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SUSCEPTIBILITY OF CHICK BRAIN L-GLUTAMIC ACID DECARBOXYLASE AND OTHER NEUROTRANSMITTER ENZYMES TO HYPERBARIC OXYGEN *IN VITRO**

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Abstract—Homogenates from chick whole brain were exposed to oxygen at 30 psi gauge pressure for 20 min at 25°. Controls were similarly incubated, but under a continuous flow of nitrogen at ambient pressure. The activities of the following neurotransmitter enzymes were then assayed in the homogenates: L-glutamic acid decarboxylase (GAD), 4-aminobutyrate-α-oxoglutarate aminotransferase (GABA-T), choline acetyltransferase (ChAc), acetylcholinesterase (AChE), tyrosine hydroxylase, dihydroxyphenylalanine decarboxylase (dopa decarboxylase), tryptophan hydroxylase, 5-hydroxytryptophan decarboxylase (5-HTP decarboxylase), monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT). The activity of each of the biosynthetic enzymes, except that of the tyrosine and tryptophan hydroxylases, was inhibited by the high pressure oxygen (OHP), with that of GAD displaying the largest percentage decrease (GAD, 51 per cent; Dopa decarboxylase, 12 per cent; 5-HTP decarboxylase, 10 per cent; ChAc, 7 per cent inhibition). The four degradative enzymes were not inhibited by OHP. In a second experiment, homogenates were exposed to oxygen at 90 psi gauge pressure. Again GAD showed the greatest decrease in activity (GAD, 66 per cent; Dopa decarboxylase, 18 per cent; 5-HTP decarboxylase, 24 per cent; and ChAc, 12 per cent inhibition). The relatively high sensitivity of GAD to inhibition by OHP was in harmony with the hypothesis that a deranged γ-aminobutyric acid metabolism plays a major role in the etiology of OHP-induced seizures.

FOR ALMOST a century it has been known that animals exhibit clonic–tonic convulsions when exposed to oxygen at high pressure (OHP).[1] Only comparatively recently, however, has it been shown that a concomitant effect is produced by OHP on the metabolism of certain neurotransmitters.[2–5] The γ-aminobutyric acid (GABA) system appears to be the most strongly implicated in OHP-induced seizures, since Wood *et al.*[6] have shown that an extensive correlation exists between reduction in brain GABA levels and time to onset of convulsions. Previous studies[7,8] have indicated that the major cause of lowered brain GABA levels during exposure to OHP is an inhibition of the activity of L-glutamic acid decarboxylase (GAD; EC 4.1.1.15), the enzyme directly responsible for GABA synthesis.

It is known that many enzymes are inhibited by OHP, and that these OHP-sensitive enzymes possess essential sulphydryl groups.[9] Since many enzymes contain sulphydryl groups necessary for their activity, including several neurotransmitter enzymes,[10,11] it is to be expected that enzymes other than GAD that are directly involved in neurotransmitter metabolism will be inhibited by hyperbaric oxygen. However, if low GABA

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levels are the prime cause of seizures induced by OHP, it might be expected that GAD activity is somehow selectively inhibited. This selectivity could simply be due to GAD being extremely sensitive to inhibition by OHP.

The present investigation tested this supposition by comparing the relative susceptibilities of the activity of several chick brain enzymes to OHP *in vitro*. These enzymes are all directly involved in the metabolism of putative neurotransmitters, namely GABA, acetylcholine, dopamine, noradrenaline and 5-hydroxytryptamine. We now show that, of the ten enzymes studied, the activity of GAD is the most sensitive to inhibition by oxygen.

MATERIALS AND METHODS

Animals. Day-old White Leghorn cockerels were obtained from a local commercial hatchery and maintained on chick starter ration until required for experimental purposes. For this study five 21-day-old chicks were used for each enzyme assay.

Exposure of homogenates to OHP. The brain was quickly removed from the decapitated head of each bird and homogenized in 10 vol. of ice-cold 0·25% Triton-X 100. Two 2-ml portions of homogenate were transferred to 50-ml beakers and exposed to oxygen at a pressure of 30 psi gauge pressure (or 90 psi gauge pressure) for 20 min at 25° with continuous shaking. Two other 2-ml portions were used as controls by incubating for 20 min at 25° under a flow of nitrogen at ambient pressure. Enzyme assays (in duplicate) were then performed immediately on the homogenates.

Enzyme assays. L-Glutamic acid decarboxylase activity was measured using the procedure of Roberts and Simonsen[12] except that no mercaptoethanol was included in the reaction mixture which consisted of 0·4 ml homogenate and 0·4 ml buffer–substrate (pH 6·5) containing 250 mM glutamic acid and 350 μM pyridoxal-5′-phosphate.

4-Aminobutyrate-α-oxoglutarate aminotransferase (GABA-T; EC 2.6.1.19) activity was determined by the method of Salvador and Albers,[13] as modified by Salganicoff and De Robertis.[14] A 0·2-ml portion of homogenate was added to 5 ml of a buffer solution, pH 8·4, containing 0·1 M α-oxoglutarate and 0·25 M GABA. Samples (0·5 ml) were removed from the incubation mixture at zero time and at 60 min for determination of the succinic semialdehyde content with 0·25 M 3,5-diaminobenzoic acid.

Choline acetyltransferase (ChAc; EC 2.3.1.6) activity was measured using the method of McCaman and Hunt,[15] as modified by Goldberg *et al.*[16]

Acetylcholinesterase (AChE; EC 3.1.1.7) activity was determined by the method of McCaman *et al.*[17]

Dihydroxphenylalanine decarboxylase (Dopa decarboxylase; EC 4.1.1.26) was assayed by a method adapted from Pscheidt and Haber.[18] A 0·4-ml portion of homogenate was added to 0·4 ml buffer–substrate consisting of 0·1 M phosphate buffer (pH 6·8), 200 μM pyridoxal-5′-phosphate and 5 mM ^{14}C-dihydroxyphenylalanine. The incubation was carried out anaerobically for 30 min at 38°.

5-Hydroxytryptophan decarboxylase (5-HTP decarboxylase; EC 4.1.1.28) activity was monitored by the method of McCaman *et al.*[19] except that the incubation mixture contained the monoamine oxidase (MAO) inhibitor, pargyline (5×10^{-4} M). MAO (EC 1.4.3.4) was assayed using the procedure of McCaman *et al*,[20] employing 5-hydroxytryptamine as substrate.

Catechol-*O*-methyl transferase (COMT; EC 2.1.1.b) activity was measured by the method of McCaman.[21]

Tyrosine hydroxylase (EC 1.10.3.1) was determined by the procedure of Lloyd *et al.*,[22] except that two of the compounds were omitted from the incubation medium. Since the reduced pterin cofactor was itself susceptible to oxidation and since we were primarily interested in the effect of OHP on the total enzyme system, this cofactor was not added to the incubation medium in order to avoid possible masking of the OHP effect. Likewise, the sulphydryl protecting agent, mercaptoethanol, was omitted.

Tryptophan hydroxylase (EC 1.99.1.5) activity was assayed by the method of Schmidt and Sanders-Bush.[23] Again reduced pterin cofactor was omitted from the reaction mixture.

RESULTS

Of the biosynthetic enzymes studied, only tyrosine hydroxylase and tryptophan hydroxylase were unaffected by OHP. The activities of GAD, Dopa decarboxylase, 5-HTP decarboxylase and ChAc were significantly inhibited by exposure of the brain homogenates to 30 psi gauge pressure of O_2, but the percentage inhibition of the GAD enzyme system was five to seven times greater than that of the other three enzymes (Table 1). Exposure of the homogenates to a higher oxygen pressure (90 psi gauge pressure) increased this inhibitory effect, but the percentage inhibition of GAD activity was still three to five times greater than that of the other oxygen-sensitive enzymes (Table 2). Even at this higher pressure, no significant inhibition of tyrosine hydroxylase or 5-HTP hydroxylase was detected.

TABLE 1. EFFECTS OF OHP AT 30 psi gauge pressure ON NEUROTRANSMITTER BIOSYNTHETIC ENZYMES*

Enzyme	Enzyme activity (nmoles/mg tissue/hr)		Inhibition (%)
	Nitrogen	OHP	
GAD	35·42 ± 3·35	16·99 ± 1·34†	51·71
ChAc	0·35 ± 0·02	0·33 ± 0·01†	7·62
Dopa decarboxylase	5·72 ± 0·11	5·29 ± 0·11†	11·45
5-HTP decarboxylase	1·13 ± 0·08	1·00 ± 0·06†	11·35
Tyrosine hydroxylase	0·060 ± 0·0015	0·058 ± 0·0017	2·86
Tryptophan hydroxylase	0·013 ± 0·0013	0·013 ± 0·0014	1·55

* Each value represents the mean (±S. E.) of five animals.

† Significantly different from nitrogen controls ($P < 0·001$), using Student's *t*-test applied to paired values. See Materials and Methods for preparation of paired samples.

The degradative enzymes were extremely resistant to OHP (Table 3). The activities of GABA-T, MAO and COMT were not significantly changed by exposure of the brain homogenate to 30 psi gauge pressure of O_2, while the activity of AChE was actually enhanced to a small but significant extent.

In all cases, the control values for the activities of the various enzymes (Tables 1 and 3) were within the range of that previously published.[16–18,20,21,24–26]

TABLE 2. EFFECTS OF OHP AT 90 psi gauge pressure ON NEUROTRANSMITTER BIOSYNTHETIC ENZYMES*

Enzyme	Enzyme activity (nmoles/mg tissue/hr)		Inhibition (%)
	Nitrogen	OHP	
GAD	36·76 ± 2·74	12·37 ± 0·78†	66·15
ChAc	5·39 ± 0·12	4·74 ± 0·11†	12·03
Dopa decarboxylase	1·12 ± 0·04	0·94 ± 0·02†	17·56
5-HTP decarboxylase	0·37 ± 0·01	0·28 ± 0·01†	24·24
Tyrosine hydroxylase	0·060 ± 0·0015	0·062 ± 0·0017	−4·03
Tryptophan hydroxylase	0·013 ± 0·0016	0·012 ± 0·0017	4·93

* Each value represents the mean (±S. E.) of five animals.
† Significantly different from nitrogen controls ($P < 0·001$) using Student's *t*-test applied to paired values.

TABLE 3. EFFECTS OF OHP AT 30 psi GAUGE PRESSURE ON NEUROTRANSMITTER DEGRADATIVE ENZYMES*

Enzyme	Enzyme activity (nmoles/mg tissue/hr)	
	Nitrogen	OHP
GABA-T	115·03 ± 1·92	117·62 ± 2·84
AChE	541·10 ± 2·19	551·41 ± 2·34†
MAO	6·40 ± 0·09	6·53 ± 0·08
COMT	0·043 ± 0·002	0·043 ± 0·014

* Each value represents the mean (±S. E.) of five animals.
† Significantly different from nitrogen controls ($P < 0·001$) using Student's *t*-test applied to paired values.

DISCUSSION

Although tyrosine hydroxylase and tryptophan hydroxylase are not inhibited by OHP, at least one stage in the synthesis of each of the neurotransmitters—GABA, acetylcholine, dopamine, noradrenaline and 5-hydroxytryptamine—is sensitive to the hyperbaric oxygen. In contrast, none of the enzymes degrading these neurotransmitters is inhibited by OHP. If our present results reflect the situation *in vivo*, a possible consequence of the specific inhibition of the neurotransmitter synthetic enzymes could be a reduction in brain levels of the transmitters. It is significant that both GABA levels[2] and monoamine levels[4,5] have previously been demonstrated to decrease in mouse brain on exposure of the animal to OHP. In this investigation we have shown that GAD is the most susceptible of the transmitter enzymes to inactivation by oxygen. Therefore, it could reasonably be expected that *in vivo* the effect of OHP on neurotransmitter levels would be greatest with respect to GABA. Since the GABA system appears to be the most sensitive to the influence of OHP, it is possible that under certain pressures of oxygen, the amino acid might be the only transmitter affected. Thus we feel that these results support the view that alterations in GABA metabolism are the prime cause of oxygen-induced seizures.

The OHP-induced inhibitions in enzyme activities reported here are probably a result of oxidation of essential sulphydryl groups,[9] since it is known that at least GAD[12] and ChAc[10] contain thiol groups necessary for their activity. Moreover the sulphydryl protective agent, dithiothreitol, has been shown to protect the activity of GAD from the deleterious effects of OHP.[27] The mechanism of the slight but statistically significant stimulation of AChE activity by OHP is at present unknown.

The present observation that tyrosine hydroxylase is unaffected by OHP contrasts with the finding of Fisher and Kaufman[28] that bovine brain tyrosine hydroxylase is inhibited by high levels of oxygen in the assay system. This discrepancy probably arises from the radically different experimental conditions used in the two studies. For example, Fisher and Kaufman[28] showed that the degree of inhibition of tyrosine hydroxylase varied with the type of cofactor added to the medium and in some cases the high oxygen levels had minimal effect. Since cofactor was omitted from our incubation medium (see Materials and Methods), this may be the source of the discrepancy. Further studies are therefore necessary to clarify the situation and are now underway.

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