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Lack of effect on glutamate dehydrogenase activity after *in vivo* administration of pharmacological doses of haloperidol

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Certain drugs which affect behaviour have been proven *in vitro* to be inhibitors of glutamate dehydrogenase (EC 1.4.1.2, GDH). Of them, haloperidol was the most potent [1]. The inhibition seems to be related to a conformational change of the enzyme structure [1]. Brain and liver enzymes are both affected by the drug with small differences [2]. Chee *et al.*, working with the purified enzyme [2] showed that the brain enzyme is more sensitive than the liver enzyme to low levels of haloperidol, which might be of pharmacological significance. Another report [3], however, mentions that the association of brain glutamate dehydrogenase with another functionally related mitochondrial enzyme, L-aspartate:2-oxoglutarate aminotransferase, prevents the inhibition of haloperidol; the mechanism involved in the *in vivo* effect would depend upon the mitochondrial content of these two enzymes.

Our studies were conducted to explore the *in vivo* effect on GDH activity in rats during acute and chronic treatment with pharmacological doses of haloperidol.

Materials and methods

In vivo *studies.* Sprague–Dawley rats weighing 300–400 g were used in these studies. Haloperidol was Haldol (Janssen Pharmaceutica, Beerse, Belgium). The working solution was prepared by dissolving the drug with sterile distilled water. The drug was administered i.p. on a milligram per kilogram base. For the acute treatment, a dose of 1 mg/kg was used as a single injection, and the animal was killed 1 or 2 hr after the administration of the drug. In the chronic experiments, haloperidol was administered daily at a dose of 0.5 mg/kg for 4 days and the animals were killed on day 5. Chronic treatment was not extended, to avoid the reported tolerance phenomenon to the effects of haloperidol [4, 5]. Control animals received only distilled water injections.

After decapitation, brains and livers were rapidly removed; brains were dissected into cortex and striatum according to Glowinski and Iversen [6], and the left and right portions from a single brain were combined. Tissue samples were homogenized in 10 vol. of 0.32 M sucrose, 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, and the crude mitochondrial fraction was isolated after centrifugation of the homogenate first at 1,100 *g* for 5 min and finally at 17,000 *g* for 10 min. The final precipitate was resuspended in 5 vol. of the homogenization buffer and treated with Triton X-100 (final concentration 0.5%).

Glutamate dehydrogenase was assayed spectrophotometrically [7] by measuring the changes in optical density of the pyridine nucleotide at 340 nm and 25° with a Gilford model 2400-2 spectrophotometer.

Protein concentration was determined by the method of Lowry *et al.* [8].

In vitro *studies.* Crude mitochondrial fractions isolated from control animals were assayed in the presence of 20 μM haloperidol, using the standard assay mixture, and with either NADH or NADPH as cofactor at the same final concentration of 0.11 mM.

Statistical treatment of the data was performed by using Student's *t*-test, results were considered significant if $P < 0.05$.

Results

In vivo *studies.* Table 1 summarizes the activities of glutamate dehydrogenase in control and haloperidol-treated animals. GDH activities in control animals in both brain regions were similar ($P > 0.05$), and in liver its activity was found to be almost five times higher ($P < 0.001$). Haloperidol produced no inhibition of brain or liver mitochondrial glutamate dehydrogenase during acute or chronic treatments.

Figure 1 shows the substrate saturation curves for the brain enzyme, performed with the fractions obtained from control and treated animals after 1 hr of a single injection of 1 mg/kg of haloperidol. No inhibition was observed even

Table 1. Effect of the *in vivo* administration of haloperidol upon the activity of glutamate dehydrogenase *

	Glutamate dehydrogenase activity [nmoles·min^{-1}·(mg protein)$^{-1}$]		
	Brain		
	Cortex	Striatum	Liver
Control	58.41 ± 7.88	41.2 ± 6.21	273 ± 60.6
Acute effect			
Haloperidol, 1 hr	60.6 ± 6.44 NS†	38.8 ± 6.20 NS	215 ± 51.5 NS
Haloperidol, 2 hr	60.5 ± 8.04 NS	38.6 ± 5.6 NS	310 ± 97.9 NS
Chronic effect			
Haloperidol, 4 days	61.21 ± 9.09 NS	42.58 ± 6.57 NS	243 ± 64.1 NS

* During the acute treatment each rat received a single i.p. injection of haloperidol at a dose of 1 mg/kg and was killed 1–2 hr after injection. In the chronic studies the animals received a daily i.p. injection of 0.5 mg/kg for 4 days and were killed on day 5. Each result is the mean ± S.E. of seven to nine experiments.

† Statistical analysis revealed non-significant differences between any treatment and its own control group.

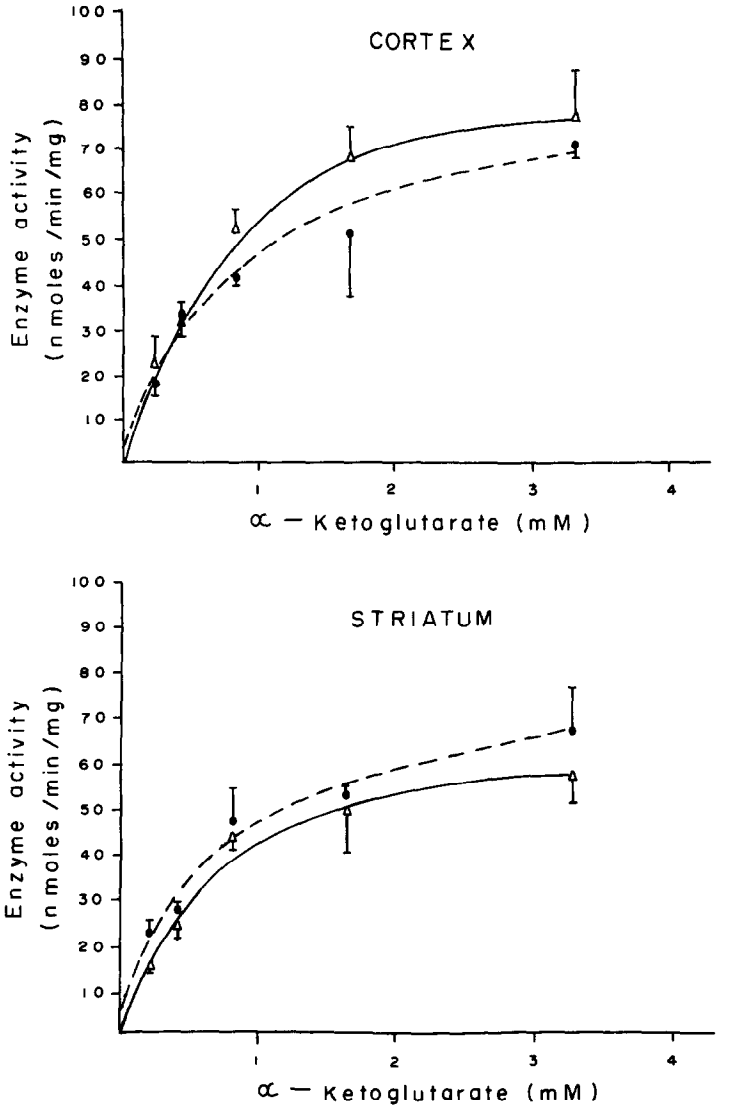

Fig. 1. Substrate saturation curve for rat brain glutamate dehydrogenase in control (△——△) and 1 hr after i.p. administration of a dose of 1 mg/kg of haloperidol (●---●). Each point is the mean ±S.E. of at least three independent determinations done in triplicate.

at low levels of the substrate, alpha-ketoglutarate ($P > 0.05$ at any level of substrate). In these studies, the standard assay with NADH as cofactor was used.

In vitro *studies.* Brain mitochondrial fractions obtained from control animals were tested for an *in vitro* inhibition by haloperidol. The drug was added to the standard assay mixture, and the reaction was followed for at least 6 min; NADH or NADPH was used as cofactor.

Table 2 confirms that haloperidol produces *in vitro* a strong inhibition of GDH activity. This inhibition was higher (60%) ($P < 0.0001$) when NADH was used as cofactor. Using NADPH, the inhibition was 35% ($P < 0.03$). This effect was observed equally in both regions tested. It is noteworthy that the enzyme reaction in the presence of NADH was 1.5 to 1.9 times ($P < 0.01$) more active than with NADPH, in the cortex as well as in the striatum.

Discussion

In this study we have confirmed the potent *in vitro* effect of low levels of haloperidol on glutamate dehydrogenase, using crude brain mitochondrial fractions, described previously by other authors for the purified enzyme [2]. Both regions studied (cortex and striatum) behave similarly, although Laduron *et al.* [4] have suggested that the frontal cortex may be a more specific target for haloperidol than the striatal system, at least when homovanillic acid (HVA) increase is being followed.

In addition, our results show that the brain enzyme was more active with NADH as cofactor and that a more pronounced inhibition by haloperidol was observed when this coenzyme was used.

No effect was observed, however, in either of the two regions after the *in vivo* administration of pharmacological doses of the drug. The doses used have been shown by other authors [9] to be effective in increasing the levels of HVA and dihydroxyphenylacetic acid (DOPAC). Acute or chronic treatments were equally ineffective.

Collins [10] has observed a small but significant fall in brain glutamate levels 1 hr after administration of 1 mg/kg of haloperidol. This author, however, was not able to measure GDH activity in brain extracts, not giving results on this matter. He only reported the *in vitro* effect of haloperidol on commercially purified GDH; using very

Table 2. *In vitro* effect of haloperidol on brain glutamate dehydrogenase*

	Enzyme activity [nmoles·min^{-1}·(mg protein)$^{-1}$]	
	Cortex	Striatum
NADPH	50.50 ± 6.30	36.48 ± 3.34†
NADPH + haloperidol	35.56 ± 2.79‡	21.87 ± 4.47‡
NADH	82.2 ± 9.50§	67.2 ± 7.0†,§
NADH + haloperidol	42.3 ± 3.13‖	23.68 ± 6.5‖

* All results are expressed in terms of means ± S.E. of five to seven independent determinations performed in triplicate. The final concentration of the drug was 20 μM and that of the cofactor was 0.11 mM.

† NS vs cortex values.

‡ $P < 0.03$ vs values in the absence of the drug.

§ $P < 0.01$ vs values obtained with NADPH as coenzyme.

‖ $P < 0.0001$ vs values in the absence of the drug.

high concentrations of haloperidol (100 μM), he obtained a 40% inhibition.

The lack of an inhibitory effect *in vivo* might have been due to the association of glutamate dehydrogenase with other mitochondrial enzymes. It is known that the binding of haloperidol to the purified enzyme produces a conformational change responsible for the diminished activity [2]. Formation of an enzyme–enzyme complex between L-aspartate 2-oxoglutarate aminotransferase and GDH prevents this inhibition [3]. Probably the GDH, as a complex, cannot undergo conformational changes, with resulting inhibition of the free enzyme. Shemisa and Fahien [3] postulated that the mechanism *in vivo* would depend upon the mitochondrial content of these two enzymes, which are located in the same region in the mitochondria.

Our preparation of the crude mitochondrial fraction demonstrated that the same potent inhibitory effect was produced by incubation *in vitro* with the drug as was described for the purified enzyme. If we assume that the proportion of the enzyme–enzyme complex was not altered by the preparative procedure of isolating the mitochondria, we cannot explain why this inhibitory effect occurred. It has to be taken into consideration, however, that in order to assay GDH activity the preparation was treated with detergent which probably provoked disruption of the mitochondrial architecture and released GDH from the complex, allowing the drug to produce a conformational change.

Preliminary experiments conducted in our laboratory, based on the work of Campbell *et al.* [11], have revealed that, under the experimental conditions reported in the present paper, the concentration of the drug inside the mitochondrial compartment, although similar to the level found in the postmitochondrial fraction, is probably too low (less than 1 μM in the mitochondrial preparation) to produce a significant inhibition of the enzyme.

Campbell *et al.* [11] have shown an almost linear increase in the concentration of haloperidol per gram of brain using doses from 1 to 10 mg/kg. Therefore, in order to obtain an inhibitory effect it would be necessary to increase the dose of haloperidol quite out of the physiological range.

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