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# Trans-synaptic induction of adrenomedullary tyrosine hydroxylase activity by choline: Evidence that choline administration can increase cholinergic transmission

(acetylcholine/tyrosine 3-monooxygenase/adrenal medulla)

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ABSTRACT **Twenty-four hours after rats receive choline chloride (20 mmol/kg, by stomach tube) the activity of tyrosine hydroxylase [tyrosine 3-monooxygenase; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] increases by 31% within adrenomedullary chromaffin cells. This treatment also causes major elevations in the levels of choline and acetylcholine within the adrenal gland; however, acetylcholine levels return to normal by 16 hr after the choline is given. The daily administration of 10 or 20 mmol/kg of choline for 4 days elevates adrenal tyrosine hydroxylase activity by 29% or 51%, respectively. Such increases in tyrosine hydroxylase activity are not observed in animals given ammonium chloride, another basic chloride-containing compound, by stomach tube or in animals treated with cycloheximide, an inhibitor of adrenal protein synthesis. They are also absent in denervated adrenals. These observations demonstrate that the increase in presynaptic acetylcholine *levels* produced by giving animals the neurotransmitter's precursor (choline) can be associated with parallel changes in the transmission of signals across cholinergic synapses, probably because more of the transmitter is released per nerve impulse.**

The administration of choline by injection (1), stomach tube (2), or diet (3) raises the level of the neurotransmitter acetylcholine (AcCh) in rat brain, and, in 2 hr, the activity of the enzyme tyrosine hydroxylase within the caudate nucleus (4). [Tyrosine hydroxylase—tyrosine 3-monooxygenase, EC 1.14.16.2; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating)—is the enzyme that catalyzes the first step in catecholamine biosynthesis.] Because this enzyme is absent from cholinergic neurons but is present in dopaminergic neurons that receive a cholinergic input, its increase after choline administration suggests that the resulting rise in AcCh levels can also be associated with an increase in cholinergic transmission, probably because more of the transmitter is released per nerve impulse (4).

The present study explores the relationship between choline-induced changes in AcCh levels and the release of the transmitter at a peripheral synapse—i.e., that of preganglionic cholinergic neurons on adrenomedullary chromaffin cells. The adrenal medulla is a better tool than the caudate nucleus for examining cholinergic transmission because its *sole* innervation is cholinergic, and its presynaptic cholinergic neurons can readily be removed surgically so that direct postsynaptic effects of choline can be distinguished from those requiring its presynaptic conversion to AcCh.

## MATERIALS AND METHODS

**Animals.** Male Sprague–Dawley rats weighing 150–200 g (Charles River Breeding Laboratories) were housed in groups of eight in a controlled environment (23–24°) for 3–5 days before an experiment. Animals had free access to food and water and were exposed to light (Vita-Lite, Duro-Test Corp.) daily between 8 a.m. and 8 p.m.

**Adrenal Denervation.** The nerve fibers leading from the left main splanchnic nerve to the left adrenal gland were carefully transected under sodium pentobarbital anesthesia 4 days before choline administration was begun. The right adrenal was also exposed, but its nerves were not severed; this adrenal served as a control for the denervated left adrenal.

**Assay of Tyrosine Hydroxylase.** The animals were killed by a blow on the head; the adrenal glands were then rapidly removed, dissected free of fat on an ice-cooled glass plate, and frozen on dry ice. Each adrenal was homogenized in 0.5 ml of ice-cold 50 mM Tris–acetate buffer (pH 6) containing 0.2% Triton X-100. Two 50 μl aliquots from each homogenate were used to measure tyrosine hydroxylase activity, which was assayed as described by Waymire *et al.* (5). In some experiments, the choline and AcCh contents of the adrenals were assayed radioenzymatically (1, 6).

**Chemicals.** Choline chloride (Aldrich Chemical Co.) was dissolved in water and administered by stomach tube in a volume of 5 ml/kg. Control animals received an equal volume of water. Ammonium chloride was dissolved in water and administered in the same manner. Cycloheximide (Nutritional Biochemicals Corp.) was dissolved in saline and injected intraperitoneally.

## RESULTS

**Effect of Choline on Adrenal Tyrosine Hydroxylase Activity.** A single dose of choline (20 mmol/kg) did not significantly affect adrenal tyrosine hydroxylase activity for the first 16 hr after its administration; after 24 hr, however, the adrenal enzyme activity was 31% higher in rats given choline than in control animals ($P < 0.05$). The activity of the catecholamine-synthesizing enzyme remained elevated after 48 hr (by 23% $P < 0.05$), but had returned to control values by 72 hr (Fig. 1). The daily administration of choline (20 mmol/kg for 4 days) elevated adrenal tyrosine hydroxylase activity by 51% (Fig. 2). Although a single administration of lower choline doses (5 or 10 mmol/kg) failed to elevate adrenal tyrosine hydroxylase activity, the administration of 10 mmol/kg per day for 4 days did significantly affect the enzyme ($P < 0.05$) (Fig. 2). In preliminary studies, we have observed a similar 40% increase in the tyrosine hydroxylase activity of the superior cervical ganglia among animals receiving choline by stomach tube for 4 days.

The 20 mmol/kg dose of choline caused marked but transient elevations in the levels of AcCh and choline in the adrenal gland

Abbreviation: AcCh, acetylcholine.

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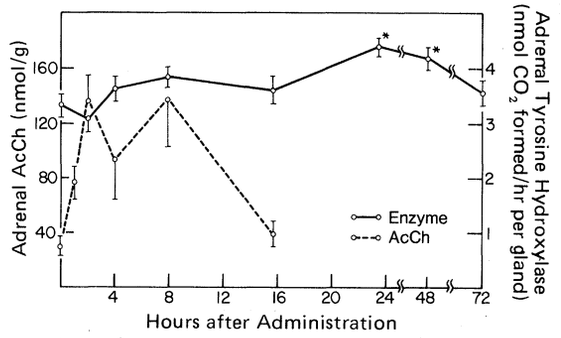


FIG. 1. Effect of choline chloride (20 mmol/kg, by stomach tube) on adrenal AcCh content and tyrosine hydroxylase activity. Each point represents the mean ± SEM of four to six adrenals; similar data were also obtained in a second experiment using five to six rats per group. *$P < 0.05$ compared with tyrosine hydroxylase activity in adrenals from control animals.

(Fig. 1). Adrenal AcCh increased by a factor of three or four for 8 hr but returned to normal levels within 16 hr of choline administration (i.e., long before a significant increase in tyrosine hydroxylase could be detected).

**Effect of Prior Denervation on the Induction of Tyrosine Hydroxylase by Choline.** Denervation 4 days before an experiment failed to modify adrenal tyrosine hydroxylase activity but completely blocked the induction of the enzyme by choline (20 mmol/kg per day for 4 days) (Fig. 3). The responses to choline administration of the innervated right adrenals (in animals with denervated left adrenals) were similar to those of adrenals in control animals: the hydroxylase activities were increased by 53% and 51%, respectively (Figs. 2 and 3).

**Effect of Protein Synthesis Inhibition on the Induction of Adrenal Tyrosine Hydroxylase by Choline.** The observation that adrenal tyrosine hydroxylase activity was elevated 24–48 hr after rats received choline, but not before this time (Fig. 1), suggested that the elevation was not caused by a change in the kinetic state of the enzyme, but by the synthesis of additional molecules of enzyme protein. To examine the importance of protein synthesis in this effect, we treated a group of animals with cycloheximide (1 mg/kg, intraperitoneally) concurrently with the choline dose, and then at 6-hr intervals until sacrifice (24 hr after choline administration). Cycloheximide alone failed to affect adrenal tyrosine hydroxylase activity; however, it completely blocked the response to choline (Fig. 4).

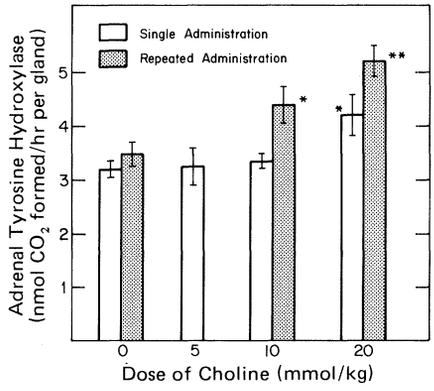


FIG. 2. Effect of choline chloride, given daily by stomach tube for 1 or 4 days, on adrenal tyrosine hydroxylase activity. (Control rats were given the same volume of water.) Animals were killed 24 hr after the last treatment. Each point represents the mean ± SEM of four to six adrenals. * $P < 0.05$; ** $P < 0.01$ when compared with tissues from control animals.

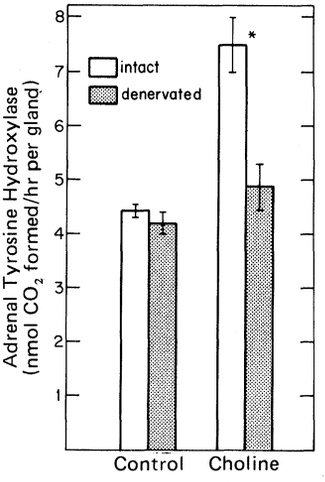


FIG. 3. Effect of prior denervation on the response of adrenal tyrosine hydroxylase activity to choline administration. The left adrenal was denervated 4 days before animals received the first of four daily injections of choline chloride (20 mmol/kg, by stomach tube). Animals were killed 24 hr after the last injection. Each point represents the mean ± SEM of five to seven adrenals. * $P < 0.01$ when compared with enzyme activities in denervated glands or in innervated adrenals from animals receiving water.

**Effect of Ammonium Chloride Administration on Adrenal Tyrosine Hydroxylase Activity.** To investigate the possibility that the induction of adrenal tyrosine hydroxylase by choline was a nonspecific consequence of the stress associated with intubation, or with the administration of a large amount of a basic compound or of the chloride ion, we examined the enzyme's activity in control animals given 20 mmol/kg of ammonium chloride. Unlike choline administration, this treatment had no effect on adrenal tyrosine hydroxylase activity; the enzyme activity was 6.47 ± 0.13 nmol of $CO_2$ formed/hr per gland in untreated animals and 6.65 ± 0.50 nmol in animals receiving the ammonium chloride.

## DISCUSSION

Other studies (7, 8) have shown that treatments that increase the release of AcCh from presynaptic cholinergic terminals cause a delayed increase in adrenomedullary tyrosine hydroxylase activity. The treatments used to produce this response

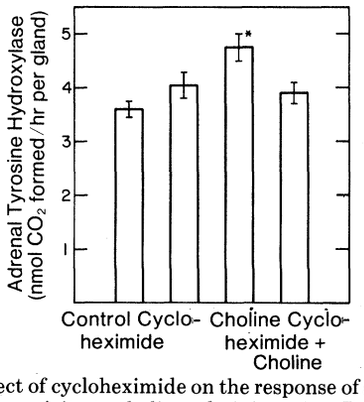


FIG. 4. Effect of cycloheximide on the response of adrenal tyrosine hydroxylase activity to choline administration. Rats received a single dose of choline chloride (20 mmol/kg, by stomach tube) and, concurrently, cycloheximide (1 mg/kg, intraperitoneally). Cycloheximide injections were repeated at 6 hr intervals, and animals were killed 24 hr after receiving the choline. Each point represents the mean ± SEM of five to six adrenals. * $P < 0.05$ when compared with control or (cycloheximide + choline) groups.

[e.g., hypotensive drugs (9–11), stress (12)] have all presumably acted centrally to increase the number of impulses flowing along the splanchnic nerves, rather than to change the number of AcCh molecules released per depolarization. The delay in the enzyme's response to these treatments reflects the time required to synthesize additional tyrosine hydroxylase molecules; this correlation has been affirmed by blocking the rise with inhibitors of protein synthesis (13) and by direct immunochemical assays of the enzyme protein in treated and untreated animals (14). The trans-synaptic induction of tyrosine hydroxylase by hypotensive drugs or stress has been mimicked by administering cholinergic agonists that act directly on nicotinic receptors on the chromaffin cell surface (15–17).

Choline administration by stomach tube can also elevate adrenal tyrosine hydroxylase activity (Figs. 1–4); the time-course of this effect is similar to that seen after the treatments, described above, that increase impulse flow along presynaptic cholinergic neurons. That the effect of choline is presynaptic is shown by its failure to increase enzyme activity in previously denervated adrenals (Fig. 3); that it involves the synthesis of additional molecules of enzyme protein is shown both by its time-course (Fig. 1), which differs from the rapid activation of tyrosine hydroxylase in the caudate nucleus (4), and by its failure to occur in animals given cycloheximide (Fig. 4), a drug that inhibits adrenal protein synthesis (13). The most likely explanation of choline's action on adrenal tyrosine hydroxylase is that, by increasing the AcCh levels in presynaptic terminals, it also increases the amount of transmitter released by each nerve impulse. The rise in enzyme activity fails to occur after the administration of a large dose of ammonium chloride by stomach tube—a treatment that presumably is as stressful as choline administration. This failure shows that the rise in tyrosine hydroxylase activity is not simply a centrally mediated stress response. It is, of course, possible that choline has a *specific* central action, i.e., to enhance transmission across central cholinergic synapses that control the rate at which impulses flow along the preganglionic sympathetic nerves to the adrenal medulla. However, in preliminary studies, we observed that choline also potentiates the effects on adrenal tyrosine hydroxylase of large doses of reserpine—a drug known to increase impulse flow along these nerves (18). This observation argues against a primary central action of the precursor. Similarly, choline administration fails to increase the activity of choline acetyltransferase in the adrenal—a change one would expect to find (19) if it acted centrally to enhance sympathetic impulse flow.

It is interesting that the choline-induced rise in adrenal AcCh levels (and thus, presumably, in AcCh release) terminates many hours before the first detectable increase in tyrosine hydroxylase activity (Fig. 1). This observation is compatible with the known half-life of the enzyme protein (12) and tends to support the view (20) that one or more "second messenger" processes, each with its own latency, mediates the transmission of signals from the nicotinic receptors on the chromaffin cell surface to the protein-synthetic apparatus within the cell. Choline administration may provide a useful and relatively specific experimental tool for characterizing these intermediate processes.

The present observations also provide strong evidence that precursor availability can control the transmission of signals across the synapse by changing the levels of the neurotransmitter within nerve terminals. They suggest, but do not prove, that the mechanism by which the precursor acts involves changing the number of transmitter molecules released when the neuron is depolarized (i.e., by increasing the number of quanta, or the number of molecules present in each quantum). Since the levels of choline and AcCh in brain (3) and, probably, peripheral neurons normally vary with the amount of choline in the diet, it seems possible that nutritional state might significantly influence the transmission of signals across some or all cholinergic synapses. The levels in mammalian brain of another neurotransmitter, serotonin, have also been shown to be controlled by the diet (21). Unfortunately, the lack of an easily identifiable serotoninergic synapse in peripheral organs has, to date, precluded the performance of studies similar to those described here for cholinergic neurons.

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