp. II) after the last injection the animals were killed. In another experiment rats (ca 235 g) received a single injection of 2 mg kg^{-1} haloperidol and were killed 30 min later.

Rats were decapitated, brains were removed and cerebral cortex and/or striata were dissected on an ice-cooled plate. Tissues were homogenized using a Potter-Elvehjem homogenizer, the cortex immediately in 19 volumes of ice-cold 0·32 M sucrose, striata, after having been kept frozen in liquid nitrogen, in 2 ml ice-cold water. From the cortical homogenates a crude mitochondrial fraction was isolated by differential centrifugation essentially as described by De Robertis et al (1962). The preparations were frozen at −20 °C until used for assay.

MAO activity was determined using kynuramine as a substrate by a modification of the method of Kraml (1965). The assay medium (final volume 1·5 ml) contained in addition to the brain tissue preparation 0·026 mM kynuramine, 1 mM EDTA and 33 mM sodium borate-HCl buffer pH 8·2. For measuring the in vitro effect of haloperidol various concentrations of the neuroleptic were added to the incubation medium. After incubation at 37 °C for 30 min, 0·5 ml of 4 M NaOH was added and the amount of 4-hydroxyquinoline formed was measured fluorimetrically. Protein was quantified by the method of Lowry et al (1951), using bovine serum albumin as a standard.

Binding of [^{3}H]spiperone to striatal membranes was measured by a modification of the method of Burt et al (1976). Striatal homogenates were mixed with an equal volume of ice-cold 100 mM Tris-HCl buffer pH 7·7 (20 °C) and centrifuged three times for 10 min at 50 000 *g* and at 4 °C, with rehomogenization of the intermediate pellets in cold 50 mM Tris-HCl buffer pH 7·7. The final pellet was homogenized in 100 volumes 50 mM Tris-HCl buffer, containing (mM) NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, 0·1% ascorbic acid and 5 μM pargyline. After preincubation of this membranal preparation at 37 °C for 10 min, 500 μl of it was put in incubation tubes, followed by 50 μl haloperidol in 0·1% ascorbic acid (final haloperidol concentration 0·1 μM, for measuring non-specific binding) or 0·1% ascorbic acid (for measuring total binding), and 50 μl [^{3}H]spiperone in 0·1% ascorbic acid (final concentration ranging from 0·1 to 1·5 nM). Tubes were incubated at 37 °C for 15 min, after which the contents were rapidly filtered under vacuum through Whatman GF/B filters, with two 5 ml rinses of cold 50 mM Tris-HCl buffer pH 7·7. The filters were counted after addition of 5 ml Emulsifier 299. Sources of chemicals: [Benzene ring-^{3}H]spiperone (spec. act. 35·9 Ci $mmol^{-1}$): New England Nuclear; haloperidol: Janssen Pharmaceutica; Emulsifier 299: Packard; kynuramine-diHBr: Sigma; 4-hydroxyquinoline.$3H_2O$: Fluka. Haloperidol was dissolved in an equimolar amount of 0·01 M HCl, brought to volume with 0·9% NaCl (saline) and adjusted to pH 5·5 with NaOH. This solution (and a similarly prepared vehicle) was used both in the in vivo and in vitro experiments.

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Results and discussion

In the first chronic experiment the rats were given a rather high dose of 5 mg kg^{-1} of haloperidol daily for 3 weeks. Seven days after the drug was stopped MAO activity towards kynuramine as substrate was measured in striatal homogenates. No change in the activity of the enzyme was found, compared with animals repeatedly injected with the vehicle (Table 1, exp. I).

In the second chronic experiment the haloperidol pretreatment schedule was brought more in line with that used by Leelavathi & Smith (1980), the haloperidol dose being 2 mg instead of 5 mg kg^{-1}, the pretreatment period 4 instead of 3 weeks, and the drug-free period 9 instead of 7 days. To check whether the absence of an effect of haloperidol on MAO activity was related to the brain region studied, the cortical MAO activity, as well as the striatal activity, was measured. As to the cortex, the enzyme activity was determined in the crude mitochondrial fraction according to Leelavathi & Smith (1980), although they also observed the effect of neuroleptics when using homogenates (of hippocampus). In the crude mitochondrial fraction of cerebral cortex about 65% of total MAO activity is localized (results not shown), but since less than half of total protein is present in this fraction, the MAO activity expressed per mg protein is much higher than in homogenates (Table 1, exp. II). Again striatal MAO activity was found not to be affected by previous repeated haloperidol treatment, but in our hands neither was MAO activity of cortical mitochondrial preparations.

The effectiveness of the repeated haloperidol treatment was verified by determining the binding of a dopaminergic radioligand to striatal membranes. Membranal preparations prepared from striatal homogenates which showed no change in MAO acitivity did show a significant increase in maximal specific [^{3}H]spiperone binding by about 27% (Table 1). The dissociation constant K_d did not change. Similar results have been reported before (Muller & Seeman 1978).

Some neuroleptics, viz. the tricyclic compounds chlorpromazine and chlorprothixene, have been reported to inhibit brain MAO activity in vitro (Roth & Gillis 1975). Whether this was the case for haloperidol too was also examined. In vitro inhibition of striatal MAO activity by this neuroleptic was found, but a statistical significant effect was reached only at high concentrations (activities in nmol mg^{-1} protein h^{-1} ± s.e.m. (n = 3) and P values by Student's t-test: controls 73·0 ± 1·6; 3×10^{-5} M haloperidol 59·0 ± 1·8, $P < 0{\cdot}01$; 10^{-4} M haloperidol 44·9 ± 1·7, $P < 0{\cdot}001$), so extrapolation to the in vivo situation is of doubtful value. For completion of data inhibition of striatal MAO activity was sought shortly after injection of haloperidol. The enzyme was assayed in striatal homogenates of rats killed 30 min after administration of 2 mg kg^{-1} haloperidol, but no effect of the treatment was observed (vehicle injected animals: 71·7 ± 1·5, haloperidol injected animals: 73·4 ± 1·5 nmol mg^{-1} protein h^{-1}, n = 12).

The reason why the results of Leelavathi & Smith (1980) could not be confirmed in the present experiments is not clear. There are some methodological differences between their experiments and ours. Leelavathi & Smith used two other substrates for measuring MAO activity, viz. 5-hydroxytryptamine and phenethylamine, preferred substrates for MAO subtypes A and B respectively. Kynuramine, the substrate we used, most probably is converted by both types of MAO (Fowler et al 1981), and since Leelavathi & Smith found a decrease in MAO activity measured with both their substrates, this cannot explain the discrepancy in results. In addition, in contrast to the absence of an effect of in vivo haloperidol administration, an in vitro effect of the neuroleptic on MAO activity could be measured by the kynuramine method. We therefore did not deem it necessary to study more specific MAO substrates. Another difference is the pretreatment history of the rats. Before the 4-week haloperidol treatment (identical to the procedure used in our second experiment), Leelavathi & Smith had used the rats for behavioural experiments, for which they had already been injected with haloperidol for two months, followed by various injections with apomorphine during 8 weeks. Whether this 'additional' treatment (and the greater age of the rats) has anything to do with the MAO inhibitory effect reported is not known. Lastly, the different strains of rats used (Leelavathi & Smith used Fisher 344 rats, we used Wistar rats) should not be overlooked.

In conclusion, our data indicate that daily administration of haloperidol during 3 to 4 weeks followed by a drug-free period of about a week, a treatment which induces the well-known increase in neuroleptic receptor binding, does not cause inhibition of MAO activity in striatum, nor in cerebral cortex. Our results in the cortex disagree with those of Leelavathi & Smith (1980), but they are in line with studies of Schwartz et al (1974) and of Mann et al (1980), who found that MAO activity in human brain is not influenced by their neuroleptic drug history.

Table 1. Effects of repeated administration of haloperidol on MAO activity (towards kynuramine as substrate) of rat striatal homogenates and cortical mitochondrial preparations and on [^{3}H]spiperone binding to rat striatal membranes. Haloperidol administration schedules: exp. I: 5 mg kg^{-1} i.p. daily for 3 weeks, drug-free period 7 days; exp. II: 2 mg kg^{-1} i.p. daily for 4 weeks, drug-free period 9 days.

A. MAO activity (nmol mg^{-1} protein h^{-1} ± s.e.m.(n))

		Controls	Haloperidol
Exp. I	Striatal homogenates	71·7 ± 0·8 (38)	71·6 ± 0·9 (38)
Exp. II	Striatal homogenates	66·4 ± 0·9 (11)	68·5 ± 0·9 (11)
	Cortical mitochondrial preparations	115·9 ± 1·7 (11)	114·8 ± 1·3 (11)

B. Specific [^{3}H]spiperone binding to striatal membranes prepared from striatal homogenates of exp. II

Kd (nM ± s.e.m.(n))	0·104 ± 0·010 (3)	0·106 ± 0·008 (3)
B_{max} (pmol g^{-1} ± s.e.m.)	21·4 ± 0·8 (3)	27·2 ± 0·4 (3)*

* Significantly greater than controls ($P < 0{\cdot}01$ by Student's t-test).

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The differential effects of amphetamine and methylphenidate on the biosynthesis of [^{3}H]dopa from [^{3}H]tyrosine in mouse striata in vivo

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Recently, we found that EGTA, which decreases the stimulation of dopamine (DA) synthesis produced by amphetamine, inhibited the circling behaviour elicited by amphetamine but not that by methylphenidate in mice lesioned unilaterally in one striatum with 6-hydroxydopamine (Fung & Uretsky 1980a, b, c). While amphetamine stimulates DA synthesis in striatal slices and synaptosome preparations (Harris et al 1975; Kuczenski 1975; Patrick et al 1975; Uretsky & Snodgrass 1977; Fung & Uretsky 1980c), and in vivo (Costa et al 1972; Kuczenski 1978), the effects of methylphenidate on DA biosynthesis remain unclear. Kuczenski & Segal (1975) reported that methylphenidate increased tyrosine hydroxylase activity in a synaptosome preparation, while Snodgrass & Uretsky (1979) could not demonstrate a stimulatory role for this compound on DA formation in striatal slices. As the in vitro condition may not accurately reflect the biochemical effects of methylphenidate in vivo, and since in vivo effects have not received attention, we set out to clarify the role of methylphenidate on the striatal dopaminergic system in vivo by examining the effects of amphetamine and methylphenidate on the biosynthesis of [^{3}H]dopa from [^{3}H]tyrosine in mice pretreated with a dopa decarboxylase inhibitor, seryltrihydroxybenzylhydrazine (RO4-4062), using a dose and time in which we found both drugs to induce marked circling behaviour (Fung & Uretsky 1980b).

Material and methods

Male Swiss-Webster mice (Laboratory Supply), 23–29 g, were anaesthetized with chloral hydrate (430 mg kg^{-1} i.p.) and the skull exposed by a longitudinal incision. A hole was made on the right side (1·2 mm lateral to the bregma) for the intraventricular administration of [^{3}H]tyrosine. On the day of the experiment, mice received either 0·9% NaCl (saline), amphetamine (4 mg kg^{-1} i.p.) or methylphenidate (20 or 50 mg kg^{-1} i.p.). Ten min later RO4-4602 (800 mg kg^{-1} i.p.) was given. After another 15 min, the mice under halothane anaesthesia were given [^{3}H]tyrosine (5 μl, 12·5 μCi) intraventricularly, using a 10 μl Hamilton syringe fitted with a polyethylene cuff that allowed the distal 2·5 mm of the needle to be exposed. The injection was made over 20 s, and the needle held in place for an additional 10–15 s. The incision was then closed with a wound clip. Animals recovered from the halothane anaesthesia in 2–3 min, and 10 min after the injection they were killed and the levels of [^{3}H]dopa, [^{3}H]tyrosine and endogenous tyrosine in the striata analysed. The injection site was verified by examining the stain produced by a 10% methylene blue solution given intraventricularly. Drugs were dissolved in saline and administered intraperitoneally in a 0·1 ml/10 g weight. [^{3}H]Dopa was assayed according to Fung & Uretsky (1980c). [^{3}H]Tyrosine and endogenous tyrosine in the effluent and 1 ml water wash were collected from the alumina columns used for the dopa assay. The pH was adjusted to 2 with 1 M HCl, and the samples then applied to Dowex 50 × 4 columns. Tyrosine was eluted with 4 ml of 1 M NH_4OH, the eluate dried under vacuum, and the residue dissolved in 1·02 ml 5% trichloroacetic acid. An aliquot (20 μl) was taken for the determination of [^{3}H]tyrosine by liquid scintillation counting (Fung & Uretsky 1980c). The remaining solution was used for the spectrofluorimetric assay of endogenous tyrosine (Udenfriend 1962). The rate of formation of [^{3}H]dopa was expressed as a conversion index, i.e. the amount of [^{3}H]dopa formed divided by the specific activity of tyrosine in the tissue.

Results and discussion

[^{3}H]Dopa accumulation was used to measure DA biosynthesis instead of [^{3}H]DA, since [^{3}H]DA is rapidly released

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