Norepinephrine: Release from Brain by d-Amphetamine in vivo

Abstract. *After injection of H^3-norepinephrine into the left lateral cerebral ventricle of cats, the lateral and third cerebral ventricles were perfused with an artificial cerebrospinal fluid. Addition of* d-*amphetamine to the perfusion fluid caused a significant increase in the concentration of H^3-norepinephrine in the effluent.*

Many drugs exert their actions on effector organs innervated by the sympathetic nervous system by releasing or blocking uptake of norepinephrine at the nerve terminal or by blocking the actions of this amine at receptors on the effector organ (*1*). Some drugs may produce their effects in the central nervous system by acting in a similar manner at noradrenergic synapses within the brain (*2*).

Amphetamine, which exhibits both peripheral and central actions, acts in the periphery by releasing norepinephrine from postganglionic sympathetic nerve endings (*3*) and by blocking reuptake of norepinephrine by the nerve terminals (*4*); it thereby increases the concentration of transmitter available at the postjunctional receptors. It has been suggested that these same mechanisms are responsible for the striking excitatory effects of amphetamine in the central nervous system (*2, 5*). Steady-state concentrations of norepinephrine, but not of dopamine, are reduced in the brain by large doses of amphetamine (*6*). In addition, the relative norepinephrine-depleting abilities of *d*- and *l*-amphetamine coincide with the relative central stimulating potency of these two isomers (*7*). α-Methyltyrosine, which blocks catecholamine synthesis, prevents the central stimulant actions of amphetamine which suggests that a small functionally active pool of norepinephrine maintained by synthesis is required for *d*-amphetamine to produce its central effects (*8*). Although this evidence suggests that the central stimulating actions of amphetamine are causally related to the ability of this drug to release brain norepinephrine, the actual release of this amine from neurons in intact brain by amphetamine has not been demonstrated.

Since norepinephrine does not readily enter or leave the brain by way of the vascular system, it has been necessary to study the functions of this amine in the central nervous system by indirect methods. For example, a decrease in the brain amine concentrations and an increase in the concentration of *O*-methylated metabolites after the administration of various drugs has been taken as evidence of release (*2*). Two recent developments have permitted a more direct approach for studying the release of these suspected neurotransmitters from brain. First, labeled catecholamines, introduced into the brain by intraventricular or intracisternal injections, mix with endogenous stores of norepinephrine and dopamine (*9*). Second, a cerebroventricular perfusion technique with inflow and outflow cannulae inserted in various parts of the cerebroventricular system has been developed (*10*) and used to detect the release of dopamine and homovanillic acid from the caudate nucleus (*11*). By using intraventricular injection and perfusion techniques, we have been able to demonstrate the release in vivo of norepinephrine in the brain by *d*-amphetamine.

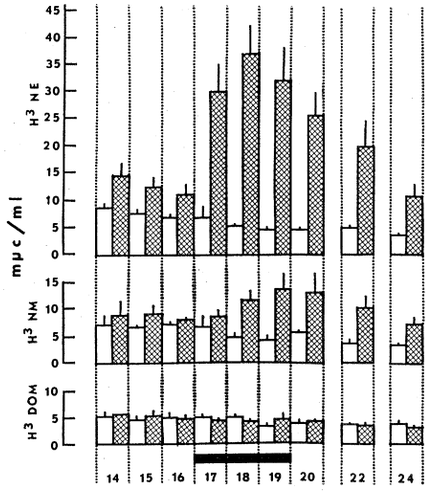

Fig. 1. Effects of *d*-amphetamine sulfate on the concentration of H^3-norepinephrine and its metabolites in cerebroventricular effluent. The height of each bar represents the mean concentration (vertical lines denote 1 standard error) of H^3-norepinephrine (H^3NE), H^3-normetanephrine (H^3NM), and deaminated-*O*-methyl metabolites (H^3DOM) in effluent collected over a 10-minute period. In three cats (open bars) the brains were perfused only with artificial cerebrospinal fluid. In five cats (shaded bars) the brains were perfused in a similar fashion, except during the time period indicated by the solid horizontal bar below the graph when *d*-amphetamine sulfate (100 μg/ml) was added to the perfusing fluid. Numbers on the abscissa indicate the successive 10-minute collection periods.

Cats were briefly anesthetized with methoxyfluorane, and the spinal cord was sectioned at C_1. Respiration was maintained with a respirator pump, and rectal temperature was maintained at 37.5° ± 0.5°C with a heating pad. A self-tapping screw-type cannula was stereotaxically implanted in the left cerebral ventricle (*12*). The cisterna magna was surgically exposed, and a polyethylene cannula (4 cm by 2 mm, outer diameter) was passed along the floor of the fourth ventricle and into the cerebral aqueduct. *dl*-Norepinephrine-7-H^3 hydrochloride (5 μc; 9.71 c/mmole), in an effective volume of 10 μl, was injected into the left cerebral ventricle. This volume is immediately distributed to the left lateral ventricle and to the ventral portion of the third ventricle (*13*). One hour later the lateral and third cerebral ventricles were perfused with an artificial cerebrospinal fluid (*14*). This fluid was pumped into the lateral ventricular cannula at a rate of 0.1 ml/min and collected continuously from the aqueduct cannula at 10-minute intervals in tubes containing 0.1 ml of 5*N* acetic acid. Total radioactivity in the effluent declined progressively so that by 2 hours it had reached a stable level. At this time, various amounts of *d*-amphetamine sulfate were added to the artificial cerebrospinal fluid, and the ventricles were perfused with this mixture for 30 minutes; the perfusion was then continued with artificial cerebrospinal fluid without the drug.

The effluent samples were analyzed for H^3-norepinephrine, H^3-normetanephrine, and H^3-deaminated-*O*-methyl metabolites by modifications of known methods (*15*). Briefly, the effluent from the ventricles was adjusted to *p*H 8.6 and shaken in 2.5-ml glass-stoppered centrifuge tubes containing 100 mg of aluminum oxide. The supernatant, containing *O*-methylated metabolites, was adjusted to *p*H 6 and placed on columns of Dowex 50W-X8 (H^+ form, 6 by 40 mm). The columns were washed with 5 ml of water, and the radioactivity in the combined effluent wash represented *O*-methylated deaminated products. H^3-Normetanephrine was eluted from the ion-exchange columns with 5 ml of a mixture of 5*N* HCl and ethanol (1:1). H^3-Catechols were eluted from the alumina with 0.5*N* acetic acid. Analysis of this acid

eluate by thin-layer chromatography indicated that the radioactivity represented H^3-norepinephrine; negligible amounts of deaminated catechol metabolites could be detected. A portion (100 μl) of each chromatographic fraction was placed in scintillation vials containing 1 ml of water and 10 ml of modified Bray's solution (*16*) and counted in a Beckman DPM-100 liquid-scintillation spectrometer. The relative concentrations of H^3-norepinephrine and its metabolites in the control effluent were similar to that found in cat brain tissues after intraventricular injection of this amine (*13*).

The effects of a 30-minute perfusion of *d*-amphetamine (100 μg/ml; total dose of 300 μg) are shown in Fig. 1. There was an immediate and sustained increase in the concentration of H^3-norepinephrine appearing in the effluent when amphetamine was added to the perfusing solution; 30 minutes after the amphetamine perfusion was discontinued, the concentration of H^3-norepinephrine in the effluent was still elevated. Concentrations as low as 6.25 μg/ml (total dose of 18.75 μg) caused a significant increase in the amount of H^3-norepinephrine in the effluent; a maximum response was obtained with 200 μg of *d*-amphetamine per milliliter of perfusing fluid.

After a latent period of 10 to 20 minutes, *d*-amphetamine also increased the concentration of H^3-normetanephrine but not of deaminated-*O*-methyl metabolites appearing in the effluent. The delayed increase of H^3-normetanephrine suggests that H^3-norepinephrine is released (*17*) from brain tissue adjacent to the ventricular system and then partially metabolized by extraneuronal catechol-*O*-methyl transferase (*2*).

After the intraventricular injection of C^{14}-inulin, the addition of *d*-amphetamine to the perfusing fluid did not alter the concentration of this substance in the effluent. Thus, the capacity of *d*-amphetamine to release H^3-norepinephrine from the brain is more specific than that reported in a previous study in which odor stimuli increased the efflux of metabolically inert substances as well as H^3-norepinephrine from the olfactory bulb of rats (*18*).

In contrast to other methods for studying the dynamics of brain catecholamines, which involve chemical or histochemical analysis of these amines and their metabolites at fixed points in time, the cerebral perfusion technique provides an opportunity for continuously monitoring the effects of various stimuli or drugs on the release of amines from the brain. This method has a distinct advantage over the use of push-pull cannulae because the latter significantly damage tissue in the perfused area (*19*). To date, because of limitations imposed by the lack of sensitivity of our analytical methods, we have been unable to detect endogenous catecholamines or their metabolites in the ventricular effluent. Accordingly, it has been necessary to resort to radioactive tracer techniques. Although the regional distribution of intraventricular H^3-norepinephrine is related to that of endogenous catecholamines (*15*), the uptake of H^3-norepinephrine in brain is probably not confined to neurons containing catecholamine so that the norepinephrine which appears in the effluent may not arise exclusively from these structures. This problem may be overcome by labeling brain catecholamines with C^{14}-tyrosine so that C^{14}-catecholamines appearing in the effluent will have been synthesized by and released from noradrenergic or dopaminergic neurons.

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17. The term release is used here to denote release from the brain and does not imply any specific releasing mechanism at the neuronal level. It is not possible here to distinguish between active release from the nerve terminal and blockade of reuptake of norepinephrine by *d*-amphetamine.
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Juvenile Hormone: Activity of Aromatic Terpenoid Ethers

Abstract. *Several aromatic terpenoid ethers possess a high degree of morphogenetic activity when assayed on the yellow mealworm* Tenebrio molitor *L. and the milkweed bug* Oncopeltus fasciatus *(Dallas). The most active compounds were the 3,4-methylenedioxyphenyl ethers of 6,7-epoxygeraniol and the corresponding ethyl-branched homologs.*

Certain commercial insecticide synergists possess significant juvenile hormone activity for several insect species (*1*). The most active synergists were 3,4-methylenedioxybenzyl and -phenyl ethers. In addition, the side chain substituents can be replaced by the sesquiterpenoid alcohol farnesol and its terminal epoxide (I).

In extending these studies I have found that the juvenile hormone (JH) activity of methylenedioxyphenyl terpenoid ethers is significantly greater than that of the corresponding methylenedioxybenzyl analogs and that substitution of 6,7-epoxygeraniol (IIa) for 10,11-epoxyfarnesol as the side chain resulted in a further increase in activity. Various homologs of the geranyl moiety, IIb, IIc, and III, possess an even higher order of JH activity.

Three aromatic terpenoid ethers, V, VI, and VII, were prepared which lack the methylenedioxy moiety. One of