Presynaptic Alpha-Receptor Subsensitivity After Long-Term Antidepressant Treatment

Abstract. *After 3 weeks of twice-daily administration of desipramine to rats, the frequency-response curve for field stimulation of adrenergic neurons in isolated left atrial strips was shifted markedly to the left and the efflux of [^{3}H]norepinephrine was enhanced greatly. After 1 day of treatment, only slight shifts in the frequency-response curve and small increases in [^{3}H]norepinephrine efflux occurred although inhibition of [^{3}H]norepinephrine uptake was already maximal, and phenoxybenzamine caused a further shift to the left in the frequency-response curve similar to that which occurred after 3 weeks of desipramine treatment alone. A gradual decrease in the sensitivity of the presynaptic α receptor would explain the delay in the onset of the clinical effect of the tricyclic antidepressants.*

Several weeks of treatment with tricyclic antidepressants usually are required for the clinical reversal of depression (*1*). Some investigators have suggested that these drugs exert their clinical effects by inhibiting norepinephrine uptake into adrenergic neurons in the central nervous system (*2*). However, this inhibition of uptake occurs shortly after the onset of tricyclic antidepressant administration (*3*). A possible explanation for the delayed clinical response is that during long-term administration of tricyclic antidepressants the amount of norepinephrine released from adrenergic neurons during nerve transmission increases gradually. Recently, norepinephrine was shown to regulate its own release by stimulating a presynaptic inhibitory α receptor (*4*). The purpose of the present study was to determine whether the long-term administration of desipramine, a prototype of the tricyclic antidepressants, potentiates adrenergic nerve transmission by increasing the release of norepinephrine and whether such increases in release are mediated by changes in the sensitivity of the presynaptic α receptor to norepinephrine.

Adrenergic nerve transmission was studied with strips of left atrial muscle isolated from male Sprague-Dawley rats. Rats were treated with desipramine (10 mg/kg, intraperitoneally) every 12 hours either for 1 day or for periods up to 6 weeks and then were killed 12 hours after the last injection. Frequency-response curves for field stimulation of adrenergic neurons were obtained from strips of left atrial muscle suspended between a small plastic clamp and a strain-gauge transducer in organ baths which contained 10 ml of Krebs-Henseleit solution at 37°C. Atrial strips were driven by electrical stimuli (two per second) through a punctate platinum electrode in contact with the tissue. Two platinum electrodes, one on either side of the muscle strip, were used to stimulate adrenergic nerve endings (field stimulation) during the refractory period of the muscle at frequencies ranging from one stimulus every 60 seconds (17 mHz) to eight stimuli per second (8 Hz) (*5*). Field stimulation of adrenergic nerve endings caused frequency-dependent increases in the force of contraction of the left atrial strips (Fig. 1). Frequency-response curves were compared by determining the lowest frequency which produces 50 percent of the maximum response, that is, the EF_{50} (50 percent effective frequency) (*6*). In control experiments atria reached a maximum response at a frequency of 4 Hz, and the EF_{50} was 688 ± 110 mHz. In the presence of a high concentration of desipramine, $10^{-5}M$, the frequency-response curve was shifted 4.5-fold to the left (EF_{50}, 150 ± 43 mHz, $P < .01$). After 1 day of desipramine administration, a shift to the left occurred (EF_{50}, 188 ± 32 mHz, $P < .005$) similar to that seen when desipramine, $10^{-5}M$, was added to the bath (Fig. 1A). After treatment with desipramine for 3 weeks, the frequency-response curve was shifted 17-fold to the left of control curves (EF_{50}, 40.1 ± 7.3 mHz, $P < .001$) and 4.7-fold to the left of curves from animals treated for only 1 day with desipramine ($P < .001$). This shift in the frequency-response curve developed gradually and was maximal after 3 weeks of treatment. These shifts to the left in the frequency-response curves could be due either to an increase in the sensitivity of the cardiac β receptor to norepinephrine or to an increase in the synaptic concentration of norepinephrine in contact with the cardiac β receptors.

To ascertain whether the sensitivity of the cardiac β receptor increased during long-term desipramine administration, concentration-response curves for exog-

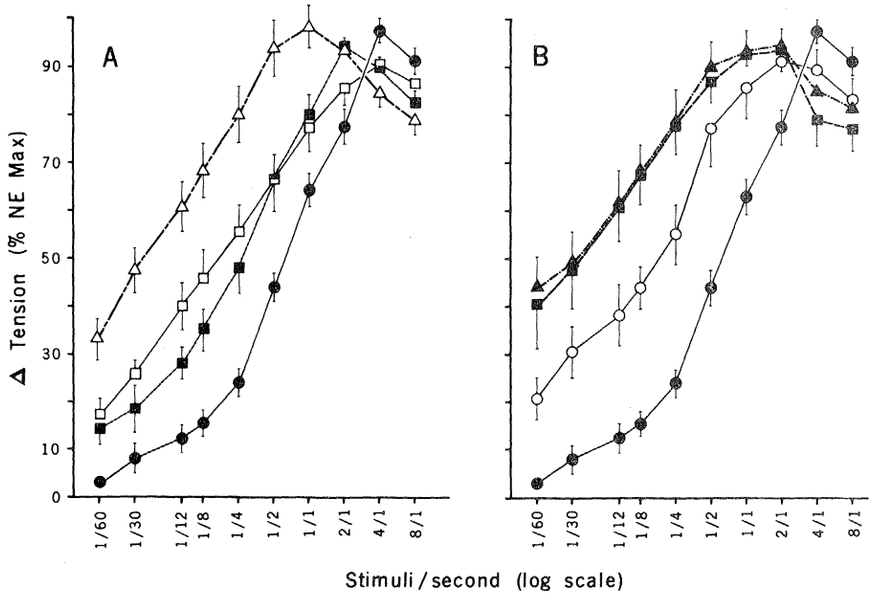

Fig. 1. Frequency-response curves for field stimulation of left atrial strips isolated from rats given short- or long-term treatment with desipramine and the effects of desipramine and phenoxybenzamine in vitro. Given are the mean values; vertical lines represent standard errors of the mean. The ordinates show the change in tension developed, expressed as a percentage of the maximum response to norepinephrine (*NE*) ($3 \times 10^{-5}M$). (A) Frequency-response curves for left atrial strips from 25 control animals (●); seven normal animals with desipramine, $10^{-5}M$, added in vitro prior to stimulation (□); 15 animals treated with desipramine for 1 day, 10 mg/kg every 12 hours (■); and 14 animals treated with desipramine for 3 weeks, 10 mg/kg every 12 hours (△). (B) Frequency-response curves of left atrial strips from 25 control animals (●); eight normal animals with phenoxybenzamine, $10^{-7}M$, added in vitro 30 minutes prior to stimulation (○); eight animals treated with desipramine for 1 day, 10 mg/kg every 12 hours, and with phenoxybenzamine, $10^{-7}M$, added in vitro 30 minutes prior to stimulation (■); and nine animals treated with desipramine for 3 weeks, 10 mg/kg every 12 hours, and with phenoxybenzamine, $10^{-7}M$, added in vitro 30 minutes prior to stimulation (▲).

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enous *l*-norepinephrine were determined, and EC_{50}'s (50 percent effective concentrations) were calculated. Left atrial strips were prepared in the same manner as described above. *l*-Norepinephrine was added in a stepwise manner to increase the concentration in the bath approximately threefold with each addition of drug (*7*). Concentration-response curves to exogenous *l*-norepinephrine revealed that the responses of control atria (EC_{50}, $3.4 \pm 0.5 \times 10^{-8} M$) were not shifted by the addition of desipramine, $10^{-5} M$ (EC_{50}, $2.9 \pm 0.4 \times 10^{-8} M$), to the organ bath. Furthermore, desipramine administration either for 1 day (EC_{50}, $4.4 \pm 0.5 \times 10^{-8} M$) or for 3 weeks ($EC_{50}$, $3.0 \pm 0.7 \times 10^{-8} M$) did not alter significantly the sensitivity of atria to *l*-norepinephrine. Thus, the sensitivity of the cardiac β receptor to *l*-norepinephrine is not changed during desipramine administration. Therefore, the shifts to the left in the frequency-response curve must be due to increases in the synaptic concentration of norepinephrine.

Increases in the synaptic concentration of norepinephrine could result from increases in the neuronal release of norepinephrine or from decreases in the removal of norepinephrine from the synapse by neuronal reuptake which is the major mechanism for removing norepinephrine from the synapse (*8*). To determine whether there were changes in the removal of norepinephrine from the synapse, we studied the uptake and retention of [^{3}H]norepinephrine by isolated left atrial strips. [^{3}H]Norepinephrine was added to the bath for 10 minutes; the tissues then were washed and rinsed several times; and the [^{3}H]norepinephrine retained by the tissues was adsorbed onto alumina and subsequently eluted into glass scintillation vials with 4.5 ml of 0.2*N* acetic acid (*9*).

These studies showed that the retention of [^{3}H]norepinephrine was reduced from a control value, per 10 minutes, of 255 ± 23 pmole/g to 59 ± 9 pmole/g after 1 day of desipramine administration and to 56 ± 6 pmole/g after 3 weeks of desipramine administration. The inhibition of norepinephrine uptake and retention could explain the shift to the left of the frequency-response curve after 1 day of desipramine administration. However, [^{3}H]norepinephrine uptake and retention were inhibited equally after either 1 day or 3 weeks of desipramine treatment while the frequency-response curve of atria from rats previously treated for 3 weeks with desipramine was further shifted 4.7-fold to the left of the 1-day desipramine treatment curve (Fig. 1A). These data suggest that desipramine administration for 1 day might increase slightly the synaptic concentration of norepinephrine by blocking norepinephrine reuptake, but that long-term desipramine administration for 3 weeks greatly increases the synaptic concentration of norepinephrine by some additional mechanism such as by increasing the amount of norepinephrine released from adrenergic neurons during electrical stimulation.

To determine more directly whether the amount of norepinephrine released after long-term desipramine administration was increased, we incubated isolated left atria with [^{3}H]dihydroxyphenylalanine ([^{3}H]dopa, specific activity 13 Ci/mmole, $1.5 \times 10^{-6} M$) for 1 hour in the presence of pargyline ($3 \times 10^{-5} M$). After the incubation with [^{3}H]dopa, the atria were washed thoroughly every 5 minutes for 45 minutes. At the end of the wash period, the efflux of [^{3}H]norepinephrine was determined for a 10-minute period prior to stimulation and a 10-minute period during field stimulation at 0.5 Hz (*10*). When calcium was omitted from the buffer, the effluxes of [^{3}H]norepinephrine prior to and during field stimulation were identical (Fig. 2). In contrast, the efflux of [^{3}H]norepinephrine during field stimulation in a calcium-containing buffer was always significantly greater than the efflux prior to stimulation. This observation indicates that in the presence of calcium the differences between the efflux of [^{3}H]norepinephrine prior to and during field stimulation were due to [^{3}H]norepinephrine released from adrenergic neurons (*11*).

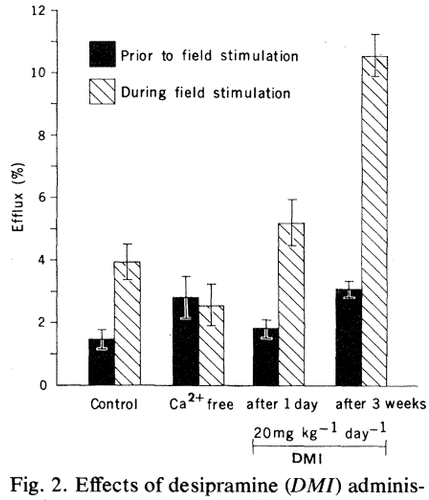

Fig. 2. Effects of desipramine (*DMI*) administration on the efflux of [^{3}H]norepinephrine during field stimulation. Atria were incubated with [^{3}H]dopa for 1 hour and then washed for 45 minutes (*10*). Solid bars, efflux of [^{3}H]norepinephrine during the 10-minute period prior to field stimulation; striped bars, efflux of [^{3}H]norepinephrine during the 10-minute period of field stimulation. Bars represent mean values ± standard errors (vertical lines) for eight controls (168.0 percent increase, $P < .005$); three calcium-free preparations (no significant change); six preparations from rats treated for 1 day with desipramine (186.8 percent increase, $P < .005$); and six preparations from rats treated for 3 weeks with desipramine (236.6 percent increase, $P < .0005$).

The efflux of [^{3}H]norepinephrine during field stimulation of control atria was 3.9 ± 0.6 percent of the total disintegrations per minute for the tissue; after 1 day of desipramine administration, the efflux during field stimulation increased by 32 percent to 5.2 ± 0.7 percent of the total disintegrations per minute. This moderate increase in efflux corresponds well with the moderate shift in the frequency-response curve for field stimulation after 1 day of desipramine administration. After 3 weeks of desipramine administration, the efflux of [^{3}H]norepinephrine during field stimulation was increased markedly by 168 percent ($P < .001$) of the control efflux and by 102 percent ($P < .001$) of the efflux during stimulation after 1 day of desipramine treatment. After 3 weeks of desipramine treatment, the efflux during field stimulation was 10.6 ± 0.7 percent of the total disintegrations per minute. Thus, both the increased efflux of [^{3}H]norepinephrine during field stimulation and the shifts to the left of the frequency-response curves indicate that after long-term desipramine administration the amount of norepinephrine released by adrenergic nerves during electrical stimulation is increased markedly.

Recent data suggest that norepinephrine regulates its own release (*4*). Norepinephrine within the synapse may stimulate presynaptic α receptors which subsequently reduce the amount of norepinephrine released per nerve impulse. To assess whether the increases in norepinephrine release after long-term desipramine administration were related to changes in the sensitivity of the presynaptic α receptor, we obtained frequency-response curves in the presence of phenoxybenzamine, an α-receptor antagonist. Phenoxybenzamine, $10^{-7} M$, was added to the organ bath 30 minutes prior to field stimulation. In preliminary experiments this concentration of phenoxybenzamine had no effect on the uptake of [^{3}H]norepinephrine by isolated left atrial strips. In addition, it is a concentration which other investigators have reported to be effective in blocking the presynaptic α receptor (*4*). In control

experiments, phenoxybenzamine shifted the frequency-response curve 3.4-fold to the left (EF_{50}, 201 ± 79 mHz, $P < .0125$, Fig. 1B). When phenoxybenzamine was added to the organ bath with atria from rats treated with desipramine for 1 day, it further shifted the frequency-response curve 5.7-fold to the left (EF_{50}, 32.9 ± 10.9 mHz, $P < .001$). Since norepinephrine uptake and retention are maximally blocked after 1 day of desipramine administration, this further shift to the left in the frequency-response curve caused by phenoxybenzamine is most probably due to an increase in the amount of norepinephrine released per nerve impulse. In addition, it is important to note that in the presence of phenoxybenzamine, $10^{-7}M$, the frequency-response curve for atria from rats treated with desipramine for 3 weeks (EF_{50}, 40.1 ± 7.3 mHz) was almost identical to the frequency-response curve for atria from rats treated with desipramine for 1 day in the presence of phenoxybenzamine, $10^{-7}M$. Thus, the inhibition of presynaptic α receptors by phenoxybenzamine increases the release of norepinephrine from control atria and from atria of rats treated for 1 day with desipramine, but not from atria of rats given long-term treatment with desipramine. These observations suggest that after 3 weeks of desipramine administration presynaptic α receptors are "subsensitive" to norepinephrine and therefore no longer inhibit neurotransmitter release. This subsensitivity results in a net increase in the amount of norepinephrine released per nerve impulse.

Numerous investigators have suggested that the tricyclic antidepressants act by enhancing adrenergic nerve transmission in the central nervous system (*12*). However, it is difficult to reconcile the rapid inhibition of norepinephrine uptake with the delayed onset of antidepressant action. Our data indicate that short-term desipramine administration maximally blocks norepinephrine uptake but only slightly potentiates adrenergic nerve transmission. However, during long-term desipramine administration, the release of norepinephrine is increased because of the loss of presynaptic α-receptor inhibition. This increase in norepinephrine release during long-term desipramine administration is consistent with the findings of Schildkraut *et al.* that during long-term tricyclic antidepressants the rate of disappearance of [^{3}H]norepinephrine from the rat brain gradually increases (*13*). It is also consistent with clinical studies showing that in the cerebral spinal fluid the concentration of 3-methoxy-4-hydroxyphenylglycol, a metabolite of norepinephrine, increases in patients who respond to therapy (*14*). Thus, the increase in norepinephrine release caused by presynaptic α-receptor subsensitivity appears to represent the clinically important mechanism of action of the tricyclic antidepressants.

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Neural Lateralization of Species-Specific Vocalizations by Japanese Macaques (*Macaca fuscata*)

Abstract. *Five Japanese macaques and five other Old World monkeys were trained to discriminate among field-recorded Japanese macaque vocalizations. One task required discrimination of a communicatively relevant acoustic feature ("peak"), and a second required discrimination of an orthogonal feature of the same vocalizations ("pitch"). The Japanese animals more proficiently discriminated the peak feature when stimuli were presented to the right ear (primarily left cerebral hemisphere), as opposed to the left ear (primarily right hemisphere). In discriminating the pitch feature, the Japanese animals either showed (i) a left-ear processing advantage or (ii) no ear advantage. The comparison animals, with one exception, showed no ear advantage in processing either feature of the vocalizations. The results suggest that Japanese macaques engage left-hemisphere processors for the analysis of communicatively significant sounds that are analogous to the lateralized mechanisms used by humans listening to speech.*

Considerable evidence from anatomical, electrophysiological, and behavioral studies suggests that, in humans, the left cerebral hemisphere is more critically involved than the right in the production and perception of speech sounds (*1-6*). The question of whether other animals possess neurally lateralized mechanisms for the production or analysis of communication sounds is of inherent interest to biologists concerned with the neural specializations evolved by species for vocal communication, and, moreover, any answers would carry important implications for nearly any theory of the origin of human speech and language. Nottebohm and his associates (*7*) have found in several species of birds that projections from vocal control areas in the left hemisphere play a more crucial role in species-specific song production than corresponding right-hemisphere projections. Several other investigators have examined a variety of noncommunicative behaviors to determine whether they are under the influence of lateralized neural networks (*8*). We report now what is, to our knowledge, the first evidence that nonhuman primates employ neurally lateralized processors for the perception of conspecific vocalizations.

With operant techniques, we have

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