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

Ismail H. Ulus; Richard J. Wurtman

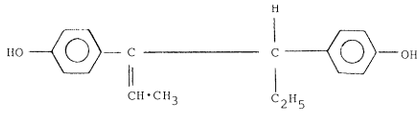
Science, New Series, Vol. 194, No. 4269 (Dec. 3, 1976), 1060-1061.

Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819761203%293%3A194%3A4269%3C1060%3ACAAOTH%3E2.0.CO%3B2-7

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

137, whereas DES gave only low-intensity signals at *m/e* 133 and 135 and no signal at *m/e* 137.

The residue from the second ethanol solution, containing about 190 ng of the unknown, was dissolved in 5 μl of bis(trimethylsilyl)acetamide, and one-half was examined by mass spectrometry after a reaction time of 2 minutes. The spectrum exhibited signals at the *m/e* values required by conversion of the compound to a di-trimethylsilyl derivative: 270 → 414; 253/255 → 397/399; 239/241 → 383/385; and 133/135/137 → 205/207/209. The appropriate shifts also occurred after trimethylsilylation of DES. These data suggest that the unknown is an isomer of DES, and one possibility is pseudo-DES.



This compound should display an intense signal for loss of an ethyl group, as the resulting ion with *m/e* 239 is both benzylic and allylic. Scission of the C-C bond between the two benzyl carbons results in the formation of ions of *m/e* 133 ($^{12}C_9H_9O$), 135 ($^{12}C_9H_{11}O$ and $^{12}C_8{}^{14}CH_9O$), and 137 ($^{12}C_8{}^{14}CH_{11}O$). Analogous ions for the trimethylsilyl derivative will appear at *m/e* 205, 207 and 209. The spectra obtained for an authentic sample of pseudo-DES (*5*) (free and derivative) match those for the unknown when the ^{14}C atom in the latter is taken into account (see Fig. 1), and the two substances behave identically in thin-layer chromatography.

Radiochemical purity is of transcending importance in tissue residue studies, particularly in view of current emphasis on analytical sensitivities and identifications at picogram levels. We have identified only one of several impurities present in the isotopic DES implants, and this was not apparent in the routine analytical procedure used for initial reporting of radiochemical purity. The labeled impurities found here may arise not only from the initial radioactive synthesis, but also from self-radiolysis (*6*) in the solid state during storage and during the experimental period.

D. M. TENNENT
R. F. KOUBA, W. H. RAY
Rhodia Inc., Hess and Clark Division, Ashland, Ohio 44805
W. J. A. VANDENHEUVEL
F. J. WOLF
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

References and Notes

1. P. W. Aschbacher and E. J. Thacker, *J. Anim. Sci.* **39**, 1185 (1974).
2. P. W. Aschbacher, E. J. Thacker, T. S. Rumsey, *ibid.* **40**, 530 (1975).
3. T. S. Rumsey, R. R. Oltjen, F. L. Daniels, A. S. Kozak, *ibid.*, p. 539.
4. T. S. Rumsey, R. R. Oltjen, A. S. Kozak, F. L. Daniels, P. W. Aschbacher, *ibid.*, p. 550.
5. The *cis* isomer was obtained from Chemetron Corporation.
6. P. Rochlin, *Chem. Rev.* **65**, 685 (1965).

15 June 1976; revised 8 September 1976

Choline Administration: Activation of Tyrosine Hydroxylase 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 hydroxylase 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, Duro-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 m*M* tris-acetate buffer, *p*H 6, containing 0.2 percent Triton X-100. The homogenates were placed in an ice bath for 20 to 30 minutes and were 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 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. Histochemical (*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 (*17*). 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 caudate.

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 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 kinetics of the enzyme: its Michaelis constant, K_m, 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 released. The evidence is indirect and involves the demonstration that choline administration activates postsynaptic cholinergic receptors (that is, the muscarinic receptors that are blocked by atropine), which then modify an enzyme (TOH) present in the postsynaptic neurons. Because variations in dietary choline content have also been shown to influence striatal ACh levels (*2*), the possibility exists that, by modifying ACh release, dietary factors can also affect the activities of catecholaminergic neurons and of other cells receiving cholinergic inputs.

Table 1. Tyrosine hydroxylase activity (nanomoles CO_2 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 $^{14}CO_2$ formation from L-[carboxy-^{14}C]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-5,6,7,8-tetrahydropterine (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 the [^{14}C]tyrosine (specific activity, 12 mc/nmole); the reaction was terminated by adding 0.5 ml of 10 percent trichloroacetic acid to the mixture. 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 ($P < .05$).

Dose of ChCl (mg/kg)	TOH activity (nmoles hour^{-1} mg^{-1})	
0	1.91 ± 0.11	(14)
30	2.26 ± 0.18	(6)
60	2.28 ± 0.09*	(9)
120	2.60 ± 0.20†	(5)

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

Table 2. Effect of atropine on the choline-induced increase in striatal TOH activity. Animals 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.

Treatment	TOH activity (nmole hour^{-1} mg^{-1})	
Control	2.23 ± 0.09	(8)
Choline chloride	2.68 ± 0.10*	(9)
Atropine sulfate	2.29 ± 0.08	(8)
Atropine sulfate + choline chloride	2.36 ± 0.05	(7)

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

ISMAIL H. ULUS
RICHARD J. WURTMAN
Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge

References and Notes

1. E. L. Cohen and R. J. Wurtman, *Life Sci.* **16**, 1095 (1975).
2. ———, *Science* **191**, 561 (1976).
3. F. E. Bloom, E. Costa, G. C. Salmoiraghi, *J. Pharmacol. Exp. Ther.* **150**, 244 (1965); N. E. Anden, *J. Pharm. Pharmacol.* **26**, 738 (1974); G. Bartholini, H. Stadler, K. G. Lloyd, in *Cholinergic Mechanisms*, P. G. Waser, Ed. (Raven, New York, 1975), p. 411.
4. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
5. J. C. Waymire, R. Bjur, N. Weiner. *Anal. Biochem.* **43**, 558 (1971).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
7. F. Javoy, Y. Agid, J. Glowinski, *J. Pharm. Pharmacol.* **27**, 677 (1975).
8. Dopa (dihydroxyphenylalanine) is the product of the hydroxylation of tyrosine and is the immediate precursor of dopamine.
9. We estimated the rate at which the catechol was formed from tyrosine by measuring its accumulation in brains of animals pretreated with a central decarboxylase inhibitor [A. Carlsson, M. Kehr, M. Lindqvist, T. Magnusson, C. Atac, *Pharmacol. Rev.* **24**, 371 (1972)] such as RO-4-4602 [R. J. Wurtman, F. Larin, S. Mostafapour, J. D. Fernstrom, *Science* **185**, 183 (1974)].
10. G. S. Lynch, P. A. Lucas, S. A. Deadwyler, *Brain Res.* **45**, 617 (1972).
11. S. G. Butcher and L. L. Butcher, *ibid.* **71**, 167 (1974).
12. T. Hattori, V. K. Singh, E. G. McGeer, P. L. McGeer, *ibid.* **102**, 164 (1976).
13. H. Stadler, K. G. Lloyd, G. Bartholini, *Arch. Pharmacol.* **283**, 129 (1974); H. Stadler, K. G. Lloyd, M. Cadea-Ciria, G. Bartholini, *Brain Res.* **55**, 476 (1973).
14. H. Corrodi, K. Fuxe, W. Hammer, F. Sjoqvist, U. Ungerstedt, *Life Sci.* **6**, 2557 (1967); J. Perez-Cruet, G. L. Gessa, A. Tagliamonte, P. Tagliamonte, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 216 (1971).
15. B. H. C. Westerink and J. Korf, *Eur. J. Pharmacol.* **33**, 31 (1975).
16. N. E. Anden and P. Bedard, *J. Pharm. Pharmacol.* **23**, 460 (1971); G. Bartholini and A. Pletscher, *Experientia* **27**, 1302 (1971).
17. N. E. Anden, *J. Pharm. Pharmacol.* **24**, 905 (1972); R. O'Keeffe, D. F. Sharman, M. Vogt, *Br. J. Pharmacol.* **38**, 287 (1970); N. E. Anden, S. G. Butcher, H. Corrodi, K. Fuxe, U. Ungerstedt, *Eur. J. Pharmacol.* **11**, 307 (1970).
18. M. F. Giorguieff, M. L. Le Floc'h, T. C. Westfall, J. Glowinski, M. J. Besson, *Brain Res.* **106**, 117 (1976).
19. R. A. Mueller, H. Thoenen, J. Axelrod, *J. Pharmacol. Exp. Ther.* **169**, 74 (1969).
20. H. Thoenen, *Life Sci.* **14**, 223 (1974).
21. T. Lewander, T. H. Joh, D. J. Reis, *Nature (London)* **258**, 440 (1975).
22. We thank M. C. Scally for his valuable assistance. These studies were supported in part by grants from the U.S. Public Health Service and the National Aeronautics and Space Administration. I.H.U. is the Catherine R. Alphonso Fellow of the Parkinson's Disease Foundation.

4 June 1976; revised 13 August 1976