Norepinephrine Turnover and Metabolism in Rat Brain after Long-Term Administration of Imipramine

Abstract. *The rate of disappearance of intracisternally administered tritiated norepinephrine from rat brain is decreased after a single dose of the tricyclic antidepressant imipramine. During long-term administration of imipramine, however, the rate of disappearance of tritiated norepinephrine from brain gradually increases, and there is a concurrent decrease in the content of endogenous norepinephrine in brain. These findings may help to explain why antidepressant effects are observed clinically only after long-term treatment with imipramine.*

A relative deficiency of norepinephrine or other biogenic amines at critical receptors in brain may occur in some depressive disorders, and it has been suggested that the changes in biogenic amine metabolism produced by antidepressant drugs may account for their clinical effects (*1*). Imipramine and various other tricyclic antidepressants inhibit the uptake of [^{3}H]norepinephrine and alter the turnover and metabolism of this amine in rat brain (*2–5*). These findings, however, have been observed after the acute administration of a single dose of tricyclic drugs, whereas long-term administration (for about 3 weeks) is generally required before the initial antidepressant effects are observed in depressed patients. We report here that there are differences between the effects of short-term (a single dose) and long-term administration of imipramine on norepinephrine turnover which may account for some of the clinical actions of this drug (*6*).

In one series of experiments, imipramine hydrochloride (10 mg/kg) or isotonic saline (1 ml) was administered by intraperitoneal injection twice daily for 3 weeks to male Sprague-Dawley rats. In another series of experiments, a single dose of imipramine hydrochloride (10 mg/kg) or isotonic saline (1 ml) was administered by intraperitoneal injection. Six hours after the intraperitoneal injection (the last intraperitoneal injection in experiments on long-term drug administration), *d,l*-[7-^{3}H]norepinephrine (100 to 128 ng; 6.6 to 8.5 c/mmole) was injected into the cisterna magna of the brain (*7*). One group of animals was killed by cervical fracture 6 minutes after the intracisternal injection to examine the effects of imipramine on the initial uptake and metabolism of [^{3}H]norepinephrine. Another group was killed 270 minutes after the intracisternal injection to examine the effects of the drug on the subsequent release and metabolism of [^{3}H]norepinephrine in rat brain. [^{3}H]Norepinephrine and its metabolites as well as endogenous norepinephrine were determined in whole rat brain (*8*). Student's *t*-test was used to determine the statistical significance of the differences between the mean concentrations of endogenous norepinephrine, [^{3}H]norepinephrine, and the various metabolites of [^{3}H]norepinephrine in the brains of animals treated with imipramine or saline.

A single dose of imipramine (Table 1) inhibited the uptake of [^{3}H]norepinephrine in brains of animals killed 6 minutes after the intracisternal injection, as reflected by the lower concentrations of [^{3}H]norepinephrine. Animals treated with a single dose of imipramine and killed 270 minutes after the administration of [^{3}H]norepinephrine, however, had higher concentrations of [^{3}H]norepinephrine in the brain than did the controls. A single dose of imipramine thus appeared to slow the rate of disappearance of [^{3}H]norepinephrine from the brain since animals treated with a single dose of imipramine had lower concentrations of [^{3}H]norepinephrine in the brain than did controls at the earlier time, but higher concentrations of [^{3}H]norepinephrine than did controls when examined at a later time. Concentrations of [^{3}H]normetanephrine were increased, whereas ^{3}H-labeled deaminated catechol metabolites were decreased in animals treated with imipramine. The content of endogenous norepinephrine in the brain was not altered by a single dose of imipramine (Table 1).

After long-term administration of imipramine (Table 2), the uptake of [^{3}H]norepinephrine into brain was inhibited as evidenced by the lower concentrations of [^{3}H]norepinephrine in animals treated with imipramine and killed 6 minutes after intracisternal injection. Residual concentrations of [^{3}H]-norepinephrine in brain were even lower in animals given long-term treatment with imipramine and killed 270 minutes after the intracisternal injection, an indication that the rate of disappearance of [^{3}H]norepinephrine from the brain was definitely not slowed and possibly even accelerated. Concentrations of [^{3}H]normetanephrine in the brain were increased, whereas ^{3}H-labeled deaminated catechol metabolites were profoundly decreased and ^{3}H-labeled deaminated *O*-methylated metabolites moderately decreased. The content of endogenous norepinephrine in brain was significantly lower after long-term administration of imipramine than after long-term administration of saline (Table 2).

The inhibition of [^{3}H]norepinephrine uptake into brain and the changes in concentrations of its metabolites (increased [^{3}H]normetanephrine and decreased ^{3}H-labeled deaminated catechol metabolites) were qualitatively similar after short-term and long-term administration of imipramine, although some of these effects were more pronounced after long-term administration. In contrast, however, the rate of disappearance of [^{3}H]norepinephrine from brain was slowed after a single dose of imi-

Table 1. The effects of a single dose of imipramine on the uptake, release, and metabolism of norepinephrine (NE) in rat brain. Imipramine hydrochloride (10 mg/kg) or isotonic saline (1 ml) was administered intraperitoneally. Six hours later, [^{3}H]NE was administered intracisternally. Animals were killed 6 or 270 minutes after the intracisternal injection of [^{3}H]NE. Results represent the mean of 13 to 16 determinations and are expressed as percentages of the control means (100 percent) ± standard error of the means. Control mean values (uncorrected for recoveries) from animals killed 6 minutes after the [^{3}H]NE injection were: [^{3}H]NE, 570 nc/brain; [^{3}H]NMN, 240 nc/brain; [^{3}H]DCM, 16 nc/brain; total [^{3}H]DOM, 220 nc/brain; free [^{3}H]DOM, 51 nc/brain; and endogenous NE, 600 ng/brain. Control mean values (uncorrected for recoveries) from animals killed 270 minutes after the [^{3}H]NE injection were: [^{3}H]NE, 88 nc/brain; [^{3}H]NMN, 5 nc/brain; [^{3}H]DCM, 2 nc/brain; total [^{3}H]DOM, 99 nc/brain; free [^{3}H]DOM, 7 nc/brain; and endogenous NE, 590 ng/brain. Abbreviations are: NMN, normetanephrine; DCM, deaminated catechol metabolites—that is, 3,4-dihydroxyphenyl glycol and 3,4-dihydroxymandelic acid; and DOM, deaminated *O*-methylated metabolites—that is, 3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenyl glycol, and the sulfate conjugate of 3-methoxy-4-hydroxyphenyl glycol.

Treatment group	Time killed (min)	[^{3}H]NE	[^{3}H]NMN	[^{3}H]DCM	Total [^{3}H]DOM	Free [^{3}H]DOM	Endogenous NE
Control	6	100 ± 2	100 ± 3	100 ± 4	100 ± 4	100 ± 4	100 ± 2
Imipramine	6	89 ± 3*	121 ± 6*	60 ± 3*	84 ± 4*	85 ± 5†	104 ± 3
Control	270	100 ± 3	100 ± 7	100 ± 7	100 ± 3	100 ± 4	100 ± 2
Imipramine	270	112 ± 4†	139 ± 10*	71 ± 4*	106 ± 4	96 ± 4	101 ± 2

* $P < .01$. † $P < .05$.

Table 2. The effects of long-term administration of imipramine on the uptake, release, and metabolism of NE in rat brain. Imipramine hydrochloride (10 mg/kg) or isotonic saline (1 ml) was injected twice daily for 3 weeks. Six hours after the last drug injection, [^{3}H]NE was administered by intracisternal injection. Animals were killed 6 or 270 minutes after the intracisternal injection of [^{3}H]NE. Results represent the mean of 17 to 20 determinations and are expressed as percentages of the control means (100 percent) ± standard error of the means. Control mean values (uncorrected for recoveries) from animals killed 6 minutes after the [^{3}H]NE injection were: [^{3}H]NE, 650 nc/brain; [^{3}H]NMN, 290 nc/brain; [^{3}H]DCM, 15 nc/brain; total [^{3}H]DOM, 280 nc/brain; free [^{3}H]DOM, 79 nc/brain; and endogenous NE, 690 ng/brain. Control mean values (uncorrected for recoveries) from animals killed 270 minutes after the [^{3}H]NE injection were: [^{3}H]NE, 136 nc/brain; [^{3}H]NMN, 7 nc/brain; [^{3}H]DCM, 2 nc/brain; total [^{3}H]DOM, 105 nc/brain; free [^{3}H]DOM, 8 nc/brain; and endogenous NE, 660 ng/brain.

Treatment group	Time killed (min)	[^{3}H]NE	[^{3}H]NMN	[^{3}H]DCM	Total [^{3}H]DOM	Free [^{3}H]DOM	Endogenous NE
Control	6	100 ± 3	100 ± 3	100 ± 8	100 ± 3	100 ± 3	100 ± 2
Imipramine	6	85 ± 4*	132 ± 6*	44 ± 4*	86 ± 3*	78 ± 5*	85 ± 3*
Control	270	100 ± 3	100 ± 8	100 ± 8	100 ± 4	100 ± 5	100 ± 2
Imipramine	270	80 ± 6*	118 ± 8	45 ± 5*	93 ± 4	83 ± 4†	84 ± 2*

* $P < .01$. † $P < .05$.

pramine, but not after long-term administration. Moreover, the content of endogenous norepinephrine in brain was lower after long-term administration of imipramine than after long-term administration of saline, whereas the content of endogenous norepinephrine in brain was not altered by a single dose of imipramine. We have observed similar differences in experiments comparing the effects of short-term and long-term administration of the tricyclic antidepressant protriptyline (*9*).

The effects on the turnover of norepinephrine in brain have also been found to be different after short-term and long-term administration of other drugs (*10*). Differences in the duration of drug administration, therefore, may possibly account for some of the apparent discrepancies in the findings of various studies of the effects of tricyclic antidepressants on the turnover of norepinephrine in brain (*3, 4, 11*).

The inhibition of uptake, decreased rate of disappearance, and alterations in metabolism of [^{3}H]norepinephrine in rat brain observed after a single dose of imipramine confirm previous findings (*2–5*). Decrease in the deamination of [^{3}H]norepinephrine could contribute to the decreased rate of disappearance of [^{3}H]norepinephrine from brain after administration of a single dose of imipramine (*4, 5, 12, 13*). The possibility of a feedback inhibition from the postsynaptic neuron must also be considered, however, inasmuch as drugs which block receptors increase turnover or synthesis of one or another of the biogenic amines in brain, presumably through feedback mechanisms (*11, 14*). Since uptake into the presynaptic neuron is thought to be the major process for terminating the physiological activity of extraneuronal norepinephrine, the inhibition of uptake produced by a single dose of imipramine may, thereby, increase concentrations of norepinephrine at receptors (*15*) and thus, by a feedback mechanism, decrease the rate of discharge of the presynaptic neuron. With long-term administration of imipramine, however, further changes seem to occur since the rate of disappearance of [^{3}H]norepinephrine from brain increases (that is, returns to at least control values) and the content of endogenous norepinephrine in brain decreases.

On the basis of data from studies on depressed patients treated with imipramine, it has been suggested that this drug may decrease norepinephrine biosynthesis (*12, 13*). Because the rate of disappearance of [^{3}H]norepinephrine from the brain, however, may not necessarily reflect the rate of synthesis of norepinephrine (*16*), the effects of imipramine on norepinephrine synthesis in brain cannot be determined from our data.

The effects of long-term administration of imipramine appear to develop gradually since imipramine hydrochloride (10 mg/kg), administered by intraperitoneal injection twice daily for 10 days, caused changes in the turnover and content of norepinephrine in the brain which were similar to, but less pronounced than the changes seen after 3 weeks of treatment with this drug. Small doses of thyroid hormone, when combined with imipramine, seem to produce more rapid clinical improvement in depressed patients than does imipramine alone (*17*). Thyroid hormone may also accelerate the development of the changes in turnover and content of norepinephrine in brain which are produced by long-term administration of imipramine. [^{3}H]Norepinephrine disappeared from brain more rapidly and the content of endogenous norepinephrine in brain was lower after 10 days of treatment with imipramine hydrochloride (10 mg/kg, twice daily) in combination with a relatively small nontoxic dose of thyroxine (375 μg/kg, daily) than after 10 days of treatment with imipramine alone. These changes were approximately equal to or greater than the changes seen after 3 weeks of treatment with imipramine (*18*).

The changes in norepinephrine uptake, turnover, and metabolism in rat brain after long-term administration of imipramine suggest that more norepinephrine may be made available to receptors (*15*) despite the reduction in brain concentrations of this amine. In depressed patients (who may have a relative deficiency of norepinephrine at critical receptors in brain) (*1*) long-term administration of imipramine may, therefore, facilitate the restoration of normal functioning in spite of relatively reduced concentrations of endogenous norepinephrine or reduced presynaptic noradrenergic neuronal activity. The decrease in the rate of disappearance of [^{3}H]norepinephrine from rat brain after a single dose but not after long-term administration of imipramine may also help to account for such side effects as sedation and postural hypotension which often occur during the initial period of administration of this drug and which gradually diminish with continuing treatment.

Our findings indicate that there are differences between the effects of a single dose and long-term administration of imipramine on norepinephrine turnover. Moreover, these findings suggest that, in addition to thyroxine (*17*), other hormones and pharmacological agents or physiological and behavioral techniques (*13, 19*), which increase the turnover of norepinephrine in brain when administered in combination with tricyclic antidepressant drugs, may also accelerate and enhance the clinical antidepressant effects of these drugs.

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20. Supported in part by PHS grant MH 15,413 from the National Institute of Mental Health, by a grant from Merck Sharp & Dohme, and by the Supreme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U.S., fellowships to medical students A.W. and C.W.A. We thank Geigy Pharmaceuticals for the imipramine and Merck Sharp & Dohme for the protriptyline used in these studies.

22 December 1969; revised 24 February 1970 ■

Visual Experience Modifies Distribution of Horizontally and Vertically Oriented Receptive Fields in Cats

Abstract. *Cats were raised from birth with one eye viewing horizontal lines and one eye viewing vertical lines. Elongated receptive fields of cells in the visual cortex were horizontally or vertically oriented—no oblique fields were found. Units with horizontal fields were activated only by the eye exposed to horizontal lines; units with vertical fields only by the eye exposed to vertical lines.*

Many investigators have studied the response characteristics of single cells in the visual system in an effort to understand the neural mechanisms of perception (*1–4*). In the visual cortex of the cat and the monkey, there are units with elongated receptive fields which respond vigorously to elongated stimuli of the same orientation as the receptive field (*2–5*). It is frequently assumed that such units are important in the perception of form (*3, 6*), but no direct test of this hypothesis has been made (*7*). Ideally, one might remove all cortical units with receptive fields of a given orientation and observe the subject's visual capabilities. This would require ablation of cells on a physiological rather than an anatomical basis.

We have developed a technique for rearing kittens which results in all of the elongated receptive fields being oriented either vertically or horizontally, in contrast to the random arrangement present in normal cats (*3, 8*). Moreover, the units with horizontal fields respond only to stimulation of one eye, and the units with vertical fields respond only to stimulation of the other eye. Therefore, it should be possible to test the behavioral function of either class of cells by simply occluding one eye or the other.

We controlled the visual experience of kittens from birth until 10 to 12 weeks of age. Each animal's total visual experience consisted of viewing a white field containing three black vertical lines with one eye and, simultaneously viewing a field containing three horizontal lines with the other eye. The lines were 1 degree wide and their center points were 6 degrees apart. These conditions were used in order to produce discordant sensory input to the binocularly activated cortical cells.

The stimuli were mounted in a mask which provided a 50- to 60-degree field of view for each eye. Beginning at 3 weeks of age the animals wore these devices for approximately 8 hours a day. Masks were put on and removed inside a darkroom in which the animals were housed from birth whenever they were not wearing the masks. Each set of lines in the mask was positioned at the focal plane of a lens so that small changes in the position of the mask would not affect the sharpness of focus. The kittens soon became accustomed to the masks and were active and playful during the exposure periods. To insure that the animals could not pull or rub the masks off they wore a large neck ruff similar to that used by Hein and Held (*9*). The animals were checked repeatedly while they were wearing the masks. We estimate that slippage of the mask did not exceed 10 degrees and in most cases was less than 5 degrees; eye movements were not measured during exposure periods. It is clear from the positive results obtained that any rotations of the eyes or the mask did not interfere with the aim of the experiment.

Single unit recordings were made from the visual cortex of these animals between 10 and 12 weeks of age. We used the preparation, recording, and mapping technique developed by Spinelli (*10*). In brief, thiopental sodium was injected intravenously to obtain general anesthesia and a small opening was made in the skin, bone, and dura above the visual cortex in one hemisphere. Subsequently the animal was paralyzed with Flaxedil, artificially ventilated, and held in a stereotaxic instrument. All pressure points and incisions were infiltrated with a long-acting local anesthetic (Zyljectin). The cat was positioned at 57 cm from a white tangent screen; at this distance 1 cm on the screen is equal to 1 degree of arc at the eye. Contact lenses were used to correct for accommodation and to protect the cornea of the eye. The projection of the optic disk onto the screen was determined with a reversable ophthalmoscope, and the position of the area centralis was inferred (*11*). The estimated projections of the area centralis were centered at or near the top of the mapped region in four animals and about 5 degrees above in the remaining animal. The units were recorded from primary visual cortex between stereotaxic coordinates anterior-posterior −1.0 to +1.0 mm and medial-lateral 0.5 to 1.5 mm. In adult cats this corresponds