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THE EFFECTS OF PSYCHOACTIVE DRUGS ON NOREPINEPHRINE-^{3}H METABOLISM IN BRAIN

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Abstract—The effects of imipramine, desmethylimipramine, chlorpromazine, and lithium chloride on the metabolism of intracisternally administered norepinephrine-^{3}H was studied in rat brain. When drugs were administered prior to the norepinephrine-^{3}H, it was found that desmethylimipramine, imipramine, and chlorpromazine all lowered the brain content of norepinephrine-^{3}H but only the antidepressants, imipramine and desmethylimipramine, simultaneously increased the brain level of normetanephrine-^{3}H. In experiments in which psychoactive drugs were injected after intracisternal administration of norepinephrine-^{3}H, imipramine and desmethylimipramine slowed the disappearance of norepinephrine-^{3}H from brain tissue and concurrently increased the brain content of normetanephrine-^{3}H. In contrast, chlorpromazine had no apparent effect in brain upon the rate of disappearance of norepinephrine-^{3}H or upon the content of normetanephrine-^{3}H. Lithium chloride decreased normetanephrine-^{3}H levels and increased the deaminated catechols. These findings suggest that while antidepressants may increase physiologically available norepinephrine, lithium, a drug effective in the treatment of mania, may decrease levels of norepinephrine available to central adrenergic receptors.

VARIOUS psychoactive drugs alter norepinephrine metabolism in brain[1] as well as in peripheral tissues. In peripheral sympathetic neurons, norepinephrine discharged from nerve endings appears to be inactivated by neuronal re-uptake or by O-methylation, whereas norepinephrine released intraneuronally is largely inactivated by deamination.[2] Study of catecholamine metabolism in brain has been facilitated by the application of techniques which circumvent the blood–brain barrier and make possible the introduction of tritiated norepinephrine into brain. Radioactive norepinephrine injected into the lateral ventricle is taken up into brain tissue, mixes with endogenous norepinephrine, and appears to be a valid tracer for this amine.[1] The pattern of norepinephrine-^{3}H metabolism in brain tissue[3, 4] is similar to that observed in peripheral sympathetic nerves.

After intracisternal administration of norepinephrine-^{3}H, the disposition of the labeled catecholamine in brain has been found to be similar to that following intraventricular administration.[5] Intracisternal injection provides a convenient and reproducible method for introducing norepinephrine-^{3}H into the brain.† With this technique the effects of imipramine, desmethylimipramine, chlorpromazine, and lithium chloride on the metabolism of tritiated norepinephrine in rat brain have been studied.

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† S. M. Schanberg, J. J. Schildkraut and I. J. Kopin, in preparation.

METHODS

By means of a technique described elsewhere,* 25 μl of Elliott's "B" irrigating solution (Baxter) containing 0·14 μg *dl*-norepinephrine-7-^{3}H (7·1 c/m-mole, obtained from New England Nuclear Corp.) was injected into the cisterna magna of Sprague–Dawley rats weighing 190–210 g. All animals were anesthetized lightly with ether during the procedure and were fully recovered within 4 min. At various times after intracisternal injection of norepinephrine-^{3}H, groups of animals were killed by cervical fracture and decapitated. Whole brains including 3 to 5 mm of spinal cords were removed rapidly, rinsed three times with water, and immediately frozen in liquid nitrogen. The frozen brains were weighed and homogenized in 8 ml of cold 0·4 N perchloric acid in an all-glass homogenizer. The homogenates were frozen and thawed, before centrifugation, to ensure extraction of particulate-bound norepinephrine.

A 0·3-ml aliquot of the supernatant fluid was added to 15 ml toluene:ethanol (10:4) solution containing phosphor;[6] total radioactivity was assayed in a Packard liquid scintillation spectrometer. Aliquots of the remaining supernatant fluid were assayed for norepinephrine-^{3}H by a modification of the method of Whitby *et al.*[7] Tritiated normetanephrine, deaminated catechols (dihydroxymandelic acid and dihydroxyphenylglycol), and O-methylated deaminated metabolites (3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol) were determined as previously described by Kopin *et al.*[8] Internal standards of toluene-^{3}H were used to correct for efficiency of counting.

Imipramine hydrochloride, 25 mg/kg; desmethylimipramine hydrochloride, 25 mg/kg; chlorpromazine hydrochloride, 25 mg/kg; and lithium chloride, 50 mg/kg × 2 (or 3)† were administered by i.p. injection. All dosages are expressed as the salts. Control animals received isotonic saline (1 ml).

RESULTS

Effect of pretreatment with psychoactive drugs on norepinephrine-^{3}H metabolism in brain

Imipramine, desmethylimipramine, chlorpromazine, or isotonic saline was injected i.p. 1·5 hr before the intracisternal administration of norepinephrine-^{3}H. Lithium chloride was administered i.p. 1·5 and 0·5 hr prior to intracisternal injection of the labeled amine. Animals were killed 6 min after injection of tritiated norepinephrine.

Norepinephrine-^{3}H content of brain was significantly lower in animals pretreated with imipramine, desmethylimipramine and chlorpromazine; lithium chloride, however, did not alter norepinephrine-^{3}H levels. Normetanephrine-^{3}H levels were significantly higher in the brains of animals receiving imipramine or desmethylimipramine. In the brains of animals pretreated with chlorpromazine or lithium chloride, normetanephrine-^{3}H levels were similar to those of untreated controls (Table 1).

Effects of psychoactive drugs on the metabolism of previously administered norepinephrine-^{3}H in brain

Norepinephrine-^{3}H was administered intracisternally. Imipramine, desmethylimipramine, chlorpromazine, or isotonic saline was injected i.p. 1·5 hr later or lithium

* S. M. SCHANBERG, J. J. SCHILDKRAUT and I. J. KOPIN, in preparation.

† This dose of lithium chloride represents 2·6 (or 3·9) mEq lithium/kg; clinically, in the treatment of mania, the dose range for chronic administration of lithium is 0·8 to 1·6 mEq/kg/day.[9]

chloride was given 1, 2, and 3 hr after injection of the tritiated amine. All animals were killed 4·5 hr after the administration of norepinephrine-^{3}H.

Norepinephrine-^{3}H content was significantly higher in the brains of animals receiving imipramine or desmethylimipramine; chlorpromazine or lithium chloride,

TABLE 1. EFFECT OF PRETREATMENT WITH PSYCHOACTIVE DRUGS ON THE CONTENT OF NOREPINEPHRINE-^{3}H IN THE RAT BRAIN

Drug	Norepinephrine (% control ± S.E.M.)	Normetanephrine-^{3}H (% control ± S.E.M.)
Experiment 1		
Control (NaCl)	100 ± 3	100 ± 8
Imipramine	81 ± 2*	152 ± 8*
DMI	70 ± 3*	205 ± 9*
Chlorpromazine	81 ± 4†	110 ± 11
Lithium chloride	106 ± 2	105 ± 9
Experiment 2		
Control (NaCl)	100 ± 3	
Chlorpromazine	80 ± 5†	

* $P = <0{\cdot}001$ when compared with control values.

† $P = <0{\cdot}01$.

Drugs were administered i.p. 1·5 hr before the intracisternal injection of norepinephrine-^{3}H. A second injection of lithium chloride was also given 0·5 hr before the norepinephrine-^{3}H. Animals were killed 6 min after the administration of norepinephrine-^{3}H and the brains analyzed for tritiated norepinephrine and metabolites as described in the text. Groups of six animals were used in experiment 1, and five in experiment 2. Drug doses: imipramine, desmethylimipramine, and chlorpromazine, 25 mg/kg; lithium chloride, 50 mg/kg × 2. Control mean norepinephrine-^{3}H = 2300 mμc/brain; normetanephrine-^{3}H = 400 mμc/brain.

however, did not alter norepinephrine-^{3}H levels (Table 2, Fig. 1). Imipramine and desmethylimipramine increased noremetanephrine-^{3}H levels, but chlorpromazine caused no significant change in levels of the O-methylated amine (Table 2, Fig. 1). Normetanephrine-^{3}H levels were significantly decreased in animals receiving lithium chloride; of the four drugs, only lithium chloride caused an increase in levels of tritiated deaminated catechol metabolites (Fig. 1). In animals treated with imipramine, desmethylimipramine, or chlorpromazine, deaminated catechol content was not significantly different from control values (Table 2, Fig. 1). Levels of O-methylated deaminated metabolites appeared to be higher than control values in animals receiving desmethylimipramine or lithium chloride, but the difference was statistically significant only for desmethylimipramine (Fig. 1).

DISCUSSION

The norepinephrine-^{3}H content of brain after intracisternal administration is determined by initial neuronal uptake, initial metabolism, and initial efflux from brain as well as subsequent release, re-uptake, and metabolism. Shortly (6 min) after the administration of norepinephrine-^{3}H, brain content of the labeled amine primarily reflects the initial events. When norepinephrine-^{3}H is injected prior to administration of drugs, the initial events are eliminated as experimental variables.

Norepinephrine discharged from the neuron in physiologically active form, by either nerve stimulation or sympathomimetic drugs, is inactivated mainly by cellular re-uptake or by conversion to normetanephrine through enzymatic O-methylation. Norepinephrine released intracellularly, either spontaneously or by reserpine-like drugs, is inactivated primarily by mitochondrial monoamine oxidase before leaving

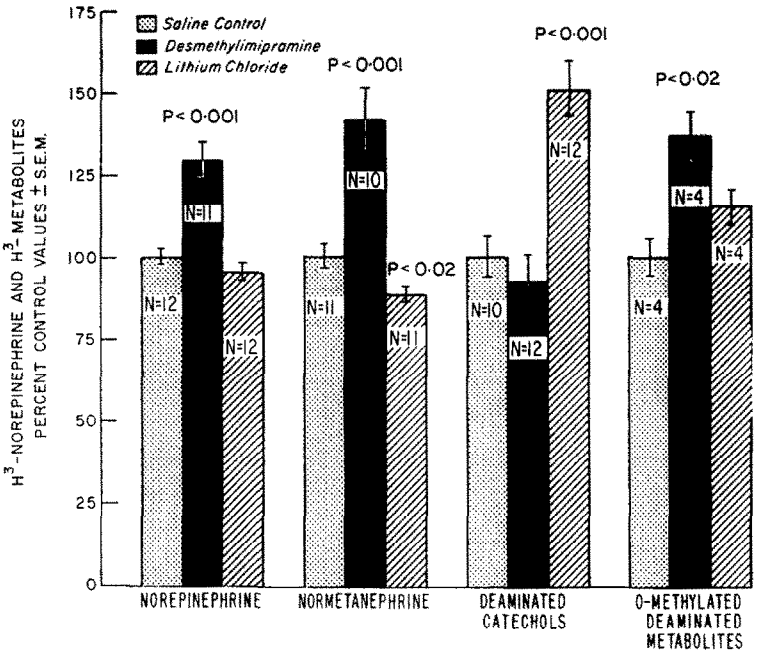

FIG. 1. Norepinephrine-^{3}H was administered intracisternally. Desmethylimipramine (25 mg/kg) was injected i.p. 1·5 hr later or lithium chloride (50 mg/kg) was given 1, 2, and 3 hr after the intracisternal injection. All animals were killed 4·5 hr after the administration of the radioactive amine and the brains analyzed for tritiated norepinephrine and metabolites. Control mean norepinephrine-^{3}H = 600 mμc/brain; normetanephrine-^{3}H = 20 mμc/brain; ^{3}H-deaminated catechols = 4 mμc/brain.

TABLE 2. EFFECTS OF IMIPRAMINE, DESMETHYLIMIPRAMINE, AND CHLORPROMAZINE ON THE METABOLISM OF NOREPINEPHRINE-^{3}H IN BRAIN

Drug	Norepinephrine-^{3}H	Normetanephrine-^{3}H	^{3}H-Deaminated catechols
		(% control ± S.E.M.)	
Control (NaCl)	100 ± 4	100 ± 3	100 ± 8
Imipramine	123 ± 4*	127 ± 14	90 ± 9
DMI	145 ± 10*	163 ± 13*	108 ± 9
Chlorpromazine	99 ± 4	104 ± 9	115 ± 15

* P = <0·001 when compared with control values.

Drugs (25 mg/kg i.p.) were administered 1·5 hr after the intracisternal administration of norepinephrine-^{3}H. The animals were killed 4·5 hr after the injection of the labeled amine and the brains analyzed for tritiated norepinephrine and metabolites as described in the text. Results expressed as per cent of control mean ± S.E.M. Each value represents the mean of at least five determinations. Control mean norepinephrine-^{3}H = 600 mμc/brain; normetanephrine-^{3}H = 20 mμc/brain; ^{3}H-deaminated catechols = 4 mμc/brain.

the cell.[2] Although monoamine oxidase does not play a major role in terminating the activity of norepinephrine at receptors, it may be largely responsible for regulating the level of norepinephrine in neurons. While most of these concepts derive from studies of the peripheral sympathetic nervous system, similar patterns of metabolism appear to occur in brain.[3, 5]

Imipramine, desmethylimipramine, and chlorpromazine block the uptake of norepinephrine-^{3}H into peripheral tissues[10] and brain slices.[11] The present findings suggest that these drugs have a similar action on norepinephrine-^{3}H uptake by brain *in vivo*. Glowinski and Axelrod[12] found that desmethylimipramine and imipramine, but not chlorpromazine, blocked uptake into brain of norepinephrine-^{3}H injected into the lateral ventricle of rats. Several factors may account for the discrepancy in the observed effects of chlorpromazine on norepinephrine-^{3}H uptake. In the studies utilizing the intraventricular method of norepinephrine-^{3}H administration, pentobarbital was used for anesthesia. This drug influences the metabolism and physiological disposition of norepinephrine-^{3}H and 5-hydroxytryptamine-^{3}H in rat brain (Schanberg *et al.*, in preparation). Furthermore, chlorpromazine, in the doses used, can cause a pronounced hypothermia which may alter the action of this drug on the disposition of norepinephrine-^{3}H in brain. In the present experiments body temperature was maintained by allowing chlorpromazine-treated rats to huddle in small enclosed cages with normally active animals (Table 3).

TABLE 3. EFFECT OF GROUPING ON CHLORPROMAZINE-INDUCED HYPOTHERMIA IN RATS

Condition	N	Rectal temperature (°C ± S.E.M.)
Isolated control	4	37·1 ± 0·1
Grouped control	4	37·8 ± 0·2
Isolated chlorpromazine-treated	4	32·8 ± 0·6
Grouped chlorpromazine-treated	4	36·3 ± 0·3

Rectal temperatures were recorded in control rats and in animals 1·5 hr after the i.p. injection of chlorpromazine (25 mg/kg). Animals were caged either singly or together with normally active rats in a ratio of one experimental to four normal rats.

Glowinski and Axelrod found that imipramine and chlorpromazine slowed the disappearance of norepinephrine-^{3}H from brain.[1] In the present study this effect was observed with imipramine and desmethylimipramine but not with chlorpromazine. The disappearance rate of norepinephrine-^{3}H is a consequence of release, re-uptake, and metabolism. Therefore, the fact that chlorpromazine did not appear to slow the net disappearance of norepinephrine-^{3}H cannot be taken to indicate that release was unaffected, especially since chlorpromazine inhibits uptake of norepinephrine-^{3}H under the conditions of the present experiments (Table 1).

Drugs that block peripheral adrenergic receptors, such as dibenzyline, phentolamine, dichloroisoproterenol, and pronethalol, diminish normetanephrine formation in isolated perfused rat hearts but do not inhibit monoamine oxidase or catechol-O-methyl transferase.[13] It has been suggested that O-methylation may be concerned with removal of norepinephrine from the receptor,[2] and drugs that interfere with norepinephrine–receptor interaction may thus diminish normetanephrine formation.

Chlorpromazine is a relatively potent adrenergic blocking agent, whereas the tricyclic antidepressants show this effect only at considerably larger doses.[14] Both the antidepressants (desmethylimipramine and imipramine) and the tranquilizer chlorpromazine appear to inhibit the uptake of norepinephrine-^{3}H; unlike the antidepressants, however, chlorpromazine does not concurrently increase normetanephrine-^{3}H in the brain (Table 1). In experiments in which the norepinephrine-^{3}H is administered prior to psychoactive drugs, the antidepressants also increase the level of normetanephrine-^{3}H, while chlorpromazine does not (Table 2). These findings are compatible with the hypothesis that chlorpromazine is a central adrenergic blocking agent and that catechol-O-methyl transferase inactivates norepinephrine which has interacted with adrenergic receptors.

Lithium chloride, a drug effective in the treatment of mania,[9, 15, 16] significantly alters the metabolism of norepinephrine-^{3}H.[17] There appears to be a shift in norepinephrine metabolism from O-methylation to deamination, as indicated by a decrease in normetanephrine-^{3}H with a concurrent increase in deaminated catechols. There is, however, no apparent change in the level of norepinephrine-^{3}H (Fig. 1).

When measured *in vitro*, lithium causes no alteration in monoamine oxidase activity; it seems unlikely that the effects of lithium are secondary to an inhibition of catechol-O-methyl transferase, since lithium does not inhibit this enzyme *in vitro* (Schanberg *et al.*, in preparation). Moreover, there is no decrease in the O-methylated deaminated metabolites of norepinephrine-^{3}H in brains of lithium-treated animals (Fig. 1) as when catechol-O-methyl transferase is inhibited by tropolone.[18] These results suggest that lithium may increase intraneuronal inactivation of norepinephrine and decrease norepinephrine available to adrenergic receptors.

In contrast to lithium, drugs that are euphoriants or antidepressants may increase physiologically active norepinephrine.[19] Amphetamine, a drug that elevates mood, has been reported to increase normetanephrine-^{3}H and decrease deaminated catechol metabolites.[18] Antidepressant monoamine oxidase inhibitors decrease inactivation of norepinephrine by deamination, whereas antidepressant tricyclic compounds prevent inactivation of norepinephrine by neuronal uptake.[12] Although differing in their mechanisms of action, both monoamine oxidase inhibitors[18] and imipramine-like antidepressants (Table 2) cause an increase in brain levels of normetanephrine in experimental animals. Schildkraut and his associates[20] have found that urinary excretion of normetanephrine increases during the period of recovery in depressed patients treated with imipramine.

The findings of this study are compatible with the hypothesis that antidepressant drugs may increase physiologically available norepinephrine. It is further suggested that lithium, a drug effective in the treatment of mania, may decrease levels of norepinephrine at central adrenergic receptors.

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