Norepinephrine Pools in Rat Brain: Differences in Turnover Rates and Pathways of Metabolism

Abstract. *The rate of disappearance of intracisternally administered [^3H]norepinephrine from rat brain gradually declines as a multiphasic exponential function of time. Conversion to [^3H]normetanephrine accounts for a larger fraction of the [^3H]norepinephrine released in the brain shortly after its intracisternal injection than that released at later times. Pools of norepinephrine in the brain thus appear to differ in their turnover rates and pathways of metabolism. The pool of norepinephrine with a rapid rate of turnover and an appreciable conversion to normetanephrine, identified by the techniques reported here, may correspond to a pool of newly synthesized norepinephrine in the brain.*

Numerous findings suggest the possibility that, in the brain as well as in the peripheral sympathetic nerves, norepinephrine occurs in multiple pools or compartments (*1–3*), although this interpretation has been questioned by some investigators (*4*). Newly synthesized norepinephrine appears to be released preferentially from peripheral sympathetic nerves by nerve stimulation (*5*), and preferential release of newly synthesized norepinephrine has also been reported to occur in the brain (*6*). These findings and several other lines of evidence have thus led to the suggestion that the physiological activity of noradrenergic neurons may depend upon a small, metabolically labile pool of newly synthesized norepinephrine occurring within the nerve terminal in compartments or storage granules possibly situated in, or adjacent to, the axonal membrane in close proximity to the synaptic cleft (*7*).

In the study reported here we have attempted to differentiate pools of norepinephrine in the brain by examining the rate of disappearance and the pathways of metabolism of intracisternally administered [^{3}H]norepinephrine as a function of the time after intracisternal administration of the radioactive norepinephrine. The rate of disappearance of intracisternally administered [^{3}H]norepinephrine from brain gradually declines as a function, but not a simple exponential function, of time. Conversion to [^{3}H]normetanephrine accounts for a larger fraction of the metabolism of [^{3}H]norepinephrine released in brain shortly after its intracisternal injection than that released at later times (*8*). These findings indicate that pools of norepinephrine in the brain may differ, not only in their rates of turnover, but also in the pathways of metabolism of the released catecholamine. We suggest that the pool of norepinephrine with a rapid rate of turnover and an appreciable metabolic conversion to normetanephrine, identified by the techniques reported here, may correspond to a pool of newly synthesized norepinephrine in the brain.

Radioactive norepinephrine, when administered into the ventricular system or basal cisterns of the brain, has been shown to label stores of endogenous norepinephrine (*3, 9*). The compound *d,l*-[7-^{3}H]norepinephrine (100 to 128 ng; 6.6 to 8.5 c/mmole) was administered by intracisternal injection to male Sprague-Dawley rats (180 to 200 g) (*3*). Animals were killed by cervical fracture; [^{3}H]norepinephrine and [^{3}H]-normetanephrine were determined in whole rat brain (*10*). The rate of disappearance and the metabolism of intracisternally administered [^{3}H]norepinephrine were studied from 6 to 420 minutes after intracisternal injection.

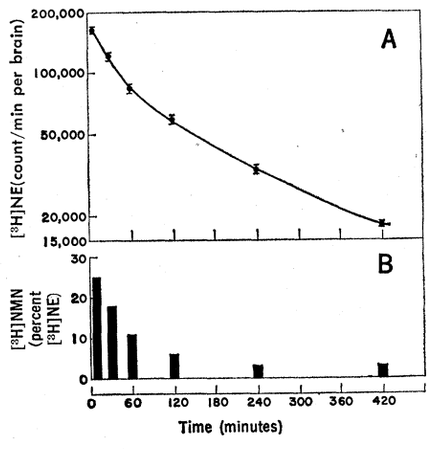

Fig. 1. (A) The disappearance of intracisternally administered [^{3}H]norepinephrine ([^3H]*NE*) from rat brain; (B) [^{3}H]normetanephrine ([^3H]*NMN*) expressed as a percentage of [^{3}H]norepinephrine at varying times after the intracisternal administration of [^{3}H]norepinephrine. Animals were killed at varying times after the intracisternal administration of [^{3}H]norepinephrine. Concentrations of [^{3}H]norepinephrine and [^{3}H]normetanephrine were determined in extracts of whole brain. Each point on the graph represents the mean ± the standard error of the mean of eight or nine determinations.

[^{3}H]Norepinephrine in the brain exhibited a multiphasic decline. The initial half-life was 54 minutes, and the half-life 4 hours after intracisternal injection was 180 minutes. [^{3}H]Normetanephrine, which reached a maximum at 6 minutes, also showed a very rapid initial rate of disappearance and a multiphasic decline. The ratio of [^{3}H]normetanephrine to [^{3}H]norepinephrine in the brain (expressed as a percentage) was 25 percent at 6 minutes after the intracisternal injection of [^{3}H]norepinephrine and progressively declined to 3 percent at 240 minutes after the intracisternal injection (see Fig. 1). When various regions of the brain were analyzed separately, we found changes that were qualitatively similar to those observed in the whole brain.

These findings could be explained in terms of a rapid initial release of [^{3}H]-norepinephrine from binding sites with conversion of the released [^{3}H]norepinephrine to [^{3}H]normetanephrine. However, since *O*-methylation of norepinephrine is thought to occur extraneuronally (*11*), it is likely that some of the intracisternally administered [^{3}H]norepinephrine may be *O*-methylated prior to the neuronal uptake and binding of the tritiated norepinephrine. This would certainly contribute to, and could possibly account for, the high ratio of [^{3}H]normetanephrine to [^{3}H]-norepinephrine observed shortly after the intracisternal injection of [^{3}H]norepinephrine. The following experiments were therefore performed in order to determine whether there was, in fact, a change in the pathways of metabolism of [^{3}H]norepinephrine released in the brain at various times after its intracisternal injection, which might contribute to the changing ratio of [^{3}H]normetanephrine to [^{3}H]norepinephrine.

It has previously been shown that electroconvulsive shock produces a small, but significant, release of intracisternally administered [^{3}H]norepinephrine in rat brain and a concurrent increase in the concentration of [^{3}H]-normetanephrine (*12*). The release of [^{3}H]norepinephrine by electroconvulsive shock and its conversion to [^{3}H]-normetanephrine were therefore examined at various times after the intracisternal administration of [^{3}H]norepinephrine. In these experiments [^{3}H]norepinephrine was first administered by intracisternal injection. At varying times thereafter electroconvulsive shock was administered to experimental animals through earclip electrodes. Electrodes

Table 1. The release of [^{3}H]norepinephrine ([^{3}H]NE) by electroconvulsive shock (ECS) and its conversion to [^{3}H]normetanephrine ([^{3}H]NMN) at various times after the intracisternal administration of [^{3}H]norepinephrine. Results are expressed in counts per minute per brain as the mean ± the standard error of the mean. In every experiment each group contained 7 to 12 animals. The mean concentration of [^{3}H]norepinephrine in shocked animals was subtracted from the mean concentration of [^{3}H]norepinephrine in unshocked control animals to give the amount of [^{3}H]norepinephrine released by electroconvulsive shock (Δ [^{3}H]NE), whereas the concentration of [^{3}H]normetanephrine in control animals subtracted from the concentration of [^{3}H]normetanephrine in shocked animals gave the increment in [^{3}H]normetanephrine produced by electroconvulsive shock (Δ [^{3}H]NMN). The increase in [^{3}H]normetanephrine was then expressed as a percentage of the released [^{3}H]norepinephrine, that is, (Δ[^{3}H]NMN)/(Δ[^{3}H]NE) × 100. This provides an index of the fraction of the released [^{3}H]norepinephrine that was converted to [^{3}H]normetanephrine.

Experiment No.	Time after [^{3}H]NE administration (min)	[^{3}H]Norepinephrine (count/min per brain)			[^{3}H]Normetanephrine (count/min per brain)			Δ [^{3}H]NMN / Δ [^{3}H]NE × 100
		Control	ECS	Δ[^{3}H]NE	Control	ECS	Δ[^{3}H]NMN	
I	20	114,500 (± 2,810)	107,000 (± 2,650)	7,500*	21,000 (± 796)	23,000 (± 814)	2,000	27
II	20	121,000 (± 7,330)	113,400 (± 2,790)	7,600	23,600 (± 784)	26,600 (± 310)	3,000*	40
III	20	113,500 (± 4,170)	102,800 (± 3,090)	10,700*	21,300 (± 741)	25,800 (± 953)	4,500†	42
IV	30	87,200 (± 2,170)	81,400 (± 1,950)	5,800*	15,200 (± 637)	17,900 (± 589)	2,700†	47
I	300	25,100 (± 890)	22,700 (± 1,050)	2,400*	1,110 (± 64)	1,170 (± 58)	60	3
II	420	22,500 (± 1,810)	18,800 (± 1,200)	3,700*	510 (± 25)	650 (± 34)	140†	4

* Probability $< .05$ (one-tailed t-test). † Probability $< .01$ (one-tailed t-test).

were applied to control animals but no current was passed. Control animals were killed 15 minutes after the application of the electrodes; experimental animals were killed 15 minutes after the electroconvulsive shock.

When electroconvulsive shock was administered 20 or 30 minutes after the intracisternal injection of [^{3}H]norepinephrine, the increase in [^{3}H]normetanephrine accounted for 27 to 47 percent of the released [^{3}H]norepinephrine. In contrast, when electroconvulsive shock was administered either 300 or 420 minutes after the intracisternal injection of [^{3}H]norepinephrine, the increase in [^{3}H]normetanephrine accounted for only 3 to 4 percent of the released [^{3}H]norepinephrine (Table 1).

The findings presented here thus suggest that a substantial fraction of the [^{3}H]norepinephrine released in the brain a short time after its intracisternal injection is metabolized by catechol *O*-methyltransferase with the formation of [^{3}H]normetanephrine. In contrast, only a much smaller fraction of the [^{3}H]norepinephrine released at later times appears to be converted to [^{3}H]normetanephrine.

A number of other possibilities must be considered in interpreting these findings. Electroconvulsive shock could have slowed the disappearance of [^{3}H]-normetanephrine from the brain or caused other alterations in the metabolism of [^{3}H]normetanephrine which might have accounted for the findings presented here. Such an explanation is unlikely, however, since we have found that electroconvulsive shock, when administered 20 or 300 minutes after the intracisternal injection of tritiated normetanephrine, does not produce a significant alteration in the rate of disappearance or in the metabolism of the intracisternally administered [^{3}H]normetanephrine.

Since *d,l*-[^{3}H]norepinephrine was used in these studies, it is not possible to determine, on the basis of our data, the extent to which differences in the metabolism of the *d*- and *l*-isomers could have contributed to the findings. However, we do not think that such an explanation will account for the large differences reported here, since other investigators did not find differences of comparable magnitude when they studied the conversion of intracisternally administered radioactive *d,l*-norepinephrine and *l*-norepinephrine to normetanephrine in rat brain (*13*).

The possibility that some of the intracisternally administered [^{3}H]norepinephrine may have been taken up into nonneuronal sites in the brain cannot be ruled out. However, preliminary experiments (*14*) suggest that most of the [^{3}H]norepinephrine released by electroconvulsive shock, under the conditions of the present study, is discharged from neurons. In these experiments, desmethylimipramine, a drug which blocks the neuronal uptake of norepinephrine in the brain (*15*), was administered prior to electroconvulsive shock. After the rats were treated with desmethylimipramine, electroconvulsive shock produced a considerably greater net decrease in the concentration of brain [^{3}H]norepinephrine than it produced in untreated control rats. This result suggests that the [^{3}H]norepinephrine released by electroconvulsive shock came mainly from neurons and that its reuptake into these neurons was blocked by the desmethylimipramine.

The fraction of the radioactive norepinephrine in the brain released by electroconvulsive shock (*16*) at the later times (when the spontaneous disappearance of [^{3}H]norepinephrine from the brain was slow) was at least as great as that released at the earlier times (when the spontaneous disappearance of [^{3}H]norepinephrine from the brain was more rapid). This result suggests that electroconvulsive shock may readily release norepinephrine from pools in the brain from which norepinephrine is not so readily released by spontaneous neuronal activity.

Our present interpretation of the findings of this study leads us to suggest that, after intracisternal administration of tritiated norepinephrine, some of the [^{3}H]norepinephrine is *O*-methylated extraneuronally prior to neuronal uptake, but more of the [^{3}H]norepinephrine is taken up into pools of norepinephrine in the brain which differ in their rates of turnover and pathways of metabolism. A substantial fraction of the [^{3}H]norepinephrine released from pools with rapid rates of turnover is converted to [^{3}H]normetanephrine in the brain, but conversion to [^{3}H]normetanephrine appears to account for only a very small fraction of the [^{3}H]norepinephrine released from pools with slower turnover rates. Similarly, it has been suggested that the more rapidly released and physiologically available norepinephrine in rat heart also undergoes preferential *O*-methylation (*1*).

In summary, the findings of the present study show that intracisternally administered [^{3}H]norepinephrine is distributed in pools which differ both in their rates of turnover and in their relative pathways of metabolism in the brain. The conversion of intracisternally administered [^{3}H]norepinephrine to [^{3}H]normetanephrine occurs to a greater extent in pools with rapid turnover rates. This may, in part, reflect the uptake of a fraction of the intra-

cisternally administered [^{3}H]norepinephrine into a superficially situated intraneuronal pool of newly synthesized norepinephrine (*17*) and its subsequent rapid release from this compartment into the extraneuronal space. Thus, the techniques of the present study may provide a means for studying, in vivo, the kinetics and metabolism of the labile pool of newly synthesized norepinephrine in the brain under various physiological and pharmacological conditions.

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16. That is, Δ[^{3}H]NE/control. See Table 1.
17. That is, a pool situated in, or adjacent to, the axonal membrane.
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Activity of an NADPH-Dependent Nitroreductase in Houseflies

Abstract. *A nitroreductase, reducing parathion to aminoparathion, was found in the soluble fraction that was obtained from abdomens of female houseflies. The reaction required reduced nicotinamide adenine dinucleotide phosphate (NADPH), but was not affected by the presence or absence of oxygen. Further degradation of aminoparathion into water-soluble compounds occurred in NADPH-fortified incubation mixtures over prolonged incubation periods. The effect of sesamex or SKF 525-A on these reactions is described.*

Oxidative enzymes in houseflies, *Musca domestica* L., were reported to "metabolize a wide variety of insecticide chemicals when the enzyme preparations were fortified with reduced nicotinamide adenine dinucleotide phosphate (NADPH)" (*1*). Considerable strain differences existed in the activity of these enzymes in vitro. Nakatsugawa and Dahm demonstrated that "rat liver microsomes degraded parathion by splitting at the aryl phosphate bond," that the system was specific for parathion, and that it did not degrade paraoxon (*2*). With the use of microsomes from houseflies and from rat and rabbit livers, it was also shown that parathion and other "P = S compounds were metabolized via two oxidative pathways, activation to P = O analogs and cleavage at the aryl phosphate bond," reactions that required oxygen and NADPH (*3*). A reductive degradation of parathion to aminoparathion has been demonstrated, after yeast had been added to parathion-treated soils or water (*4*) and also with two species of bacteria (*5*).

Degradation in vitro of parathion to aminoparathion, which is due to the presence of a nitroreductase in houseflies, is described here. Enzyme preparations were obtained from a DDT susceptible strain (CSMA-1948) of houseflies, a strain resistant to DDT and naphthalene vapors (P_2/sel), and another one resistant to DDT only (F58-W). The CSMA strain was used in all tests, while the other two were occasionally used for comparison. Female houseflies were frozen with Dry Ice, and the abdomens were removed and homogenized in grinding medium at 0° to 4°C at a ratio of ten abdomens per milliliter. The medium consisted of 0.15*M* phosphate buffer, *p*H 7.4, and 0.25*M* sucrose. Various cell fractions were obtained by differential centrifugation as described (*6*), except that the soluble fraction was obtained at 150,000*g* for 60 minutes. Pellets were resuspended in 5 to 7 ml of grinding medium. Reaction mixtures were kept in open, 10-ml Erlenmeyer flasks and consisted of 1 to 3 ml of the various cell fractions to which NADPH at $1.3 \times 10^{-4}M$ and 0.22 μc of ^{14}C–ring-labeled ethylparathion (100 μg) in 10 μl of ethanol were added. These mixtures were incubated for 60 minutes in a water bath of a gyratory shaker at 30°C and 120 rev/min. In some tests, shorter or longer incubation periods were employed. After that, the reaction was terminated by adding 3 ml of acetone, followed by extraction with 3×10 ml of a 2 : 1 mixture of hexane and acetone and separation into a hexane and water-acetone phase as described (*6*).

Portions of the hexane phase were analyzed by gas-liquid chromatography (GLC) with a Packard model 7834 gas chromatograph, the conditions being as follows: two different column packings (5 percent Dow 11 on 60-80 chromosorb W and 5 percent QF-1 on 80-90 Anakrom AS) at 175° and 190°C, respectively, helium as the carrier gas, and a hydrogen flame detector coated with potassium chloride, thus rendering the detector especially sensitive to phosphorus (*7*). Labeled metabolites in the hexane fraction were resolved by thin-layer chromatography (TLC) on silica gel, with hexane-chloroform-methanol (7 : 2 : 1) as developing solvents. The metabolites were then detected by radioautography. In addition, color reactions were utilized by spraying the thin-layer plates successively with the reagents of the Averell-Norris method (*4, 8*), except that the reduction step was eliminated. In this way, the presence of previously reduced compounds was indicated by a positive color test. Additional spraying of the plates with a palladium chloride solution followed by spraying with 5*N* NaOH resulted in spots of different colors for parathion, paraoxon, aminoparathion, *p*-nitrophenol, and *p*-aminophenol. To determine the radiocarbon content of isolated compounds, silica-gel areas which had the same R_F value as reference grade chemicals were scraped from the plates into scintillation vials containing 12 ml of dioxane scintillation solvent and 4 percent Cab-O-sil