receptors are selectively destroyed by kainic acid. Whether the neurotoxic effect of kainic acid is mediated through presynaptic elements or through direct action on postsynaptic receptors is not known. In transmission electron microscopic studies, primary auditory terminals are not altered up to 18 hours after injection of 2 μg of kainic acid (*12*).

Our data suggest that the neurotoxic effects of kainic acid are directly related to the amount of primary auditory innervation of the neurons in the cochlear nucleus. Spherical cells in the anteroventral cochlear nucleus, which degenerated early after kainic acid injection, receive large auditory nerve endings on their cell bodies (*10, 13*). Stellate cells, in the anteroventral and posteroventral cochlear nucleus, which degenerated more slowly after kainic acid injection, receive auditory nerve endings only on their dendrites (*10*). Fusiform cells were the only cells identified as degenerating in the dorsal cochlear nucleus after kainic acid injection. This degeneration did not occur until 24 hours after the injection. Correspondingly, the dorsal cochlear nucleus receives only a light innervation from the auditory nerve, mainly in the fusiform cell area, with auditory nerve terminals mostly on basal dendrites of fusiform cells (*13, 14*). Granule cells, on the surface of the anteroventral and posteroventral cochlear nucleus and also within the dorsal cochlear nucleus, receive no primary auditory innervation (*13*) and were unaffected by kainic acid.

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Feedback Inhibition of Brain Noradrenaline Neurons by Tricyclic Antidepressants: α-Receptor Mediation

Abstract. *The use of different receptor blocking agents and single-unit recording techniques indicates that feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants is mediated by presynaptic α-receptors. After chronic imipramine treatment, noradrenaline neurons in the locus coeruleus of rat brain remained partly depressed, in agreement with clinical data. They were, however, resistant to further inhibition by imipramine or clonidine.*

Inhibition of reuptake at the nerve cell membrane of central monoamine neurons appears to be a major action of tricyclic antidepressant drugs (*1*). Secondary amines such as desipramine (DMI) are particularly potent on noradrenaline (NA) neurons, whereas tertiary amines such as imipramine (IMI) are more potent blockers of serotonin (5-hydroxytryptamine) (5-HT) uptake in vivo (*1*). The monomethylated antidepressants (such as DMI) preferentially reduce

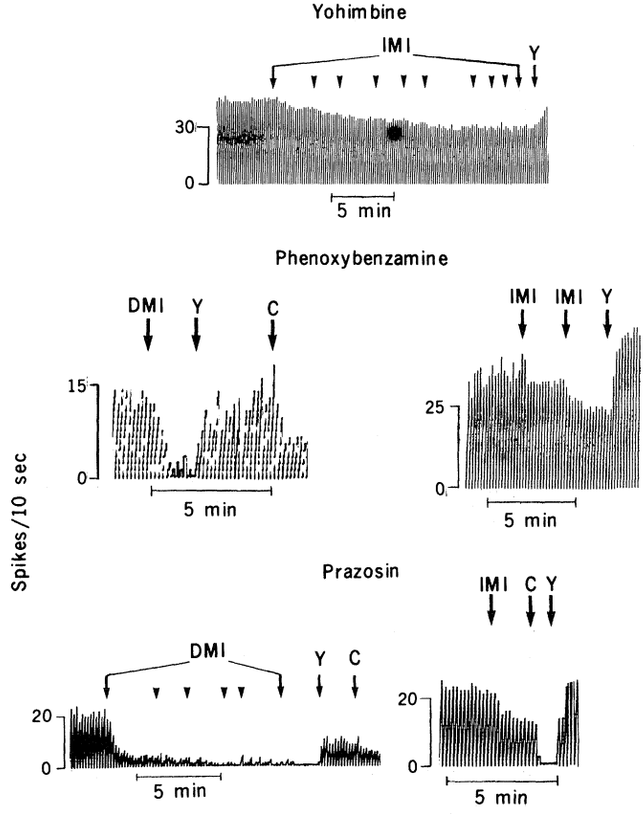

Fig. 1. Effect of various α-receptor blocking agents on the inhibition of locus coeruleus NA neurons by tricyclic antidepressants and clonidine. (Top) Treatment with yohimbine (*Y*), (10 mg/kg intraperitoneally), 30 minutes before recording almost completely blocked the inhibition by IMI (0.8 mg/kg intravenously, at arrows). Additional yohimbine (0.5 mg/kg intravenously) quickly reversed the weak inhibition ($n = 6$). (Middle) Treatment with phenoxybenzamine (10 mg/kg intraperitoneally) 30 minutes before recording did not block the inhibition by DMI (0.5 mg/kg intravenously) ($n = 4$) and only partially blocked that of IMI (0.8 mg/kg intravenously, at arrows) ($n = 3$). Both effects were rapidly reversed by yohimbine (0.5 mg/kg intravenously), after which clonidine (*C*) (20 μg/kg intravenously) only partially inhibited the firing rate of the unit. (Bottom) Treatment with prazosin (0.6 mg/kg intraperitoneally) 30 minutes before recording did not prevent the decrease in firing rate by DMI (0.20 mg/kg intravenously, at arrows) ($n = 4$), or by IMI (0.8 mg/kg intravenously) ($n = 3$) and clonidine (20 μg/kg intravenously), which completely silenced the cell. In contrast yohimbine (0.5 mg/kg intravenously) promptly restored the activity of the neuron.

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brain NA turnover, whereas their dimethylated analogs (such as IMI) preferentially reduce brain 5-HT turnover (*2*). The use of single-unit recording techniques revealed that low doses of the tricyclics decreased the firing rates of central NA and 5-HT neurons, respectively, although, as in the biochemical experiments, no absolute selectivity was found (*3*).

The inhibition of NA neurons in the locus coeruleus of the rat, obtained by all tested tricyclics, is probably mediated through a negative feedback mechanism since, for example, it can be blocked by previous depletion of NA (*3*). The receptor involved might be an α-receptor since clonidine, an antihypertensive α-receptor agonist, specifically inhibits the locus coeruleus neurons, and this action is antagonized by α-receptor blocking agents such as piperoxane or yohimbine (*4*, *5*). These agents, in contrast to other α-blocking agents such as phenoxybenzamine, also antagonize the deceleration of NA release and turnover, cardiovascular depression, and sedation after small doses of clonidine.

The receptors that mediate these effects have been termed presynaptic receptors or autoreceptors (*4*–*7*). However, there is another type of central α-receptor that is activated by high but not low doses of clonidine. These receptors mediate behavioral excitation as well as classical postsynaptic stimulation in the spinal cord; they are poorly blocked by piperoxane or yohimbine but effectively blocked by phenoxybenzamine (*7*, *8*).

The present study was undertaken to characterize the feedback inhibition by tricyclics of locus coeruleus neurons by use of several α-receptor blocking agents and single-unit recording techniques. Piperoxane was reported to block the inhibition by DMI (*5*). Hence, we included yohimbine, phenoxybenzamine, and also prazosin, which has been claimed to block classical postsynaptic α-receptors almost exclusively (*9*). We also studied the effect of chronic IMI treatment on the firing rate of NA neurons in the locus coeruleus, since biochemical data suggest that feedback inhibition of brain NA turnover gradually develops into increased turnover after chronic treatment with agents such as IMI (*10*). In view of the antagonism by tricyclics of the antihypertensive effect of clonidine (*11*), the interaction between these drugs was also explored.

Male Sprague-Dawley rats weighing about 250 g were used. The animals were anesthetized with chloral hydrate (400 mg per kilogram of body weight, intraperitoneally) and mounted in a stereotaxic apparatus; additional injections were given as needed. A 3-mm burr hole was drilled in the occipital bone 1.1 mm lateral to the midline and 1.1 mm posterior to lambda. For single-barrel experiments a micropipette with a tip diameter of 1 μm filled with 2*M* NaCl saturated with fast green (in vitro impedance, 3 to 7 megohms at 135 Hz) was lowered into the brain. The electrode potentials were passed through a high-input impedance amplifier and filters. Each spike was discriminated and fed into an integrator (reset every 10 seconds) and finally displayed on an oscilloscope, an audiomonitor, and a recorder. Drugs were administered into a tail vein. The body temperature of the animals was kept at 36° to 37°C. Only one cell was recorded in each animal.

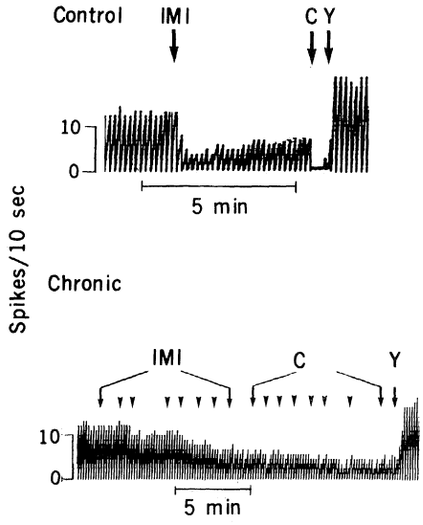

Fig. 2. Effect of IMI, clonidine, and yohimbine on locus coeruleus neurons in animals given chronic IMI treatment (bottom) and in control animals (top). The top trace shows typical depression induced by IMI (0.6 mg/kg intravenously) followed by silencing with clonidine (5 μg/kg intravenously) and excitation by yohimbine (2 mg/kg intravenously) (n = 3). Animal treated chronically with IMI shows resistance to the decrease in NA neuronal firing rate by IMI (0.2 mg/kg intravenously, at arrows) and clonidine (20 μg/kg intravenously, at arrows) (n = 11). However, the neuron was rapidly excited by yohimbine (0.5 mg/kg intravenously).

In some experiments microiontophoretic techniques were used, with circuitry essentially as described (*12*) except that a solid-state design was chosen. Micropipettes with a tip diameter of 4 to 5 μm were used; a few strands of fiberglass were inserted before pulling to facilitate direct filling of the tips by capillary action (*13*). The center barrel was filled with 2*M* NaCl solution saturated with fast green and was used for recording action potentials. The side barrels contained 4*M* NaCl solution for automatic current balancing (*12*) and a dilute solution of clonidine (0.01*M* in 0.1*M* NaCl, *p*H 4.0, to reduce its transport number) (*4*, *5*). Between ejections, which were performed frequently to avoid dead-time artifacts, a retaining current of 10 nA was maintained. The in vitro impedances were typically 2.5 to 5 megohms in the central barrel and 20 to 80 megohms in drug barrels. The agonist was always tested by application of an iontophoretic current of 6.5 nA for 1 minute (*5*). The maximum decrease from baseline was used for comparisons; baseline was defined as the average firing rate during the four 10-second intervals immediately before drug application.

Data for several trials in different units in different animals were pooled and the percentage of inhibition (mean ± standard error of mean) was calculated. Recording sites were marked at the end of each experiment by iontophoretic ejection of fast green. The rats were then perfused through the heart with 10 percent formaldehyde, and serial 50-μm frozen sections of the brain were cut, mounted, and stained with cresyl violet and counterstained with neutral red. Generally, a typical result from 3 to 11 locus coeruleus units is shown. The neurophysiological characteristics of the cells were identical to those previously described (*4*, *5*). None of the drugs caused reduction in spike amplitude.

Treatment with phenoxybenzamine or prazosin was largely ineffective in preventing the later inhibition of central NA neurons in the locus coeruleus by DMI or IMI, whereas yohimbine almost totally blocked the inhibition by IMI and also rapidly reversed the inhibition by the tricyclics obtained despite treatment with phenoxybenzamine or prazosin (Fig. 1). The dose of phenoxybenzamine used completely antagonizes the stimulation of postsynaptic α-receptors in the spinal cord by high doses of clonidine, whereas yohimbine in the dose used here was ineffective in this respect (*7*). Prazosin (*14*) was also ineffective in antagonizing the inhibitory action of a small dose of clonidine (Fig. 1).

In rats chronically treated with IMI (10 mg/kg intraperitoneally at 12-hour intervals for 11 days), the average firing rate 12 to 16 hours after the last injection was 1.3 ± 0.2 sec^{-1} (mean ± standard error of mean) (n = 34) compared with 2.6 ± 0.2 sec^{-1} (n = 37) in saline-treated control rats ($P < .001$, Student's t-test). This apparent reduction in firing rate of the NA neurons in rats chronically treated with IMI is at variance with previous biochemical data in rats (*10*) possi-

bly because of the more indirect methodology used in the earlier experiments, but is consistent with clinical reports showing lowered concentrations of NA metabolites in the cerebrospinal fluid of patients being treated with tricyclics (*15*). Neurons in chronically treated rats showed a marked resistance to further inhibition by IMI or by subsequent injections of clonidine (Fig. 2) (n = 11). The resistance to clonidine was less marked in chronically treated rats that were not given IMI immediately before clonidine (the last IMI injection was 12 hours earlier). Also, in these rats the response to microiontophoretic application of the α-agonist (44.0 ± 5.5 percent inhibition; n = 9) was significantly reduced compared to that in saline-treated controls (74.0 ± 5.5 percent inhibition, n = 10) ($P < .005$, Student's t-test). The mechanism could conceivably involve reduced sensitivity of the presynaptic receptor, but in view of the apparent dependence on dose of IMI, a receptor-blocking action of the tricyclics involved may also be implicated. Some biochemical data support the latter contention (*16*).

In any case, the reduced response of the presynaptic α-receptor may be significant in the antagonism by tricyclics of the antihypertensive effect of clonidine (*11*). The result also raises the possibility that with chronic antidepressant treatment, not only is NA reuptake inhibited but the NA system is also stabilized against changes, particularly reduction in firing rate.

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Angiotensin Regulates Release and Synthesis of Serotonin in Brain

Abstract. *Angiotensin II released serotonin from neuron terminals and accelerated synthesis of the serotonin. This increase in synthesis depended on the activation of tryptophan hydroxylase. A biphasic effect was observed: at high doses the stimulatory effect depended on conversion of angiotensin II to angiotensin III. At low doses an inhibitory effect was found, possibly dependent on an angiotensin II metabolite. These actions represent a subtle regulation of the open-loop serotonin system.*

Angiotensins I and II have been found in the nervous tissue (*1-4*). The distribution of angiotensin II in mammalian brain has been mapped (*5*). The occurrence of this polypeptide in certain neurons, fibers, and terminals of the central nervous system (*6-8*) facilitates the study of its possible participation in neurotransmission.

One of the questions we have studied is whether pressor response of angiotensin II, when injected into cerebral ventricles, is a direct action or one mediated by a particular neurotransmitter. The results of Vollmer and Buckley (*9*), showing that the intraventricular administration of phentolamine—an adrenergic receptor antagonist—potentiates the central hypertensive effect of angiotensin II, suggested that the central adrenergic system could modify the angiotensin response. Moreover, angiotensin II activates certain neurons, causing an enhanced sympathetic outflow (*10*), and can increase the biosynthesis of norepinephrine from tyrosine (*11*). We have also demonstrated that serotonin is selectively released from mast cells by angiotensin II (*12*), and others have found that serotonin plays some role in the regulation of arterial blood pressure (*13*). Since a good correlation was demonstrated between the concentration of angiotensin in cerebrospinal fluid and systolic blood pressure in patients with essential hypertension, we postulated the existence of an angiotensin-serotonin axis in the central nervous system (*14*, *15*). Thus, in the experiments reported here, we studied the pressor responses to angiotensin II injected either into the third ventricle or the cisterna magna in rats depleted of serotonin by treatment with *p*-chlorophenylalanine (*p*-CPA), a tryptophan hydroxylase inhibitor (*16*). For these experiments we used white Wistar rats weighing 300 g. The rats were anesthetized with embutal and a 21-gauge BD needle was inserted through the nose just into the third ventricle. Cisternal punctures were performed through the first vertebral space, and a polyethylene cannula was placed in the cisterna and fixed to the skin. At the end of each experiment the site of injection was verified with a color dye. The volume injected was usually 20 μl and always less than 100 μl. Arterial blood pressure was monitored throughout the experiment.

The injection of angiotensin II (from 2 to 200 ng) into the third ventricle of the brain produced significant increases in blood pressure. Both systolic and diastolic pressure increased 20 ± 3 (standard error) and 15 ± 4 mm-Hg, respectively, after the injection of 50 ng of angiotensin II. Intraventricular injection of saline (100 μl) did not elicit any significant

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