# DISSOCIATION OF AMPHETAMINE-INDUCED RELEASE OF NOREPINEPHRINE FROM INHIBITION OF NEURONAL UPTAKE IN ISOLATED BRAIN TISSUE*

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AMPHETAMINE and amphetamine analogues increase the efflux of norepinephrine from neurons in the central nervous system. The mechanism by which this effect is produced has not been satisfactorily resolved. Amphetamine has been observed to inhibit neuronal uptake of biogenic amines (ROSS and RENYI, 1964; GLOWINSKI and AXELROD, 1965; HÄGGENDAL and HAMBERGER, 1967), and thus the efflux of norepinephrine could be explained by inhibition of neuronal uptake of spontaneously released norepinephrine. Indirect measurements of uptake and release observed *in vivo* suggest that inhibition of neuronal uptake is probably not the major mechanism by which amphetamine increases the efflux of norepinephrine since the tricyclic antidepressants appear to inhibit neuronal uptake without releasing norepinephrine (CARLSSON and WALDECK, 1966; FUXE and UNGERSTEDT, 1968). Amphetamine also has been observed to release $^3$H-norepinephrine from presumed intraneuronal binding sites (GLOWINSKI and AXELROD, 1965; STEIN and WISE, 1969; ZIANCE *et al.*, 1972), and the efflux of norepinephrine from central nervous system neurons could be largely the result of this effect. In the present report, the effects of amphetamine and other drugs on release and neuronal uptake are studied in *in vitro* systems where these two processes can be measured quantitatively. The aim is to establish conditions in which the actions of drugs on these two processes can be distinguished.

Release of $^3$H-norepinephrine from isolated brain tissue was studied by methods previously described (ZIANCE and RUTLEDGE, 1972; ZIANCE *et al.*, 1972). The method involves incubating chopped rat brain tissue with $10^{-6}$ M $^3$H-norepinephrine, allowing the $^3$H-amine to be taken up into the neurons, washing the unbound and non-specifically bound $^3$H-amine from the tissue and then measuring the effect of drugs on the amount of $^3$H-norepinephrine in the incubation medium and tissue. Since a portion of the $^3$H-norepinephrine is deaminated under the conditions of this experiment, $^3$H-norepinephrine was separated from deaminated metabolites by cation exchange chromatography with Dowex 50, $Na^+$. *O*-methylation plays a minor role in the metabolism of norepinephrine in this system (ZIANCE *et al.*, 1972) and thus norepinephrine was not separated from its *O*-methylated metabolite, normetanephrine. Release is expressed as $^3$H-norepinephrine in the medium as a percentage of

* This study was supported by Grant NS90951 from the U.S.P.H.S.

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$^3$H-norepinephrine in the medium + tissue. Previous studies (Ziance *et al.*, 1972; AZZARO and RUTLEDGE, 1973) have shown that release of norepinephrine from brain tissue by amphetamine as measured by this procedure is: (1) sensitive, the threshold for release of $^3$H-norepinephrine from cerebral cortex by amphetamine is $10^{-7}$ M; (2) tissue selective, the $ED_{50}$ for release of $^3$H-norepinephrine from cerebral cortex is less than the $ED_{50}$'s for release from medulla oblongata or corpus striatum; (3) substrate specific, the concentration–effect curve for release of $^3$H-norepinephrine by amphetamine is markedly different from the curves for release of $^3$H-dopamine or $^3$H-5-hydroxytryptamine; (4) temperature dependent, maximal release occurs at 37°C and there is no release at 0°C; (5) stereoselective, *d*-amphetamine is more potent than the *l*-isomer.

Neuronal uptake of $^3$H-norepinephrine into chopped rat brain tissue was measured by a method involving a 10 min incubation of chopped rat brain tissue with $10^{-7}$ M $^3$H-norepinephrine and measuring total radioactivity in the incubation medium and tissue (ZIANCE and RUTLEDGE, 1972). Drugs were added to the incubation medium 10 min before and during the incubation with $^3$H-norepinephrine. Experimental samples were incubated at 37°C and compared to control samples incubated at 0°C. Since there is relatively little metabolism of $^3$H-norepinephrine in this 10 min exposure, $^3$H-norepinephrine was not separated from the deaminated metabolites. Results were expressed as tissue to medium ratios: (dis/min per g of tissue)/(dis/min per ml of medium). This ratio approaches 1·0 at 0°C which indicates a lack of specific uptake at this temperature. The ratio was 7·1 ± 0·4 at 37°C in the absence of drugs, and this reflects approximately a seven-fold increase in uptake and accumulation of $^3$H-norepinephrine into the tissue. From these ratios the percentage inhibition of neuronal uptake produced by drugs was determined.

TABLE 1. EFFECT OF AMPHETAMINE, COCAINE AND DESIPRAMINE ON RELEASE AND INHIBITION OF NEURONAL UPTAKE OF $^3$H-NOREPINEPHRINE IN ISOLATED CEREBRAL CORTEX TISSUE

| Drug | Inhibition of uptake* (%) | Release† (%) |
|---|---|---|
| Amphetamine $10^{-5}$ M | 85·0 ± 1·3 (8)‡ | 30·4 ± 1·3 (14) |
| Cocaine $10^{-5}$ M | 83·6 ± 2·4 (6) | 10·2 ± 1·3 (3) |
| Desipramine $10^{-5}$ M | 78·9 ± 3·5 (3) | 0·7 ± 0·7 (4) |

* All samples were incubated for 10 min with $10^{-7}$ M $^3$H-NE. Each value represents the mean ± S.E.M.

† Calculated as $\frac{\text{dis/min } ^3\text{H-NE in medium (100)}}{\text{dis/min } ^3\text{H-NE in medium + dis/min } ^3\text{H-NE in tissue.}}$ Release in the absence of drug was 11·7 ± 1·1 and was subtracted from each of the drug values. Each value is the mean ± S.E.M.

‡ Number within the parenthesis represents the number of experiments.

The effect of $10^{-5}$ M concentrations of amphetamine, cocaine and desipramine upon neuronal uptake and release of $^3$H-norepinephrine from chopped cerebral cortex tissue can be seen in Table 1. Inhibition of neuronal uptake by the three drugs was approximately the same while release of $^3$H-norepinephrine was markedly different. Release of $^3$H-norepinephrine by amphetamine was much greater than that produced by cocaine and $^3$H-norepinephrine was not released by desipramine.

The effects of various concentrations of amphetamine ($10^{-7}$–$10^{-3}$ M) on release and neuronal uptake of $^3$H-norepinephrine were measured and it was observed that amphetamine released $^3$H-norepinephrine from cerebral cortex with concentrations which were only slightly less than those required for inhibition of neuronal uptake. The $ID_{50}$ for inhibition of neuronal uptake by amphetamine was $7{\cdot}1 \times 10^{-7}$ M while the $ED_{50}$ for release was $1{\cdot}2 \times 10^{-6}$ M. Although the $ED_{50}$ for cocaine induced release of $^3$H-norepinephrine was approximately the same as that of amphetamine, the maximal release ($11{\cdot}2 \pm 2{\cdot}1\%$) produced by cocaine ($10^{-4}$ M) was much less than that of amphetamine ($10^{-3}$ M, $38{\cdot}1 \pm 1{\cdot}6\%$). Desipramine, on the other hand, was much less potent than the other drugs in releasing $^3$H-norepinephrine; the lowest concentration of desipramine which released $^3$H-norepinephrine was $10^{-4}$ M. It is not likely that the efflux of $^3$H-norepinephrine produced by amphetamine is the result of inhibition of reuptake of spontaneously released $^3$H-norepinephrine since cocaine and desipramine were equipotent with amphetamine in inhibiting uptake of $^3$H-norepinephrine but were much less efficacious in releasing the $^3$H-amine.

Results have also been obtained (RUTLEDGE *et al.*, 1972) which indicate that when neuronal uptake is blocked with cocaine or desipramine, amphetamine is still capable of releasing $^3$H-norepinephrine even though the concentration effect curve is shifted to the right. Studies on the uptake of $^3$H-amphetamine into isolated synaptosomes from rat cerebral cortex suggest that low concentrations of amphetamine ($10^{-7}$–$10^{-6}$ M) enter the neuron by the neuronal uptake mechanism, since the accumulation of $^3$H-amphetamine is inhibited by cocaine and desipramine (RUTLEDGE *et al.*, 1972). Thus, these drugs probably inhibit the release of $^3$H-norepinephrine which is observed with low concentrations of amphetamine by inhibiting the uptake of amphetamine into the neuron. Amphetamine in higher concentrations ($10^{-5}$–$10^{-3}$ M) appears to enter the neuron by a nonspecific process which is not inhibited by cocaine or desipramine and thus the release produced by high concentrations of amphetamine is not prevented by blockade of neuronal uptake.

In summary, inhibition of neuronal uptake is probably not the primary mechanism by which the efflux of $^3$H-norepinephrine is enhanced by amphetamine. However, inhibition of neuronal uptake may play a role in blocking recapture of the released amine.

*Acknowledgements*—The skillful technical assistance of Mrs. Elisabeth Dreyer is gratefully acknowledged. The authors are grateful to Smith, Kline and French Laboratories for the contribution of *d*-amphetamine sulfate and to Geigy Pharmaceuticals for the contribution of desipramine hydrochloride.

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