Biochemical Pharmacology, Vol. 22, pp. 865–868. Pergamon Press, 1973. Printed in Great Britain.

SHORT COMMUNICATION

Effect of calcium, potassium and sodium on tyrosine hydroxylase activity in different regions of the rat brain

(*Received* 9 *August* 1972; *accepted* 14 *October* 1972)

STIMULATION of sympathetic nerves is followed by an increased rate of synthesis of noradrenaline from tyrosine, but not from dihydroxyphenylalanine (Dopa).[1] This implies that the rate-limiting reaction mediated by tyrosine hydroxylase (TH) is enhanced. Two different processes can be distinguished with respect to this increased synthesis. One is due to formation of new enzyme (tyrosine hydroxylase), its onset is slow and it develops gradually over a period of days.[2] It has been coined "trans-synaptic induction" and it was abolished by cycloheximide or actinomycin D.[3] The other process is evident within less than 60 min[1] and is not accompanied by synthesis of the new enzyme.[4] It has been suggested that this enhanced synthesis of noradrenaline is due to depletion of a specific catecholamine store which controls tyrosine hydroxylase activity.[4]

Nerve impulses are accompanied by several ionic changes, most prominent are the increased permeability to sodium and the penetration of calcium.[5] The release of transmitters, adrenergic and cholinergic, is mediated through calcium ions.[6] In the adrenal medulla perfused *in vitro* it has been shown that calcium is the triggering factor which causes release of catecholamines rather than depolarization *per se*.[7]

We have, therefore, considered the possibility that changes in the ionic composition of the cell may affect the activity of tyrosine hydroxylase immediately following stimulation rather than depletion of a poorly defined catecholamine pool or in addition to depletion of this pool.

In a previous communication we have reported that tyrosine hydroxylase from the adrenal medulla was inhibited *in vitro*, when potassium replaced sodium in the incubation medium and that calcium ions in low concentrations (0·1 mM) significantly enhanced tyrosine hydroxylase activity.[8]

We have now extended our study to tyrosine hydroxylase obtained from different regions of the brain. The results are presented in this report.

Male rats of the Hebrew University strain were used, weighing 200–250 g. The rats were killed by dislocation of the neck. The skull was immediately opened and the brain was separated and put on ice. The brains were then cut, as previously described,[9] into brain stem, hypothalamus, extrapyramidal nuclei (caudate nucleus + basal ganglia) and cortex. The cerebellum was discarded. Homogenization was carried out in a medium of 0·32 M sucrose, 10 ml of medium per gram tissue. The homogenate was centrifuged at 17,000 *g* for 60 min. The supernatant was partly lyophilized (down to between one-half to one-third of the original volume) and was kept frozen until used.

Tyrosine hydroxylase activity was assayed according to the method of Nagatsu *et al.*[10] The incubation medium consisted of 100 μmoles of acetate buffer pH 6·0; 100 μmoles of 2-mercaptoethanol, 0·5 μmole ferrous–ammonium sulfate; 1 μmole pargyline, 1 μmole DOPA-decarboxylase inhibitor (RO-4-4602/1, from Hoffman–La Roche & Co.), 2 μmoles of 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride, 0·2 ml of the enzyme. The final volume was 1·1 ml. 0·5 μCi of 1-tyrosine-3,5-H^3 was added (Radiochemical Centre, Amersham, sp. act. 52 Ci/mmole). Incubation was carried out at 37° in a shaking bath for 15 min. The reaction was stopped by adding 0·1 ml of 3 M trichloroacetic acid (TCA). In the blanks TCA was added before incubation. In experiments where additional or different ions were used (Ca^{2+}, K^+) the blanks included these ions, too so as to avoid any methodological effect apart from that on the enzymatic activity. At the end of the reaction the incubation mixture was applied on a column of Dowex 50W × 4 and the effluent plus one washing with 1 ml of water were collected into a counting vial followed by addition of 10 ml of Bray's solution.[11] Packard Tri-Carb scintillation spectrometer was used for counting.

All reagents were prepared in redistilled water. In experiments with a Na^+ medium the acetate buffer consisted of sodium acetate ($[Na^+]$ = 100 mM), a K^+ medium consisted of potassium acetate buffer ($[K^+]$ = 100 mM). Ca^{2+} was added as $CaCl_2$.

The concentrations of norepinephrine and dopamine in the final incubation medium were less than 10^{-7} M, which is far below the range of inhibition of tyrosine hydroxylase. The concentration of calcium in the final incubation medium was less than 20 μM.

Figure 1 shows that the effect of replacing sodium by potassium was different in various parts of the brain. Whereas in the presence of potassium tyrosine hydroxylase activity in the hypothalamus was decreased, there was no effect of potassium on TH activity in the extra-pyramidal nuclei while TH activity in the brain stem and in the cortex was considerably enhanced by potassium. Thus, only tyrosine hydroxylase obtained from the hypothalamus resembled that of the adrenal medulla[8] in being inhibited by potassium. We have previously reported in the adrenal medulla an optimal effect of Na^+ at 30 mM in the presence of 100 mM K^+[8]; however it was far less than the effect of replacing K^+ with Na^+ completely.

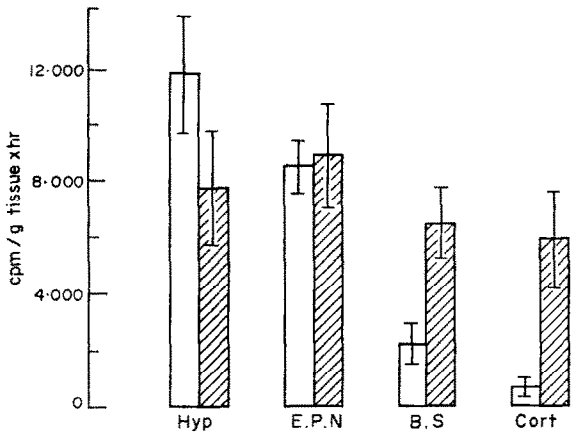

FIG. 1. Effect of sodium and potassium on tyrosine hydroxylase activity *in vitro* in different regions of the rat brain. In each pair of columns the left one is for activity with 100 mM sodium and the right one with 100 mM potassium. Vertical bars represent S.E.M. Hyp; hypothalamus; N = 11. E.P.N., striatum and caudate; N = 11. B.S., brain stem; N = 9. Cort., cortex; N = 7. The difference in activity between sodium medium and potassium medium (paired analysis), hypothalamus, $P < 0{\cdot}05$; E.P.N., n.s.; B.S., $P < 0{\cdot}01$; Cort., $P < 0{\cdot}02$.

Figure 2 shows the effect of increasing concentrations of Ca^{2+} on tyrosine hydroxylase activity in different parts of the brain. In all four regions of the brain (cortex, brain stem, extrapyramidal nuclei and hypothalamus) tyrosine hydroxylase activity was enhanced by calcium ions. The hypothalamus again resembled the adrenal medulla in that peak activation occurred at a calcium concentration of 0·1 mM. In the extrapyramidal nuclei and the brain stem peak activation occurred at 0·05 mM but tyrosine hydroxylase activity was still significantly enhanced at 0·1 mM. The cortex showed increasing activation even at 0·2 mM while in the hypothalamus, brain stem and extrapyramidal nuclei the activation was declining at this concentration of calcium.

From the results presented here and in the previous communication[8] it seems that calcium ions at certain concentrations can enhance tyrosine hydroxylase activity. The relatively low concentrations of calcium (0·05 and 0·1 mM) necessary for activation suggest that such enhancement may be of physiological significance. Calcium is necessary for the release of transmitters,[6] calcium influx increases during nerve stimulation[5] and free calcium in the cell may also increase through release from intracellular binding sites during stimulation. Increased free calcium in the cell may, therefore, cause an immediate increase in the rate of catecholamine synthesis by enhancing the rate-limiting reaction, that of tyrosine hydroxylation.

It is more difficult to envisage a physiological role for sodium and/or potassium on tyrosine hydroxylase activity. Whereas sodium increased the activity of the enzyme (compared to potassium) in the adrenal medulla and the hypothalamus it was the other way round in the brain stem and cortex, while in the extra-pyramidal nuclei there was no effect. The main intra-cellular ion is potassium and it is noteworthy that the wide differences in tyrosine hydroxylase activity observed in the various regions of the brain when a sodium medium was used for TH assay were considerably reduced when a potassium medium was used (Fig. 1). Thus, at present the possible significance of the effects of sodium and potassium on tyrosine hydroxylase activity is still obscure.

Two interesting observations have recently been reported on the effect of ions on catecholamine synthesis. Boadle-Biber *et al.*,[12] Have reported enhanced synthesis of norepinephrine from tyrosine in the *vas deferens* upon exposure to high potassium concentrations. This enhancement could have been

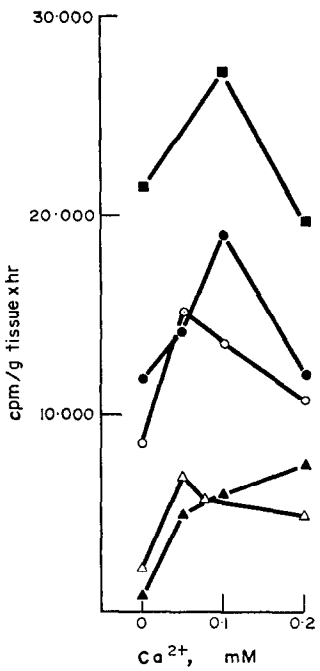

FIG. 2. Effect of calcium on tyrosine hydroxylase activity *in vitro* in different regions of the rat brain. ●——● hypothalamus, N = 12; ○——○, EPN (= striatum and caudate nucleus), N = 10; △——△brain stem, N = 9; ▲——▲, cortex, N = 7; ■——■, adrenal, N = 12. Abscissa, final calcium concentration in incubation medium (mM). Assay performed in sodium medium. Differences between activity with different calcium concentrations and activity in the absence of calcium: EPN, Ca = 0·05 mM, $P < 0·01$; Ca = 0·1 mM, $P < 0·02$. Brain stem, Ca = 0·05 mM, $P < 0·05$; Ca = 0·1 mM, $P < 0·05$. Cortex, Ca = 0·1 mM, $P < 0·05$; Ca = 0·2 mM, $P < 0·01$.

produced by the depolarization caused by the elevated $[K^+]$. It is noteworthy that when $[K^+]$ in the incubation medium was elevated beyond 70 mM the enhancement of norepinephrine synthesis was abolished.[12] This may corroborate our finding that high $[K^+]$ inhibits tyrosine hydroxylase activity.[8] However, since the experiment of Boadle-Biber *et al.*[12] was carried out on a tissue with intact cells and our experiment was on the isolated enzyme no direct comparison seems valid. It is interesting, however, that the enhanced synthesis due to elevated $[K^+]$ was evident only in the presence of Ca^{2+}.[12] Therefore calcium may have been the immediate cause of the increased synthesis of catecholamines in these experiments too and thus would corroborate our findings on the direct enhancing effect of calcium ions on tyrosine hydroxylase.

Another report by Goldstein *et al.*,[13] describes a decreased rate of catecholamine synthesis from labelled tyrosine in slices of rat striatum upon increasing the concentration of calcium in the incubation medium. Here, again, direct comparison with our results is not meaningful since slices were used and not isolated enzymes. However, the results reported by Goldstein *et al.*[13] are in disagreement with those of Boadle-Biber *et al.*,[12] who found enhancement of catecholamine synthesis when calcium was present in the incubation medium. One possible explanation for this divergence may be the different concentrations of tyrosine used in the media. Goldstein *et al.*[13] used $\sim 1·5 \times 10^{-6}$ M tyrosine, which is lower than the saturating concentration, whereas Boadle-Biber *et al.*[12] used 5×10^{-5} M tyrosine. It is possible that increased calcium in the medium may have decreased the uptake of labeled tyrosine into the slices. Thus, in the experiments of Goldstein *et al.*,[13] where tyrosine concentration was low, uptake may have become the limiting factor rather than tyrosine hydroxylase activity; it is conceivable that the effect of calcium on these two processes may be in opposite directions.

Finally, Kuczenski and Mandell[14] have reported increased activity of hypothalamic tyrosine hydroxylase *in vitro* in the presence of potassium chloride, ammonium chloride and ammonium

sulfate when compared to incubation in the absence of ions. However, no data are given for sodium ions, so no comparison can be made between K^+ and Na^+. The effect of calcium was not studied by these authors.

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