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# EFFECT OF CALCIUM ON TRYPTOPHAN HYDROXYLASE FROM RAT HIND BRAIN

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Recent evidence indicates that tyrosine hydroxylase prepared from central noradrenergic or peripheral sympathetic neurones which have been stimulated electrically or depolarized by potassium, is markedly activated when assayed in vitro with subsaturating concentrations of substrate and reduced pterin cofactor (1,2). The activation is produced by changes in the kinetic properties of the enzyme which are mimicked by the addition of calcium ions, in vitro, to enzyme isolated from untreated control tissues (1,2). The activation of the enzyme produced by depolarization of the intact sympathetic neurones appears to be a calcium dependent phenomenon (1) and it has been proposed that this activation may explain the increase in norepinephrine formation which occurs during stimulation of sympathetically innervated tissues (3).

The formation of 5-hydroxytryptamine (5-HT) from tryptophan in both mammalian central nervous system (4) and ganglia of molluscs (5, and Boadle-Biber, unpublished) is also enhanced by electrical stimulation or depolarization of the nervous tissue with potassium. The present study was carried out to determine whether tryptophan hydroxylase, the enzyme presumed to be rate limiting in the formation of 5-HT, which shares many properties in common with tyrosine hydroxylase (6,7) is also activated by calcium ions.

Hind brains were dissected from male Sprague Dawley rats (Charles River Breeding Laboratories) which had been killed by decapitation. The fresh tissue

TABLE 1. Effect of calcium on tryptophan hydroxylase from rat hind brain

| Concentration of calcium (M) | n* | Tryptophan Hydroxylase Activity+ (ng 5-HTP/mg protein/15 min) | (% Control) |
|---|---|---|---|
| 0 | 6 | 144 ± 8 | 100 |
| $1 \times 10^{-5}$ | 6 | 149 ± 9 | 104 ± 6 |
| $5 \times 10^{-5}$ | 4 | 178 ± 2 | 124 ± 7 |
| $1 \times 10^{-4}$ | 6 | 174 ± 5 | 121 ± 4 |
| $5 \times 10^{-4}$ | 4 | 220 ± 7 | 153 ± 5 |
| $1 \times 10^{-3}$ | 6 | 231 ± 5 | 160 ± 4 |
| $5 \times 10^{-3}$ | 4 | 290 ± 10 | 201 ± 7 |
| $1 \times 10^{-2}$ | 5 | 326 ± 6 | 226 ± 4 |

+ Tryptophan hydroxylase activity was determined in the 39,000 x g supernatant of homogenates of rat hind brain. Results are expressed as the mean ng 5-HTP/mg protein/15 min ± S.E.M. Assays were conducted in the presence of 200 μM tryptophan and 50 μM $6MPH_4$.

* n = number of determinations.

was homogenized in 1.5 volumes 0.05 M Tris-acetate pH 7.5 in an all glass homogenizer and the homogenate was centrifuged for 30 min at 39,000 x g (21,000 r.p.m.) in a Spinco Model L centrifuge at 4°C. Dithiothreitol (DTT), final concentration 2 mM, was added to the supernatant which was then passed over a column of Sephadex G-25 (coarse type, Pharmacia 2.5 x 100 cm) equilibrated with 0.05 M Tris-acetate pH 7.5 containing 2 mM DTT. This gel filtration step reduced the fluorescence of the blanks in the tryptophan hydroxylase assay, presumably because it removes endogenous tryptophan and 5-HT. The protein fraction (pink) was collected and frozen in 3 ml aliquots at -70°C and stored at -20°C. Tryptophan hydroxylase was assayed by formation of 5-hydroxytryptophan (5-HTP) according to the procedure of Friedman et al., (7) with the following minor changes: Glucose 6-phosphate and glucose 6-phosphate dehydro-

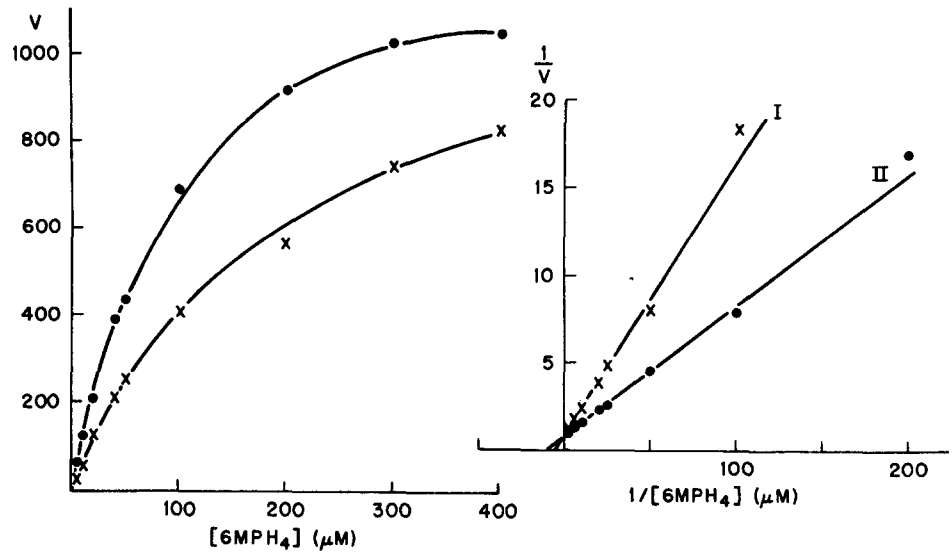


Fig. 1. Effect of 6-methyl tetrahydropterin concentration on the rate of formation of 5-hydroxytryptophan in the presence ● and absence X of 10 mM $CaCl_2$. Tryptophan concentration was 500 μM. Velocity is expressed as ng 5-hydroxytryptophan formed per mg protein in 15 min. A double reciprocal plot of the data is also shown (right). Curve I: Control. Curve II: 10 mM $CaCl_2$.

genase were omitted; sheep liver pteridine reductase was used from the first ammonium sulfate fraction following Kaufman's purification procedure (8); the aromatic amino acid decarboxylase inhibitor, NSD 1055, (Lederle) was substituted for NSD 1034; and the reaction was carried out in a 1.0 ml volume and started by addition of substrate, L-tryptophan (D-tryptophan to blanks). Calcium chloride was added 10 min before the reaction was started. The reaction was linear for 20 min and was followed routinely for 15 min. It was stopped with 100 μl 70% perchloric acid, the precipitated proteins were centrifuged out and the supernatant passed through millipore filters to remove any remaining particulate matter. Formation of 5-HTP was determined by fluorescence.

Table 1 shows the effect of different concentrations of calcium chloride on the activity of tryptophan hydroxylase. There was a 100% increase in

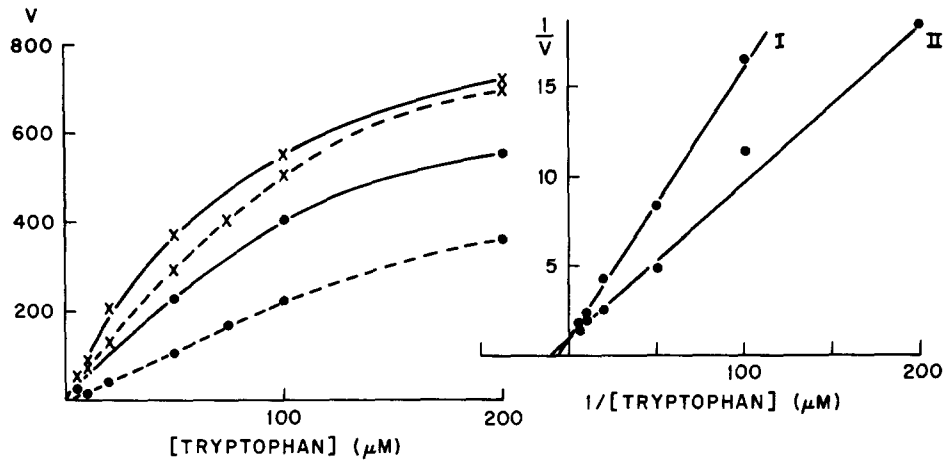


Fig. 2. Effect of L-tryptophan concentration on the rate of formation of 5-hydroxytryptophan in the presence X and absence ● of 10 mM $CaCl_2$. 6-Methyl tetrahydropterin concentration was 1 mM (solid lines) and 0.1 mM (dashed lines). Velocity is expressed as ng 5-hydroxytryptophan formed per mg protein in 15 min. A double reciprocal plot of the data obtained in the presence of 1 mM 6-methyl tetrahydropterin is also shown (right). Curve I: Control. Curve II: 10 mM $CaCl_2$.

activity at 5 and 10 mM calcium. Under identical assay conditions barium (10 mM) produced a 50% increase in activity while magnesium (10 mM) had no significant effect on enzyme activity.

The effect of calcium on the $K_m$ of tryptophan hydroxylase for the reduced pterin cofactor 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$) and for the substrate tryptophan is shown in Figs. 1 and 2 respectively. Calcium (10 mM) caused an approximately two-fold change in the apparent $K_m$ of tryptophan hydroxylase for $6MPH_4$, from 200 μM in control enzyme to 111 μM in the presence of calcium. There was no significant change in $V_{max}$ under these conditions. A change of similar magnitude was also observed with calcium for the $K_m$ of the enzyme for tryptophan. A control value of 142 μM decreased to 72 μM in the presence of calcium (10 mM). Again there was no significant change in $V_{max}$ (Fig. 2).

The effect of increasing the concentration of L-tryptophan in the presence of a subsaturating concentration of $6MPH_4$ (0.1 mM) on the rate of formation of 5-HTP is also shown in Fig. 2. The increase in activity in the presence of calcium is much more pronounced with the low concentration of $6MPH_4$ (0.1 mM) than with saturating $6MPH_4$ (1 mM).

The changes in the affinity of tryptophan hydroxylase for substrate and cofactor seen with calcium are very modest compared with the effects of this ion on the affinity of tyrosine hydroxylase for its substrate and cofactor (two-fold compared with five-fold changes) (1). Furthermore, tryptophan hydroxylase requires extremely high concentrations of calcium to produce these changes. However, since tryptophan concentrations are known to be subsaturating in mammalian nervous tissues (9,10), changes in affinity for substrate of this order would probably account for the increase in 5-HT formation known to occur in intact 5-HT containing neurones during nerve activity (4). It remains to be determined whether calcium ions or some other as yet undetermined factors play a role in regulating tryptophan hydroxylase activity *in vivo*.

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REFERENCES

1. V.H. MORGENROTH, 111, M. C. BOADLE-BIBER and R. H. ROTH. Proc. Natl. Acad. Sci. 71, 4283 (1974).
2. R. H. ROTH, P. M. SALZMAN and V. H. MORGENROTH, 111, Biochem. Pharmacol. 23, 2779 (1974).
3. N. WEINER, Ann. Rev. Pharmacol. 10, 273 (1970].
4. P. J. SHIELDS and D. ECCLESTON, J. Neurochem. 19, 265 (1972).
5. N. N. OSBORNE, Br. J. Pharmacol. 48 546 (1973].
6. W. LOVENBERG and S. J. VICTOR, Life Sci. 14, 2337 (1974).
7. P. A. FRIEDMAN, A. H. KAPPELMAN and S. KAUFMAN, J. Biol. Chem. 247, 4165

(1972).

8. S. KAUFMAN, In: Methods in Enzymology (Eds. S. P. Colowick and N. O. Kaplan) Vol. 5, p. 812, Academic Press, New York (1962).

9. D. G. GRAHAME-SMITH, J. Neurochem. 18, 1053 (1971).

10. J. D. FERNSTROM and R. J. WURTMAN, Science 173, 149 (1971).