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NORADRENERGIC NEURONS: ALLOSTERIC ACTIVATION OF HIPPOCAMPAL TYROSINE HYDROXYLASE BY STIMULATION OF THE LOCUS COERULEUS

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In peripheral noradrenergic neurons an increase in impulse flow results in an acceleration of norepinephrine biosynthesis. This increase in transmitter synthesis is due to an increase in the activity of the rate limiting enzyme tyrosine hydroxylase. Until recently this increase in tyrosine hydroxylase activity was thought to arise as a result of the removal of endproduct inhibition subsequent to the depletion of a small pool of norepinephrine which has access to tyrosine hydroxylase (1). Recent experiments in our laboratory, however, have suggested that the post-stimulation increase in tyrosine hydroxylase activity observed in sympathetic nerve endings after electrical stimulation of noradrenergic nerves occurs as a result of an allosteric activation of tyrosine hydroxylase (2). This allosteric activation appears to be mediated by an increase in affinity of the enzyme for both substrate and pteridine cofactor and a decreased affinity of the enzyme for the endproduct inhibitor norepinephrine.

It is generally assumed that noradrenergic neurons in the central nervous system behave in a fashion quite similar to the postganglionic sympathetic noradrenergic neurons in the periphery. In fact, it has recently been demonstrated that stimulation of central noradrenergic neurons results in an increase in the turnover of norepinephrine as well as an increase in the accumu-

lation of a major metabolite of norepinephrine, 3-methoxy-4-hydroxyphenethylene glycol sulfate (3-5). These studies were also suggestive that stimulation of these noradrenergic neurons resulted in an increase in norepinephrine biosynthesis. In view of these observations we attempted to determine if an increase in impulse flow in central noradrenergic neurons would result in an activation of tyrosine hydroxylase and, if so, whether this activation was mediated by a change in the kinetic properties of tyrosine hydroxylase.

For this study we chose a central noradrenergic system which has the majority of its cells of origin in the locus coeruleus. The locus coeruleus in the rat consists almost exclusively of noradrenergic cell bodies and has a bilateral localization beneath the floor of the 4th ventricle. The noradrenergic terminals in the hippocampus of the rat are largely supplied by these noradrenergic cell bodies located in the locus coeruleus (6-8). Due to the highly compact nature of this group of cells it is possible to effectively stimulate these neurons electrically by placement of the stimulating electrode in close proximity to the locus coeruleus. Since the ascending pathway from the locus to the hippocampus does not cross the midline, the hippocampus on the contralateral side serves as an excellent control tissue.

Male Sprague Dawley rats (240-280 g) obtained from Charles River Breeding laboratories were anesthetized with chloral hydrate (400 mg/kg) and placed in a Kopf stereotaxic instrument. Coaxial electrodes were placed stereotaxically within 0.5 mm of the locus coeruleus and the stimulus provided by a Grass model 5 stimulator connected to a constant current source. The stimuli were applied in monophasic pulses of 2 msec duration at a frequency of 20/sec and a current of 400μ A for periods of 15 minutes. Immediately after the termination of the stimulation rats were killed by decapitation. The hippocampi were dissected free from the remainder of the brain and frozen on dry ice. The remainder of the brain was placed in 5% glutaraldehyde-0.9% saline and saved for sectioning and staining with cresyl violet for precise localization of the stimulation electrode. The frozen hippocampi (stimulated and contra-

lateral side) were homogenized individually in 10 volumes of 0.05M Tris-acetate buffer, pH 6.0 and tyrosine hydroxylase measured in the 104,000 x g supernatant by a modification of the method of Shiman et al. (9) and Coyle (10).

Electrical stimulation of the locus coeruleus for 15 minutes results in a marked increase in tyrosine hydroxylase activity found in the hippocampus on the stimulated side. An increase in activity of about 300% was observed when compared to the activity of tyrosine hydroxylase found in the contralateral hippocampus (Table 1). Preliminary experiments indicate that this activation persists for periods up to about 20 minutes following the termination of electrical stimulation of central noradrenergic neurons. Stimulation of the locus coeruleus also resulted in a dramatic change in the kinetic properties of tyrosine hydroxylase isolated from the hippocampus on the stimulated side. The K_m for tyrosine was decreased 10 fold while the K_i for norepinephrine was increased about 20 fold (Table 2).

Table 1

Effects of Stimulation of the Locus Coeruleus on Hippocampal Tyrosine Hydroxylase Activity

	n	Tyrosine Hydroxylase Activity* (pmoles DOPA/mg protein/min)
Hippocampus (control side)	3	7.5 ± 0.9
Hippocampus (stimulated side)	3	33.2 ± 2.3

*Tyrosine hydroxylase activity was determined in the 104,000 x g supernatants of rat hippocampus. Results are expressed as the mean ± S.E.M. of three stimulated rats (assayed in triplicate) in which the electrode placement in the locus coeruleus was verified histologically. Assays were conducted in the presence of 10 μM tyrosine and 0.1 mM $DMPH_4$.

These results demonstrate that stimulation of the locus coeruleus of the rat results in a post-stimulation increase in the activity of tyrosine hydroxylase isolated from the hippocampus which receives its major noradrenergic

Table 2

Effect of Electrical Stimulation on the Kinetics of Hippocampal Tyrosine Hydroxylase*

	K_m Tyrosine (μM)+	K_i NE (mM)+
Hippocampus (control side)	53.6 ± 2.8	0.39 ± 0.05
Hippocampus (stimulated side)	5.4 ± 2.1	7.66 ± 0.18

*The locus coeruleus was stimulated for 15 minutes at 20 Hz with monophasic pulses 2msec in duration and with a constant current of 400 μA.

+The K_m for tyrosine was determined by the method of Lineweaver and Burke (12) at a $DMPH_4$ concentration of 10^{-3}M and seven tyrosine concentrations ranging from 10^{-3} to 10^{-7} M. Each value is the mean of the intercepts generated from six separate lines.

The K_i for norepinephrine was determined by the method of Dixon (13) at 6 norepinephrine concentrations (5 x 10^{-2} to 10^{-5}M) and three $DMPH_4$ concentrations (10^{-3} to 10^{-5}M).

innervation from the locus. This allosteric activation of tyrosine hydroxylase appears to be mediated in part by an increase in the affinity of the enzyme for substrate tyrosine and a decrease in the affinity for the end-product inhibitor norepinephrine. It is most likely that of these 2 alterations the most important change for in vivo modulation of enzyme activity is the change in affinity for norepinephrine. It is generally assumed that tyrosine hydroxylase in noradrenergic nerves is saturated with substrate since brain concentrations of endogenous tyrosine are in the range of 10^{-4}M (11). However, a 20 fold change in the affinity for the enzyme for the endproduct inhibitor norepinephrine could conceivably result in a significant increase in tyrosine hydroxylase activity in vivo.

The fact that it is possible to demonstrate a post-stimulation increase in the activity of tyrosine hydroxylase which withstands the freezing, thawing and homogenization of the tissue make it feasible to study this interesting

phenomenon in greater detail. It appears conceivable that the increase in hippocampal tyrosine hydroxylase activity induced by electrical stimulation of the locus coeruleus could be mediated through an as yet unidentified allosteric effector. For example, the observed post-stimulation alterations in the kinetic properties of tyrosine hydroxylase could be initiated as a result of either an increase in the formation of a positive allosteric activator or by removal of an allosteric inhibitor. Preliminary experiments in our laboratory in fact indicate that addition of calcium or cAMP to the high speed supernatant obtained from the rat hippocampus results in kinetic alterations in tyrosine hydroxylase similar to those observed upon electrical stimulation of the locus coeruleus. Thus it seems quite possible that changes in the influx of calcium or alterations in the endogenous levels of cAMP which probably occur during depolarization of noradrenergic terminals might in part be responsible for the kinetic activation of tyrosine hydroxylase which occurs during increased impulse flow in central noradrenergic neurons. Experiments are in progress to determine the mechanism responsible for this stimulus-induced activation of tyrosine hydroxylase.

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