Choline Administration: Activation of Tyrosine Hydroxylase in Dopaminergic Neurons of Rat Brain

Ismail H. Ulus; Richard J. Wurtman


Stable URL:
http://links.jstor.org/sici?sid=0036-8075%2819761203%293%3A194%3A4269%3C1060%3ACAAOTH%2E0%3E2.B%3E%3C2-7

Science is currently published by American Association for the Advancement of Science.

Your use of the JSTOR archive indicates your acceptance of JSTOR’s Terms and Conditions of Use, available at http://www.jstor.org/about/terms.html. JSTOR’s Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/journals/aaas.html.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact jstor-info@umich.edu.

http://www.jstor.org/
Wed Mar 10 14:26:19 2004
Choline Administration: Activation of Tyrosine Hydrolase in Dopaminergic Neurons of Rat Brain

Abstract. The administration of choline in doses previously shown to elevate brain acetylcholine concentrations also increases the activity of tyrosine hydrolase in rat caudate nuclei. This response can be blocked by atropine, a muscarinic antagonist. These findings indicate that choline-induced increases in acetylcholine concentrations may be associated with parallel changes in the amount of the neurotransmitter released into synapses.

The administration of choline by injection (1) or diet (2) raises the concentration of the neurotransmitter acetylcholine (ACh) in rat brain. This increase may or may not be associated with a change in the amount of transmitter actually released into synapses per unit time. Studies described in this report show that, by activating central muscarinic receptors, choline administration also elevates the activity in caudate nuclei of tyrosine hydroxylase (TOH), the enzyme that catalyzes the first step in catecholamine biosynthesis. This enzyme is absent from cholinergic neurons but is present in dopaminergic neurons that receive cholinergic inputs (3). Hence, its activation by choline suggests that choline administration actually does enhance ACh release, at least within the brain.

Male Sprague-Dawley rats (Charles River) weighing 150 to 200 g were housed in groups of eight in a controlled environment (23° to 24°C) for 2 to 4 days before use in an experiment. Animals had free access to food and water and were exposed to light (Vita-Lite, Duco-Test Corp., North Bergen, N.J.) daily between 7 a.m. and 7 p.m. Choline chloride (ChCl) was dissolved in saline and injected intraperitoneally in a total volume of 2 ml per kilogram of body weight. Rats were decapitated 2 hours after injection and their brains were quickly removed. The corpora striata were dissected (4) on an ice-cooled glass plate and immediately frozen on Dry Ice. Frozen tissues were weighed and homogenized in 10 volumes of 50 mM tris-acetate buffer, pH 6, containing 0.2 percent Triton X-100. The homogenates were placed in an ice bath for 20 to 30 minutes and then centrifuged at 10,000 × g for 10 minutes. The TOH activity in the supernatant fluid was assayed by the method of Waymire et al. (5) and the protein concentration by the method of Lowry et al. (6).

A single injection of 60 or 120 mg of ChCl per kilogram caused striatal TOH activity to increase by 19 percent (P < .05) or 36 percent (P < .01), respectively (Table 1). A smaller dose (30 mg/kg) failed to elevate TOH activity significantly. We have previously shown that this lower dose causes peak increments of 11 percent in brain ACh levels, while the 60-mg/kg dose causes brain ACh to rise by 22 percent (1). [We were unable to measure ACh and TOH activity in the same tissue samples because measurement of ACh requires that brain enzymes be inactivated—for example, by a focused microwave beam aimed at the head (1, 2).] The increase in TOH activity that follows choline administration could be blocked by treating rats concurrently with atropine sulfate, 40 mg/kg, intraperitoneally (Table 2). This muscarinic antagonist had no effect on TOH activity when injected alone (Table 2).

Javoy et al. (7) have recently shown that dopa synthesis (8) in the corpus striatum is accelerated when rats are treated with oxotremorine, a drug that stimulates central muscarinic receptors. In preliminary studies, we noted a similar acceleration of dopa synthesis in rats given choline (9). In animals killed 30 minutes after receiving RO-4-4602 (800 mg/kg, intraperitoneally), those that had also received choline reached 90 minutes before death had striatal dopa concentrations (957 ± 84 ng/g) 42 percent higher than those of rats that received only the dopa decarboxylase inhibitor (674 ± 45 ng/g, P < .01). Choline itself reportedly acts as a very weak agonist on muscarinic receptors; however, the brain choline levels attained after animals received the choline doses used here (1) were probably far too low for the choline to have any direct effect on ACh receptors. Hence, the activation of these receptors after choline administration most likely resulted from in-
creased release of ACh into synapses. This activation, in turn, was associated with, and may have resulted from, the precursor-induced increase in ACh synthesis and levels (1).

The precise anatomic mechanisms that mediate the choline-induced activation of striatal TOH are still unknown. Histological (10) and lesion (11) studies have been interpreted as showing that practically all of the ACh in striatal neurons is located within small interneurons. Considerable immunohistochemical (12) and biochemical (13) evidence suggests that at least some of these cholinergic interneurons receive presynaptic inputs from dopaminergic neurons; however, there is still no direct evidence that the inverse synaptic connections (that is, of cholinergic on dopaminergic neurons) also exist. Cholinergic drugs are known to accelerate both dopamine turnover (14) and dopamine synthesis (7, 8) and to increase the concentration of the dopamine metabolite homovanillic acid (HVA) (15) in the striatum. Conversely, such anticholinergic drugs as atropine and scopolamine diminish striatal dopamine turnover and decrease striatal HVA concentrations (16). Moreover, these drugs lessen the increase in striatal HVA content caused by giving rats drugs that block dopamine receptors—an increase thought to reflect activation of nigro-neostriatal neurons by a polysynaptic mechanism (7). Thus, increases in ACh release seem likely to cause parallel changes in the activation of nigro-neostriatal neurons. Such changes might be expected to affect TOH activity within these neurons. Dopaminergic terminals within the striatum apparently contain both muscarinic and nicotinic receptors, and the activation of these receptors facilitates dopamine release in vivo and in vitro (18). Hence, the choline-induced activation of TOH activity could be mediated either by a polysynaptic neuronal loop, which might involve axons terminating on dopaminergic cell bodies in the substantia nigra, or by axo-axonal synapses entirely within the substance.

Cholinergic activation by various pharmacological agents has also been shown to elevate TOH activity in such tissues as the adrenal medulla (19, 20), the superior cervical ganglia (19, 20), and the locus coeruleus (21). In the medulla and ganglia this increase can first be detected 16 to 24 hours after cholinergic activation, and, apparently, is mediated by accelerated synthesis of the enzyme protein (20). In the locus coeruleus, the increase in TOH activity also requires about 24 hours to become statistically

Table 1. Tyrosine hydroxylase activity (nanomoles CO₂ formed per hour per milligram of protein) in striata of rats receiving ChCl. Rats were killed 2 hours after receiving ChCl intraperitoneally. Tyrosine hydroxylase was assayed by measuring the rate of [¹⁴C]CO₂ formation from [1-carboxy-¹⁴C]tyrosine. The enzyme was incubated for 30 minutes at 37°C in a round-bottom flask containing tyrosine (0.1 mM) and 6,7-dimethyl-5,6,7-tritrihydropte-
inoine (0.5 mM) in a final volume of 120 μL. After a 2-minute incubation, the enzymatic reaction was initiated by adding 10 μL of [¹⁴C]tyrosine (specific activity, 12 mCi/nmol); the reaction was terminated by adding 0.5 mL of 10 percent trichloroacetic acid to the mix-
ture. Values are expressed as mean ± S.E.M. The number of samples assayed is indicated in parentheses. Linear regression analysis of enzyme activity against choline dose yielded a value of the correlation coefficient, r, of .55 (P < .05).

<table>
<thead>
<tr>
<th>Dose of ChCl (mg/kg)</th>
<th>TOH activity (nmole hour⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.91 ± 0.11 (14)</td>
</tr>
<tr>
<td>30</td>
<td>2.26 ± 0.18 (6)</td>
</tr>
<tr>
<td>60</td>
<td>2.28 ± 0.09* (9)</td>
</tr>
<tr>
<td>120</td>
<td>2.60 ± 0.20* (5)</td>
</tr>
</tbody>
</table>

*P < .05 compared with enzyme activity in samples from control rats by Student's t-test. \( P < .01 \).

significant; however, this increased activity does not appear to involve a change in the rate of enzyme synthesis (21). The speed with which choline increases striatal TOH activity (Table 1) strongly suggests that the increase is mediated by activation of preexisting enzyme protein. This activation is not associated with any apparent change in the properties of the enzyme: its Michaelis constant, \( Kₘ \), for tyrosine is similar in striatal homogenates from control animals (52 ± 2 μM) and from rats treated with 120-mg/kg choline (55 ± 6 μM).

As far as we know, these observations provide the first evidence that the increase in the brain level of a neurotransmitter (in this case, ACh) produced by administering its precursor (choline) can cause more of the transmitter to be re-

Table 2. Effect of atropine on the choline-in-
duced increase in striatal TOH activity. Ani-
mals received ChCl (60 mg/kg) and atropine sulfate (40 mg/kg) intraperitoneally 2 hours before they were killed. Values are expressed as mean ± S.E.M. The number of samples assayed is indicated in parentheses. Data were analyzed by analysis of variance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TOH activity (nmole hour⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.23 ± 0.09 (8)</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.68 ± 0.10* (9)</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>2.29 ± 0.08 (8)</td>
</tr>
<tr>
<td>Atropine sulfate + choline chloride</td>
<td>2.36 ± 0.05 (7)</td>
</tr>
</tbody>
</table>

*Different from control groups or from atropine-
treated groups at *P < .05.*

References and Notes

8. Dopa (dihydroxyphenylalanine) is the product of the hydroxylation of tyrosine and is the imme-
diate precursor of dopamine.
9. We estimated the rate at which the catechol was formed from tyrosine by measuring its accumu-

4 June 1976; revised 13 August 1976

Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge

Ismail H. Ulus
Richard J. Wurtman

3 DECEMBER 1976