of Hassinen[10] that this compound strongly inhibits dinitrophenol-induced ATPase of liver mitochondria.

It is clear from these experiments that dithiopyridine and disulfiram are less specific than DTNB in their effect on mitochondrial metabolism. While the action of DTNB[1-3] appears to be concerned almost entirely with the effect of inorganic phosphate on mitochondria (probably because it inhibits phosphate transport), the less charged disulfides, dithiopyridine and disulfiram, have additional effects such as inhibition of DNP-stimulated respiration.

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Release of norepinephrine and normetanephrine from cat brain by central nervous system stimulants*

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IT HAS been suggested[1-3] that the central actions of a number of psychoactive drugs are related to their ability to alter the concentration of norepinephrine at specific postsynaptic receptor sites within

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the central nervous system. For example, the stimulant or antidepressant effects of amphetamine and imipramine may be related to an increase, and the depressant properties of reserpine and α-methyltyrosine to a decrease in synaptic cleft concentrations of norepinephrine. Although, at present, it is not technically feasible to determine directly the concentration of neurotransmitters in synaptic clefts within the central nervous system, the gross release of suspected neurotransmitters (acetylcholine, 5-hydroxytryptamine, dopamine) from brain tissue lining the cerebroventricular system can be detected with the aid of a perfusion technique.[4–6]

By labeling catecholamine stores with intraventricularly administered ^{3}H-norepinephrine,[7] and subsequently perfusing the cerebroventricular system, it has been possible to continuously monitor the efflux of ^{3}H-norepinephrine and its metabolites from cat brain *in situ*. With this technique it has been demonstrated that amphetamine increases the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine.[8–9] In the present report the effects of amphetamine on the efflux of these amines are compared with the effects of several other central nervous system stimulants.

Mongrel cats of either sex weighing 2–4 kg were briefly anesthetized by open drop administration of methoxyflurane and placed in a stereotaxic apparatus (David Kopf, Inc.) where they remained for the entire experimental period. The spinal cord was sectioned at C_1 and respiration was maintained with a respirator pump. Systemic blood pressure was recorded from the right carotid artery and rectal temperature was maintained at 37·5 ± 0·5° with a heating pad.

A self-tapping screw-type cannula was implanted in the left lateral cerebral ventricle as described by McCarthy and Borison.[10] Thirty min after spinal section, 5μc *dl*-norepinephrine-7-^{3}H hydrochloride (7·45 c/m-mole, 1 μc/μl; New England Nuclear Corp.) was injected intraventricularly. This solution was washed out of the cannula (dead space, 6–8 μl) with 10 μl of artificial cerebrospinal fluid.[11] One hr after injection of the isotope, the cisterna magna was surgically exposed and a polyethylene cannula (4 cm × 2 mm o.d.) was passed along the floor of the fourth ventricle and into the cerebral aqueduct. Artificial cerebrospinal fluid was then infused into the lateral ventricle at a rate of 0·1 ml per min with a Harvard infusion pump. The perfusion effluent for the first 2 hr was discarded and then a total of seven consecutive 10-min samples were collected in 5-ml glass-stoppered centrifuge tubes containing 0·1 ml of 5·0 N acetic acid. Only artificial cerebrospinal fluid was perfused during the first three samples. After collection of these "control" (pre-drug) samples, a variety of central nervous system stimulants were dissolved in artificial cerebrospinal fluid and perfused for three additional collection periods (samples 4–6). Perfusion with the drug solutions was immediately followed by a 10-min perfusion with artificial cerebrospinal fluid (sample 7). The 1-ml samples of perfusion effluent were analyzed for ^{3}H-norepinephrine and its metabolites as described previously.[9]

In the present study, those drugs that increased the effluent concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine had a similar time course of action as did *d*-amphetamine.[8] That is, they caused an immediate increase (in the first 10 min) in the ^{3}H-norepinephrine concentration and a delayed (10–20 min) increase in the concentration of ^{3}H-normetanephrine. For this reason, the total efflux of ^{3}H-norepinephrine during the 30-min drug perfusion period (samples 4–6) was compared with the total 30-min pre-drug period (samples 1–3), while the change in efflux of ^{3}H-normetanephrine during the last 30 min of perfusion (samples 5–7) was compared with the 30-min pre-drug period. Statistical analysis of the concentration of labeled compounds in the perfusion effluent was carried out using Student's *t*-test, paired comparison.[12]

Since there is little information on the central stimulating actions of drugs which have been administered into cerebral ventricles, it was difficult to determine what concentrations of drugs should be added to the perfusing fluid. In a previous study, of identical experimental design, it was demonstrated that 100 μg/ml of *d*-amphetamine sulfate caused a maximal increase in the efflux of ^{3}H-norepinephrine.[9] The concentrations of other stimulant drugs used in the perfusion experiments were based upon their relative abilities to increase spontaneous locomotor activity in mice when determined as described by Dominic and Moore.[13] In this test, 2 mg/kg of *d*-amphetamine sulfate increased motor activity 2- to 4-fold. The dose of other stimulants required to cause an equivalent increase in motor activity was determined and the dose-ratio obtained was used to compute the perfusion concentrations of other drugs (i.e. compared with 100 μg/ml of *d*-amphetamine sulfate). Intravenous injections of *d*-amphetamine (1 mg/kg) activated the EEG in spinal cats and produced signs of behavioral stimulation;[14] the same dose of amphetamine increased the effluent concentration of ^{3}H-norepinephrine.[9] The ratio of the effective intravenous dose of amphetamine to the effective intraventricular perfusion concentration is 1000 μg/kg to 100 μg/ml. If the same ratio were applied to

the other drugs used in this study (see Table 1), the relative intravenous doses of the drugs would be: ephedrine HCl, 20 mg/kg; *p*-chloroamphetamine HCl, 3 mg/kg; methylphenidate HCl, 3·75 mg/kg; pipradrol HCl, 3 mg/kg; cocaine HCl, 7·5 mg/kg; caffeine, 5 mg/kg. Comparable doses have been reported by other workers to have central stimulant actions in cats.[14–17]

The effects of perfusing various stimulants through the cerebroventricular system on the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine are summarized in Table 1. *d*-Amphetamine, ephedrine,

TABLE 1. EFFECT OF CENTRAL NERVOUS SYSTEM STIMULANTS ON THE EFFLUX OF ^{3}H-NOREPINEPHRINE (^{3}H-NE) AND ^{3}H-NORMETANEPHRINE (^{3}H-NM)*

Drug	Concn (μg/ml)	N	^{3}H-NE (mμc ± S.E.)	^{3}H-NM (mμc ± S.E.)
Control		5	−8·6 ± 2·6	−5·4 ± 1·5
d-Amphetamine SO_4	100	5	62·1 ± 9·1†	11·9 ± 4·2†
Ephedrine HCl	2000	4	79·2 ± 11·4†	10·5 ± 4·4†
p-Chloroamphetamine HCl	300	4	114·7 ± 33·3†	18·1 ± 7·9†
Methylphenidate HCl	375	4	42·2 ± 4·1†	3·9 ± 1·4†
Pipradrol HCl	300	5	45·8 ± 25·7	−0·4 ± 0·7
Cocaine HCl	750	3	−5·4 ± 1·6	−5·9 ± 1·4
Caffeine	500	5	2·2 ± 1·8	−9·1 ± 1·2

* One hr after an injection of 5 μc ^{3}H-norepinephrine into the left lateral ventricle, the lateral and third ventricles were perfused with artificial cerebrospinal fluid and various drugs as described in Methods. Each value represents the mean change in radioactivity for ^{3}H-norepinephrine (samples 4–6) and ^{3}H-normetanephrine (samples 5–7) when compared with the 3 pre-drug samples (1–3). N = number of animals.

† Mean for drug samples significantly greater than pre-drug samples ($P < 0.05$).

p-chloroamphetamine and methylphenidate significantly increased the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine. Although pipradrol tended to increase the efflux of ^{3}H-norepinephrine, the effect was not significantly different from the pre-drug period because of the large variability in the response. Neither cocaine nor caffeine significantly altered the efflux of ^{3}H-norepinephrine or ^{3}H-normetanephrine and none of the drugs altered the effluent concentrations of ^{3}H-deaminated catechols or ^{3}H-deaminated *O*-methyl metabolites.

The increased efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine caused by some of the drugs in this study may result from any one of a number of mechanisms. For example, the drugs may act at the terminals of amine-containing neurons to actively release or to block uptake of ^{3}H-norepinephrine resulting in an overflow of the amine into extracellular spaces. On the other hand, the drugs may exchange with neuronal or extraneuronal ^{3}H-norepinephrine in a nonspecific manner. Alternatively, increased activity of amine-containing neurons resulting from direct or reflex stimulation by the drugs may cause the increased efflux of ^{3}H-norepinephrine. Regardless of the ultimate mechanism, the increase in the effluent concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine may be a gross representation of the concentration of these amines at postsynaptic receptor sites in tissues lining the cerebroventricular system.

Drugs which act on effector organs innervated by the sympathetic nervous system by releasing or blocking the uptake of norepinephrine at peripheral nerve terminals may act in a similar manner at noradrenergic synapses in the brain. For example, recent studies *in vitro*[18] and *in vivo*[8,19] suggest that amphetamine increases the extraneuronal concentration of norepinephrine in brain tissues by one or both of these mechanisms. Ephedrine, which also releases norepinephrine from peripheral sympathetic nerve terminals[20] and blocks the neuronal uptake mechanism,[21] has been reported to reduce the concentration of norepinephrine in the rat[22] but not in the cat[23] brain. In the present study, ephedrine caused an increase in the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine similar to that induced by *d*-amphetamine, suggesting that the two drugs may exert their central stimulant actions by a similar mechanism. This suggestion is supported by the finding that α-methyltyrosine, an inhibitor of catecholamine synthesis, blocks the stimulation of motor activity caused by *d*-amphetamine and

ephedrine.[13] Thus, these two drugs may act by releasing newly synthesized catecholamines from central amine-containing neurons.

The *p*-chloro analogue of amphetamine has been reported to elevate the rat brain concentration of ^{3}H-normetanephrine but to have no effect on the concentration of ^{3}H-norepinephrine.[24] These same workers indicated that the stimulation caused by this drug is related temporally to its effect on the metabolism of norepinephrine.[25] The marked increase in the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine induced by *p*-chloroamphetamine in the present study strongly suggests that it acts in a manner similar to that of amphetamine.

The mechanism of action of the two piperidine derivatives, methyphenidate and pipradrol, is not well understood. Both compounds have been reported to block the uptake of norepinephrine into peripheral sympathetic nerve terminals.[26] Results of the present study indicate that methylphenidate, and possibly pipradrol, act by increasing the synaptic concentration of norepinephrine. However, since their stimulant actions are not blocked by α-methyltyrosine,[13] it is unlikely that they act by releasing newly synthesized norepinephrine.

Previous studies concerning the central biochemical actions of cocaine are confusing. This drug does not alter brain concentrations of norepinephrine,[27] it blocks the brain uptake of intracisternally administered[28] but not intraventricularly administered[29] ^{3}H-norepinephrine, and it prevents the accumulation of ^{3}H-norepinephrine in brain slices.[30] It has been suggested[29] that cocaine may not have the same actions in the intact brain as it does in brain slices or in the peripheral nervous system. The results of the present study suggest that cocaine does not act by increasing the synaptic concentration of norepinephrine in structures lining the ventricular system.

Caffeine has no effect on the concentration of norepinephrine in the cat hypothalamus,[23] but it has been suggested that this drug releases norepinephrine from the rat heart and brain.[31] Results of the present study suggest that the central stimulating actions of caffeine are not related to an increased concentration of norepinephrine at postsynaptic receptor sites. However, the possibility exists that both caffeine and cocaine exert their effects at sites that are not adjacent to the ventricular system.

Since neither cocaine nor caffeine increases the efflux of ^{3}H-norepinephrine or ^{3}H-normetanephrine, but, like the other central stimulants studied, both increase motor activity, it would appear that the increased efflux observed during perfusion of the other drugs is not the result of generalized central nervous system stimulation. Rather, it would appear that amphetamine, ephedrine, *p*-chloroamphetamine, methylphenidate, and possibly pipradrol, act directly at the terminals of catecholamine-containing neurons.

In this study we have attempted to establish whether a number of drugs that stimulate the central nervous system are capable of increasing the efflux of ^{3}H-norepinephrine. It is beyond the scope of the present study to correlate the effects of these drugs on central stimulation and efflux of norepinephrine. In ongoing studies, however, we are attempting more complete studies utilizing different concentrations of the drugs on the efflux of ^{3}H-norepinephrine and on electroencephalographic recordings.

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