Comparison of tyrosine hydroxylase and choline acetyltransferase activity in response to sympathetic nervous system activation

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Choline acetyltransferase and tyrosine hydroxylase serve as enzyme markers for preganglionic and postganglionic sympathetic nerves, respectively. There is abundant evidence that experimental conditions which increase the activity of the peripheral sympathetic nervous system produce an increase in the activity of neuronal tyrosine hydroxylase and dopamine β-hydroxylase. The activity of these enzymes serve as indices of sympathetic neural activity. It is not known, however, if choline acetyltransferase activity also serves as a reliable index of neural activity.

Initial studies by Mueller and co-workers showed that 6-hydroxydopamine and reserpine injection increased the activity of tyrosine hydroxylase within the terminals of adrenergic neurons and in the adrenal medulla. Molinoff et al. demonstrated a neurally mediated increase in dopamine-β-hydroxylase. Subsequent studies employing a variety of conditions such as cold, immobilization, amphetamine, insulin, phenoxybenzamine, swimming and hypothalamic stimulation also increase tyrosine hydroxylase activity in the peripheral sympathetic system and adrenals. Since the increase in tyrosine hydroxylase activity is blocked by RNA and protein synthesis inhibitors, it was suggested that there is an increase in enzyme synthesis. Using immunoprecipitation, Chuang and Costa showed that there is an increase in enzyme protein in response to cold stress in rats. In addition to the increased activity requiring protein synthesis, there may be a short-term regulation of activity, associated with protein phosphorylation and changes in apparent substrate affinities.

In contrast to tyrosine hydroxylase, relatively few studies have been performed on the regulation of choline acetyltransferase activity in the sympathetic nervous system. Oesch has, for example, examined the effect of reserpine injection into rats on the activity of choline acetyltransferase in stellate and superior cervical ganglia and in the adrenal medulla. He reported that the activity increases in response to reserpine treatment. As reported in preliminary form, we find that tyrosine hydroxylase activity is more responsive than choline acetyltransferase and that large, perhaps supraphysiologic, stimuli are necessary to increase activity.
Male Sprague-Dawley rats (100–120 g or 200–250 g, as specified) were obtained from Biolabs (St. Paul, Minn.) and housed in groups of 6 in a controlled (24 °C) environment. The animals were exposed to light 12 h per day and allowed food and water ad libītum. To allow for acclimatization, no experimental manipulations were performed until one week after the animals were received.

After the specified treatment, the animals were sacrificed by a blow to the neck unless otherwise noted. The superior cervical and stellate ganglia and the adrenal medulla were removed, weighed, placed in polyethylene vials and frozen in liquid N₂ as rapidly as possible (within 10 min) and stored. After storage, the tissues were homogenized in 0.3 ml of ice-cold 5 mM potassium phosphate (pH 7.4) containing 0.2% Triton X-100 with a glass-to-glass Duall tissue grinder (Kontes; Vineland, N.J.). Duplicate portions (10 μl) were taken for choline acetyltransferase measurements by the procedure of Roskoski. After the remainder was centrifuged at 13,000 × g (5 min, 4 °C) in an Eppendorf microfuge, tyrosine hydroxylase activity was measured in 50 μl portions of the supernatant by the method of Coyle using 0.2 mM tyrosine and 0.9 mM DMPSH₄. At these concentrations, measured activity therefore approximates the Vₘₐₓ; observed changes in activity are not due to alterations in the Kᵣ of the enzyme for substrate or cofactor.

Student’s ‘t’-test was used to establish significance of differences between means. A P < 0.05 was chosen as the level of significance.

In agreement with other investigators, we find that reserpine injection (7.5 mg/kg) produces an increase in tyrosine hydroxylase activity in the adrenal gland and stellate ganglia within 24 h (Table I). After injection of this large dose, the animals

<table>
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<th>TABLE I</th>
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<td>Effect of reserpine on tyrosine hydroxylase (TH) and choline acetyltransferase (CAT) activity in sympathetic ganglia and adrenal medulla</td>
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Reserpine (7.5 mg/kg) was injected intraperitoneally into 6 rats (100–120 g); the 6 control rats received only vehicle. After 24 h, the specified tissue was removed and stored prior to assay. Aliquots of the whole homogenate were taken for choline acetyltransferase measurement (CAT). Portions of the homogenate were centrifuged (27,000 × g, 10 min) and transferase activity was determined in the supernatant fraction (CAT Sup). Tyrosine hydroxylase was measured as described in the text. Results are expressed as nmole/mg protein/h.

<table>
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<th>Superior cervical ganglia</th>
<th>Stellate ganglia</th>
<th>Adrenal medulla</th>
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<tr>
<td></td>
<td>CAT</td>
<td>CAT Sup</td>
<td>TH</td>
</tr>
<tr>
<td>Control</td>
<td>91.5 ± 5.7</td>
<td>11.4 ± 12.5</td>
<td>4.26</td>
</tr>
<tr>
<td>Reserpine</td>
<td>94.4 ± 7.2</td>
<td>13.9 ± 10.3</td>
<td>4.56</td>
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* Significantly different from control (P < 0.05).
develop ptosis, tearing, diarrhea, and become quiescent. In contrast to Oesch\textsuperscript{17}, however, we did not observe an increase in choline acetyltransferase activity in superior cervical or stellate ganglia or adrenal medulla (Table I). We also obtained similar results two days after reserpine injection. The 24 h experiment was performed 3 times with animals weighing 200–250 g and 3 times with animals weighing 100–120 g. Experiments were then performed to ascertain the difference between our findings and those of Oesch\textsuperscript{17}. In contrast to the latter investigator, who measured choline acetyltransferase activity in the 27,000 × g supernatant in a Triton X-100 extract, we routinely measure activity in the whole homogenate\textsuperscript{20}. As noted by Oesch\textsuperscript{17}, we find about 25% of enzyme activity occurs in the supernatant. He also notes that the increases observed did not consistently reach statistical significance using whole homogenate. However, we have been unable to detect a statistically significant increase.

Following the methodology of Fonnum\textsuperscript{6}, experiments were performed to ascertain the means by which the choline acetyltransferase binds to the 27,000 × g membrane fraction. On the one hand, Triton X-100 fails to solubilize the transferase; on the other hand, 0.2 M KCl completely solubilizes the enzyme (not shown). NaCl (0.2 M) also renders the enzyme soluble. We conclude, like Fonnum, that the enzyme is bound to the membrane fractions by electrostatic forces. We also conclude that reserpine injection fails to increase the total choline acetyltransferase activity at 24 (Table I) and 48 h in the adrenal or the stellate and superior cervical sympathetic ganglia.

To follow up the reserpine studies where a consistent dissociation between choline acetyltransferase and tyrosine hydroxylase activity was observed, a second method — cold exposure — was used to increase the activity of the sympathetic system. Thoenen\textsuperscript{2a} and Chuang and Costa\textsuperscript{1} have shown that maintaining rats at 4 °C for as little as 2 h produced an increase in adrenal tyrosine hydroxylase activity after 1–2 days. In our experiments, individually caged rats (100–120 g) were kept in a 4 °C cold room for 8 days. The control animals were also individually caged. Under these conditions, we find no change in superior cervical or stellate ganglion nor in adrenal medullary choline acetyltransferase activity. TH activity, however, was significantly increased in each tissue (not shown).

Choosing a third means for activating the sympathetic system, electrical stimulation of the posterior hypothalamus was performed as described by Folkow and Rubinstein\textsuperscript{5}. One group of rats was stimulated for 24 h and a second group for 48 h. At the time the stimulator was on, the rats exhibited a typical alerting and avoidance response. They usually tried to escape from the cage each time they were stimulated. Each stimulation also increased arterial pressure. During the 24 or 48 h period of stimulation, current was increased as needed in order to maintain the blood pressure increase at a constant level. Individual pressure increments averaged 24 ± 1 mm Hg in the 6 stimulated rats. Resting mean arterial pressure, however, was not significantly altered by 48 h of stimulation, averaging 120 ± 2 mm Hg at beginning and 124 ± 4 mm Hg at the end of the experiment. In addition, no change was noted in the heart rate during stimulation (396 ± 14 beats/min) when compared to the control period.
The pressure responses produced by hypothalamic stimulation appear to be produced by selective activation of sympathetic nerves to cardiovascular effectors and not by release of adrenal medullary catecholamines. Thus, responses were not altered by adrenal demedullation but were reduced significantly by the ganglionic blocker hexamethonium (not shown).

After 24 h of stimulation, there was no change in choline acetyltransferase or tyrosine hydroxylase activity in the tissues sampled. Even after 48 h of stimulation, tyrosine hydroxylase activity was significantly elevated only in the adrenal medulla (Table II). It was unchanged in the stellate and superior cervical ganglia. Again, we did not observe any increases in choline acetyltransferase activity.

The activity of enzymes mediating neurotransmitter metabolism has been used by many investigators as an index of neuronal activity. We wished to ascertain the relative sensitivity of choline acetyltransferase and tyrosine hydroxylase, respective marker enzymes of the preganglionic and postganglionic sympathetic nervous system, in response to sympathetic nerve activation. We used 3 experimental approaches to activate the sympathetic system: reserpine injection, cold exposure, and electrical stimulation of the hypothalamus. The results were consistent and readily reproducible, and indicate that tyrosine hydroxylase activity is a more sensitive indicator of sympathetic activity than choline acetyltransferase. Using similar methods, we also found that adrenal tyrosine hydroxylase activity increases in guinea pigs in response to cold exposure under conditions where choline acetyltransferase activity remains unchanged (not shown). This suggests that the finding is not species-specific.

An increase of blood pressure during hypothalamic stimulation indicates

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<tr>
<td>CAT</td>
<td>TH</td>
<td>CAT</td>
</tr>
<tr>
<td>Control</td>
<td>45.8±6.3</td>
<td>74.1±6.5</td>
</tr>
<tr>
<td>48 h Stimulation</td>
<td>54.3±3.8</td>
<td>75.4±18.2</td>
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* Significantly different from control ($P < 0.05$).
sympathetic neural system activation. The degree of this activation, however, is difficult to quantify. We consider the hypothalamic stimulation (10–20 sec/min for 24–48 h), which prompts these blood pressure and alerting responses and maintains the animal in an active state for 48 continuous hours, to be a rather potent sympathetic stimulus. We anticipated that this intervention would elevate tyrosine hydroxylase activity in ganglia and the adrenal. Since elevation was found only in the adrenal after 48 h stimulation, we feel that rather marked stimulation is required to elicit this response. With reserpine injection, the dosage routinely employed (7.5 mg/kg) incapacitates the animals. The exposure of small animals to cold also produces a marked increase in sympathetic activity.

In contrast to Oesch, we were unable to demonstrate an increase in choline acetyltransferase activity in response to reserpine. One difference in methodology partially accounts for the discrepancy. We measured activity in whole homogenate and Oesch measured activity in the Triton X-100 solubilized 27,000 × g supernatant fraction. We were unable to show a change in this fraction, even though there was a measurable increase in tyrosine hydroxylase activity. In closer agreement with our results, he states that, when activity of whole homogenate is assayed, the differences did not always approach statistical significance. In 7 separate experiments (42 experimental and 42 control animals), we have been unable to demonstrate such an increase. We have repeated his work as closely as possible and have not been able to reproduce the results. The difference might be due to a difference in rat strain or to a subtle difference in methodology. Our failure to detect an increase in choline acetyltransferase in response to reserpine injection does not alter our main thesis that tyrosine hydroxylase is a more sensitive indicator of increased sympathetic nerve activity.

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3 Dairman, W. and Udenfriend, S., Increased conversion of tyrosine to catecholamines in intact rat following elevation of tissue tyrosine hydroxylase levels by administered phenoxybenzamine, Molec. Pharmacol., 6 (1971) 350–356.