STUDIES ON THE MECHANISM OF ACTION OF COCAINE IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

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IN THE CENTRAL NERVOUS SYSTEM

By

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The purpose of this study was to investigate the ability of cocaine to influence the efflux of dopamine and/or serotonin from the brain. By comparing the effects of cocaine administration observed using a cerebroventricular perfusion technique with the results of similar studies done previously with tyramine and amphetamine, some conclusions were made concerning the mechanism of action of cocaine in the central nervous system.

A cerebroventricular perfusion technique was utilized to detect the effect of endogenously synthesized ^{3}H-dopamine or ^{3}H-serotonin from the cat brain. Dopamine or serotonin stores in the brain were labelled using a continuous intraventricular infusion of ^{3}H-tyrosine or ^{3}H-tryptophan. The perfusing cerebrospinal fluid was collected at 5 or 10 min intervals from a catheter placed at the cerebral aqueduct. Perfusates were analyzed for ^{3}H-dopamine using a combination of ion-exchange and alumina adsorption chromatography and analyzed for ^{3}H-serotonin using a combination of ion exchange resin and a resin which separates by both charge and molecular weight.

Cocaine, when administered either intraventricularly (10^{-5}M) or intravenously (5 mg/kg), significantly increased the efflux of endogenously synthesized ^{3}H-dopamine from the brain. Intraventricular perfusion of cocaine caused a concentration-related increase in the efflux of ^{3}H-dopamine.

The efflux of endogenously synthesized ^{3}H-dopamine caused by intraventricularly administered amphetamine (10^{-5}M) and tyramine (10^{-5}M) was the same before and after a mechanical lesion of the nigrostriatal pathway. The cocaine-induced release of newly synthesized ^{3}H-dopamine, on the other hand, was reduced by the lesion. These results suggest that cocaine, but not amphetamine or tyramine, requires the activity of nigrostriatal neurons in order to increase the efflux of endogenously synthesized ^{3}H-dopamine.

A continuous infusion of cocaine (10^{-5}M) did not alter the efflux of ^{3}H-dopamine caused by pulse injections of either d-amphetamine (10^{-5}M) or of tyramine (10^{-4}M). Thus, the mechanism of action of cocaine in the central nervous system does not appear to be the same as amphetamine, which is reported to act by actively releasing amines and by blocking the reuptake of released transmitter, or tyramine, which is reported to act by releasing amines. Cocaine may act by facilitating the neurogenic release or by blocking the reuptake of neurogenically released dopamine.

Cocaine, when administered intravenously at high concentrations, significantly increased the efflux of endogenously synthesized ^{3}H-serotonin from the brain. This effect did not appear to be concentration related. The magnitude of this increase was only 200% of baseline, while the magnitude of the increase of efflux of ^{3}H-dopamine was 800% of baseline for the same concentrations of cocaine. The intravenous

administration of cocaine (5 mg/kg) resulted in a slight but prolonged decrease in the efflux of ^{3}H-serotonin.

A continuous infusion of cocaine (10^{-3}M) did not alter the efflux of ^{3}H-serotonin caused by pulse injections of either d-amphetamine (10^{-4}M) or of tyramine (10^{-4}M). Because of the high concentrations of drugs used to investigate the serotonergic system in this study, it is difficult to interpret the results in terms of brain concentrations found after systemic administration of these same drugs. In spite of the extremely high concentrations of cocaine used in the drug interaction studies, neither the effect of amphetamine nor tyramine was blocked. These results suggest that either cocaine does not block the uptake of these two drugs into serotonin neurons or that uptake is not necessary for their action.

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LITERATURE REVIEW

I. A. Introduction

The recent increase in the abuse of cocaine has renewed interest in the pharmacological properties of this drug. Cocaine has three distinct actions: 1) it is a local anesthetic of high efficacy; 2) it is a sympathomimetic drug with powerful vasoconstrictor properties; 3) it is a powerful CNS stimulant of short duration with a low margin of safety.

A number of studies (Van Rossum et al., 1962; Smith, 1965; Wallach and Gershon, 1971; Scheel-Krüger, 1972; Groppetti et al., 1973) have shown that the behavioral effects following cocaine or amphetamine administration are similar. These include an increase in alertness and a decrease in food intake in mice, an increase in locomotor activity, rearing, and body temperature and a decrease in food intake in rats, and a desynchronization of the EEG and an increase in multiple reticular unit activity in cats. Clinically, cocaine and amphetamine induce a psychotic state resembling paranoid schizophrenia (Wallach and Gershon, 1971).

One question the experiments presented in this thesis will try to answer is whether cocaine and amphetamine exert their action in the central nervous system by a common mechanism.

B. Physical Properties of Cocaine

The high lipid solubility of cocaine, as indicated by the high brain/plasma ratio for this drug, allows it to rapidly enter the brain. Peak concentrations of cocaine are reached in the brain 15 minutes after an intravenous injection. The drug quickly disappears from the brains of acutely treated animals (Nayak et al., 1976).

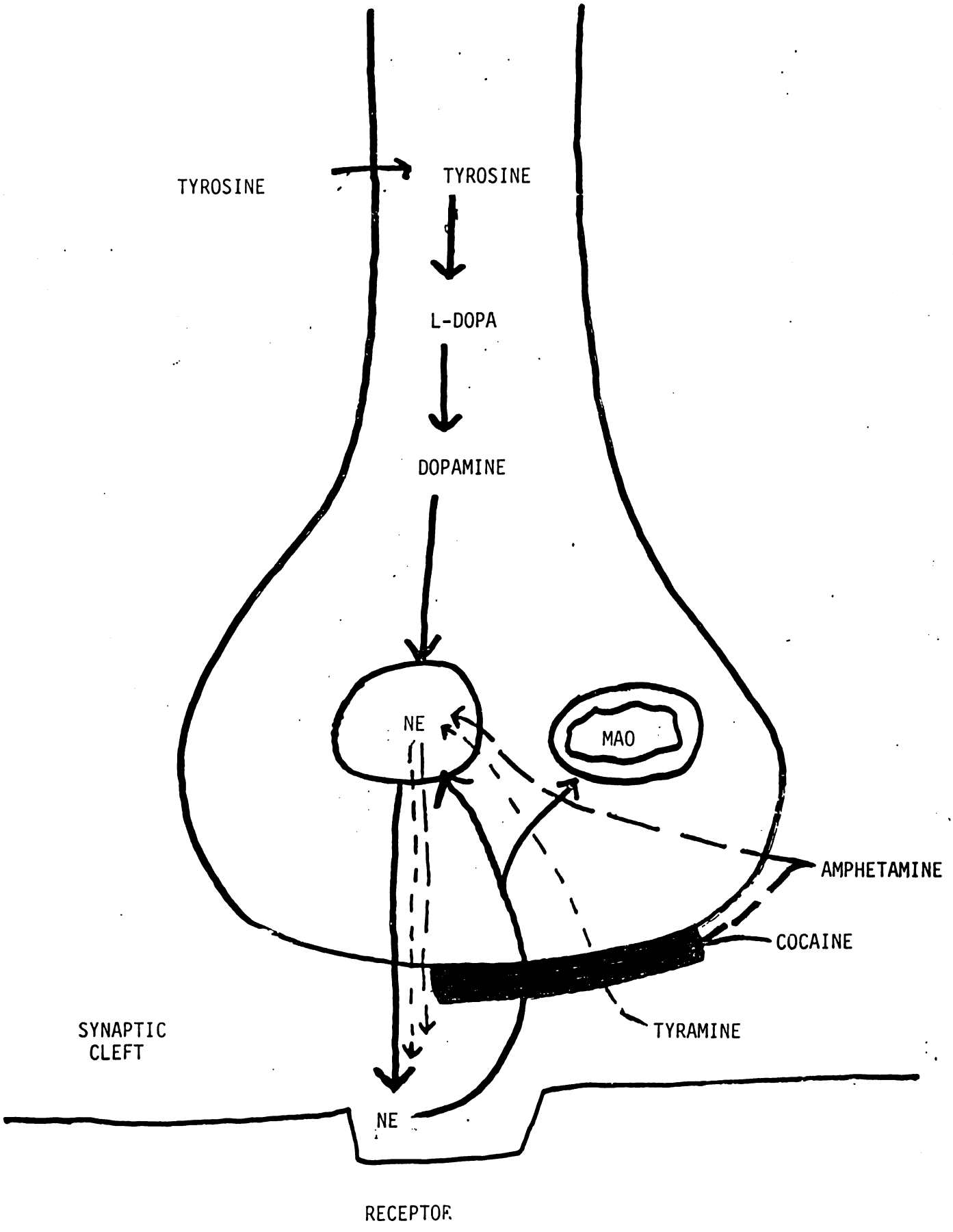
II. A. Model of a Norepinephrine Neuron

Cocaine has mostly been studied in the periphery on noradrenergic transmission processes. A model of a noradrenergic neuron is presented in Figure 1. Norepinephrine, which is stored in the neuron in synaptic vesicles, is released from the nerve terminal in response to an arriving action potential or in response to the actions of drugs. Stimulation of the neuron causes permeability changes of the membrane to calcium, allowing a transient increase in the concentration of calcium inside the cell near the membrane which serves to mobilize the synaptic vesicles and attach the vesicular membrane to the cell membrane. At the same time, the action of ATP on the granule membrane causes a conformational change which then allows the escape of the transmitter. This active ejection of transmitter is directed towards the postsynaptic membrane (Poisner, 1970). Once the transmitter has diffused across the synaptic cleft, it combines with specific receptor sites on the postsynaptic membrane and produces a permeability change.

The action of the released norepinephrine is ended when it is actively taken back up into the nerve terminal (Dengler et al., 1961; Snyder and Coyle, 1969). The norepinephrine can then enter the mitochondria and be degraded by monoamine oxidase, or re-enter the storage vesicles. The stored amine can then be released again.

Figure 1. Model of a peripheral noradrenergic nerve terminal (adapted from Iversen, 1967) depicting the postulated mechanisms of action for cocaine, amphetamine and tyramine.

Figure 1


TYROSINE
TYROSINE
L-DOPA
DOPAMINE
NE
MAO
AMPHETAMINE
COCAINE
TYRAMINE
SYNAPTIC CLEFT
NE
RECEPTOR

When Iversen (1967,1970) presented the above model he suggested 10 possible ways a drug could interact with the neuron. The following discussion will focus on only two of these, release of the neurotransmitter into the synaptic cleft and uptake back into the nerve terminal.

B. Are there Differences Between Neurons?

Is it valid to generalize from a model of a peripheral noradrenergic neuron to a central dopaminergic or serotonergic neuron? Many pharmacological studies have been carried out in the peripheral nervous system rather than in the central nervous system (CNS) because of the less complex anatomical relationship of a peripheral neuron to its surroundings, whereas the cell bodies in the CNS are generally covered with synapses and densely packed with glial cells. Because of the many varied synapses converging on a single CNS neuron, it may be influenced by more than one transmitter substance, where only a single transmitter is thought to act at a peripheral synapse. When results of peripheral experiments are considered with these reservations, they serve as a useful guideline for what may occur in the CNS, but differences should be expected.

The mechanism of action of uptake of the dopamine neuron is fundamentally different from that in the norepinephrine neuron, because the uptake is not blocked by drugs (e.g., desipramine) which block the reuptake of transmitter into the norepinephrine neuron (Fuxe et al., 1967).

Raiteri et al. (1975) demonstrated that amphetamine releases dopamine at a lower concentration than that needed to release norepinephrine. This could be due to less stringent structural and stereochemical

requirements of dopaminergic uptake sites, allowing easier accessibility of the drug to amine storage sites. Both of these reports indicate differences not only between the peripheral and central neurons, but also between dopaminergic and noradrenergic neurons.

Some similarities between catecholaminergic and serotonergic neurons are worth noting. Kinetic analysis indicates two components of serotonin accumulation, one representing a high and the other a low affinity transport system. The low affinity transport of serotonin might involve uptake by the catecholamine transport system. Serotonin administered into the brain may enter catecholaminergic neurons in significant quantities (Shaskan and Snyder, 1970). Mutual inhibition exists between dopamine and serotonin for uptake into the synaptic vesicles (Matthaei et al., 1976).

C. Two Pools of Amines

The different actions of amphetamine and reserpine on the metabolism of ^{3}H-norepinephrine indicate the presence of more than one storage pool of brain catecholamines. These pools were first described by Glowinski and Axelrod (1965) as a "reserpine resistant" pool and a "reserpine releasable" pool. These correspond to terms in use now as newly synthesized pool being the "reserpine resistant" pool and amine stored in vesicles being the "reserpine releasable" pool. The fact that reserpine will release more ^{3}H-norepinephrine if it is not given immediately after incubation with ^{3}H-norepinephrine suggests that the ^{3}H-amine is transferred from the "reserpine resistant" pool to the "reserpine releasable" pool, or that the turnover rate in the "reserpine resistant" pool is faster (Glowinski and Axelrod, 1965).

In the rat, 20% of the normal striatal dopamine content is in a rapidly turning-over pool separate from the larger stable pool (Javoy and Glowinski, 1971). The source of dopamine mobilized in response to receptor blockade is derived from newly synthesized dopamine, the large dopamine pool being relatively non-functional. Newly synthesized dopamine appears to have a considerably greater functional importance than does stored dopamine under the circumstances of compensatory activation of the nigrostriatal pathway (Shore and Dorris, 1975).

One conflicting *in vitro* study showed the concentration-effect curves for the release by amphetamine of newly formed norepinephrine and exogenous norepinephrine from tissue slices are the same. This may indicate that the newly formed amine and exogenous amine rapidly equilibrate in the same amine pools (Ziance *et al.*, 1972).

Most of this evidence indicates that newly synthesized dopamine and stored dopamine can be thought of functionally as two separate pools. Only a few reports cited in this introduction have studied the newly synthesized pool of dopamine. All of the experiments presented in the Results section will focus on newly synthesized dopamine and serotonin.

III. A. Mechanism of Action of Cocaine

Cocaine enhances the response of effector organs to exogenously administered norepinephrine by inhibiting the uptake and storage of this amine in the neuron (Dengler *et al.*, 1961). Noradrenergic nerve terminals possess sites on their exterior of a specific type with which norepinephrine molecules must first combine before being transported into the terminal. Cocaine molecules, by also combining with these

"transfer sites", competitively block the uptake of norepinephrine (Furchgott et al., 1963). Cocaine acts as a competitive inhibitor of the membrane amine pump, but ouabain (10^{-6}M) by decreasing the Na^+ gradient, acts as a noncompetitive inhibitor, suggesting that the amine pump is linked energetically with glycoside sensitive Na^+-K^+-dependent ATPase (Berti and Shore, 1967). Cocaine causes a decreased accumulation of catecholamines inside the neuron by blocking the uptake across the neuronal membrane rather than by direct amine release as observed after d-amphetamine treatment (Fuxe et al., 1967).

Cocaine will inhibit the uptake of ^{3}H-dopamine into brain slices (Ross and Renyi, 1966; Fuxe et al., 1967). In brain homogenates incubated with ^{3}H-norepinephrine, inhibition of neuronal uptake by equimolar concentrations of amphetamine and cocaine was about the same, while release of ^{3}H-norepinephrine by amphetamine was much greater than that produced by cocaine (Rutledge et al., 1973; Azzaro et al., 1974). Cocaine appears to be only an uptake inhibitor, however. It did not evoke a releasing action of ^{3}H-dopamine from brain slices at concentrations where a powerful uptake inhibition is observed (Heikkila et al., 1975b). Cocaine in a concentration that blocks the uptake of ^{3}H-dopamine, does not cause a marked release of ^{3}H-dopamine from striatal slices, while an effective uptake blocking concentration of amphetamine causes a marked release of ^{3}H-dopamine (Heikkila et al., 1975a).

These in vitro studies indicate that cocaine can block the uptake of catecholamines into neurons in the central nervous system just as it does in the periphery.

B. Mechanism of Action of d-Amphetamine

d-Amphetamine, a sympathomimetic drug, may exert its effect by displacing norepinephrine from tissue stores near sympathetic nerve endings (Dengler et al., 1961). It has been shown using histochemical techniques that d-amphetamine blocks the neuronal accumulation of dopamine and norepinephrine. The accumulation of catecholamines observed after their injection into reserpine-pretreated rats could be released by amphetamine but not by cocaine (Fuxe et al., 1967). Amphetamine will release ^{3}H-norepinephrine from synaptosomes of rat cortex but will not release ^{3}H-inulin or ^{14}C-urea. Higher concentrations of amphetamine (10^{-4}M) are required to obtain an initial release of ^{3}H-dopamine than are required for release of ^{3}H-norepinephrine, but at high amphetamine concentrations a greater proportion of ^{3}H-dopamine is released than ^{3}H-norepinephrine (Ziance et al., 1972).

Inhibition of neuronal uptake is probably not the primary mechanism by which the efflux of ^{3}H-norepinephrine is enhanced by amphetamine. But inhibition of neuronal uptake may play a role in the action of amphetamine by blocking uptake of released amine (Rutledge et al., 1973). ^{3}H-Dopamine is accumulated within specific neurons of brain tissue and selectively released by amphetamine (Azzaro and Rutledge, 1973). Because amphetamine also releases ^{3}H-norepinephrine from chopped rat brain tissue, the increase in efflux of ^{3}H-norepinephrine induced by this drug is not simply due to inhibition of neuronal uptake of spontaneously released ^{3}H-amine (Azzaro et al., 1974). In neostriatal tissue slices there is a releasing action of ^{3}H-dopamine by d-amphetamine, but the apparent blockade of uptake is of questionable significance and appears to result from the release of previously accumulated ^{3}H-dopamine

(Heikkila et al., 1975a). d-Amphetamine is a powerful releasing agent of ^{3}H-dopamine from brain slices (Heikkila et al., 1975b).

Using a technique where release is primarily measured because uptake is prevented by superfusion, d-amphetamine had a releasing effect on ^{3}H-norepinephrine from striatal synaptosomes. This released amine was artificially stored in dopamine nerve endings. Therefore, amphetamine inhibits uptake of ^{3}H-norepinephrine but releases and inhibits uptake of ^{3}H-dopamine. The newly synthesized neurotransmitter pool, which is preferentially affected by amphetamine is probably influenced more effectively by this combination of uptake inhibition and direct release than by either mechanism alone (Raiteri et al., 1975).

When the relative potencies of amphetamine on uptake and release were compared, it was found that approximately 11-fold higher concentrations were required to release ^{3}H-dopamine compared to its ability to inhibit uptake (Holmes and Rutledge, 1976). Amphetamine, an indirect acting dopamine agonist, must first be taken up into the neuron before it can release endogenous dopamine (Pycock et al., 1976).

All of this evidence obtained in vitro indicates that d-amphetamine has two actions in the central nervous system. At low concentrations it can block the uptake of amines into the neuron. At high concentrations it can cause a release of amines from the neuron. Both actions may serve to increase the concentration of amine in the synaptic cleft.

In vivo, there is probably a combination of these processes at work. Amphetamine when administered intraventricularly will increase the efflux of exogenous ^{3}H-norepinephrine or ^{3}H-dopamine from the brain (Carr and Moore, 1969a, 1970b). Acute lesions of the nigrostriatal

pathway blocked the efflux of exogenous ^{3}H-dopamine induced by d-amphetamine (Von Voigtlander and Moore, 1973). This may indicate that amphetamine acts by facilitating neurogenic release or by blocking the uptake of neurogenically released amine.

C. Mechanism of Action of Tyramine

Tyramine may exert its sympathomimetic effects by displacing norepinephrine from noradrenergic nerve endings (Dengler et al., 1961). Tyramine is a strong releasing agent and decreases the accumulation of ^{3}H-dopamine during uptake studies using rat brain tissue slices (Heikkila et al., 1975b). The amine-releasing effect of tyramine in vitro and in vivo may be due in part to interference of the drug with the size and structure of the amine-ATP aggregates inside the storage vesicles (Pletscher et al., 1970). Because it has no effect in brain tissue slices pretreated with reserpine, tyramine exerts its releasing action predominately by displacing dopamine from the synaptic vesicles (Stoof et al., 1976).

Tyramine competitively inhibits the ATP-Mg^{2+} dependent uptake of ^{14}C-dopamine, ^{14}C-serotonin and ^{14}C-norepinephrine into synaptosomes. The uptake of amines which takes place in the absence of ATP-Mg^{2+} is not impaired by tyramine. Tyramine blocks amine transport either without being transported into the synaptosomes or it is taken up but cannot be stored and is immediately released (Matthaei et al., 1976).

Tyramine will increase the efflux of exogenous ^{3}H-dopamine from the brain when administered intraventricularly. Acute lesions of the nigrostriatal pathway had no effect on this increase (Von Voigtlander and Moore, 1973). This indicates that tyramine actively releases dopamine independent of dopamine nerve activity.

Once again, a clear distinction is not made in these reports between release and blockade of uptake. For the purposes of this thesis, the mechanism of action of tyramine will be thought of as release of transmitter. The action of cocaine can now be compared with amphetamine, a drug which both releases and blocks uptake, and tyramine, a drug which only releases neurotransmitters.

IV. A. *In Vitro* Interactions Between Cocaine and Amphetamine

When incubated with brain cortex slices, cocaine had no effect on the uptake of amphetamine (Ross and Renyi, 1966). When amine uptake in brain homogenates is blocked with cocaine, amphetamine is still capable of releasing ^{3}H-norepinephrine, but the concentration effect curve is shifted to the right. This blockade of amphetamine-induced release may be explained by the ability of cocaine to block the sites of uptake of low concentrations of amphetamine into the neuron, but higher concentrations of amphetamine enter the neurons by some other non-specific way and then cause release (Rutledge *et al.*, 1973). The uptake of low concentrations of amphetamine into brain cortex synaptosomes was markedly inhibited by cocaine. The concentration-effect curve for amphetamine is shifted to the right when release of amines by amphetamine is studied in the presence of concentrations of cocaine which markedly inhibit neuronal uptake. These results indicate that amphetamine is transported by the neuronal uptake system and that cocaine inhibits the release of ^{3}H-norepinephrine induced by low concentrations of amphetamine by inhibiting the transport of amphetamine into the neuron (Azzaro *et al.*, 1974). When cocaine alone is added to neostriatal tissue slices incubated with ^{3}H-dopamine there was no change

in spontaneous efflux, but cocaine blocked the release caused by d-amphetamine. Therefore, d-amphetamine itself must be taken up to evoke a releasing action (Heikkila et al., 1975a).

These studies suggest that at least in vitro amphetamine must be taken up into the neuron to cause a release of ^{3}H-norepinephrine or ^{3}H-dopamine and cocaine interferes with this process.

B. Peripheral and In Vitro Interactions Between Cocaine and Tyramine

Based on studies done on the peripheral nervous system, cocaine was shown to shift the concentration-effect curve of tyramine to the right, i.e., cocaine appears to be a competitive inhibitor of tyramine effects. Cocaine will depress the sensitivity of a perfused rabbit ear to the actions of tyramine (Burn and Rand, 1958). Cocaine will block the increase in blood pressure caused by tyramine (Trendelenburg, 1961). It has been proposed that cocaine blocks the uptake of tyramine into the neuron. Similar studies of the interaction between amphetamine and cocaine in the periphery have not been reported, but the blockade of the tyramine-induced increase of blood pressure by cocaine, can be overcome with amphetamine (Eble and Rudzik, 1965). These investigators suggested that this occurred because amphetamine increased the circulating concentration of tyramine. But if cocaine does not block the uptake of amphetamine into the neuron in vivo, amphetamine itself, could be producing the pressor response.

In vitro work suggests that cocaine combines with the transfer site in the neuronal membrane and competitively inhibits the uptake of tyramine, thus antagonizing the release of norepinephrine by tyramine (Furchgott et al., 1963). In tissue slices the uptake of tyramine is

partly inhibited by cocaine (Ross and Renyi, 1966). Cocaine, when added before ^{3}H-tyramine to brain slices, will decrease the intraneuronal metabolism of ^{3}H-tyramine to ^{3}H-products as well as decrease the amount of ^{3}H-tyramine retained. This indicates that cocaine blocks the uptake of ^{3}H-tyramine at the neuronal membrane (Steinberg and Smith, 1970).

These studies show that cocaine interferes with the uptake of tyramine into the neuron and therefore interferes with the action of tyramine.

V. Behavioral Effects of Cocaine

Cocaine, at doses which increased the locomotor activity of mice, did not cause significant changes in brain norepinephrine, dopamine or serotonin levels (Smith, 1965). Nevertheless, biochemical studies have implicated dopamine neurons in cocaine-induced behavioral effects (Groppetti et al., 1973). Early behavioral studies (Van Rossum et al., 1962, 1964) showed that reserpine, which depletes stores of catecholamines and serotonin by disrupting the storage vesicles, will block the increase in motor activity caused by cocaine. This motor activity is restored by L-DOPA, which is metabolized to dopamine and/or norepinephrine. The increases in motor activity induced by cocaine can also be blocked by neuroleptics, a class of drugs which block dopamine receptors (Van Rossum, 1970). The neuroleptics also blocked the reinforcing action of cocaine in monkeys which were taught to bar press for an intravenous dose of cocaine (Wilson and Schuster, 1974).

Cocaine produces amphetamine-like stereotypy whereas benztropine, an in vitro blocker of dopamine uptake does not. Cocaine increased the accumulation in the brain of both normetanephrine and 3-methoxytyramine,

two metabolites correlated with catecholamine releasing properties of the central stimulant drugs. Benztropine does not produce similar increases. Therefore, the stereotypy produced by cocaine cannot be explained only by the inhibition of striatal dopamine uptake (Scheel-Krüger, 1972).

Rats lesioned bilaterally in the substantia nigra (i.e., without functional dopamine terminals in the striatum) failed to show an increase in locomotor activity following cocaine administration. The lack of locomotor activity of the substantia nigra-lesioned rats in response to both cocaine and amphetamine indicates that both drugs produced their effects through the same mechanism or that the striatum was the final common output for the locomotor activity initiated by these two drugs (Creese and Iversen, 1975).

VI. A. Cocaine and Serotonin

Reports on the interactions of cocaine with serotonergic neurons in the brain do not agree. Cocaine will decrease serotonin accumulation after pargyline and decrease 5-hydroxyindole acetic acid accumulation after probenicid, suggesting that cocaine decreases serotonin turnover in the brain. This action of cocaine may be responsible for the differences in a number of pharmacological effects between cocaine and amphetamine, and may also account for the confusing properties of cocaine which make it difficult to classify as either a stimulant or psychomimetic drugs (Friedman *et al.*, 1975). Cocaine inhibits serotonin uptake *in vitro* and reduces the accumulation of newly synthesized serotonin in mouse brain *in vivo* which could indicate a decrease of turnover rates and endogenous serotonin synthesis from ^{3}H-tryptophan (Schubert *et al.*, 1970). Cocaine has no effect on soluble

tryptophan hydroxylase within a wide range of concentrations but impairs the high affinity uptake of tryptophan into the synaptosomal biosynthetic unit, and by this mechanism decreases particulate tryptophan hydroxylase activity. Cocaine has no effect on the low affinity uptake of tryptophan. Forebrain but not midbrain serotonin turnover rates are altered by in vivo drug administration to rats (Knapp and Mandell, 1972). Cocaine, while reducing the uptake of tryptophan and its conversion to serotonin, led to an increase in tryptophan hydroxylase activity in the midbrain and the striatum. The relationship between serotonin synthesis and behavior is not yet firmly established, but serotonin appears to inhibit spontaneous motor activity, startle reflex and self stimulation, while cocaine tends to have opposite effects. Thus, it may be that experimental antagonism between serotonergic function and cocaine has similar kinds of behavioral correlates (Knapp and Mandell, 1976).

B. d-Amphetamine and Serotonin

The reports on the interactions of d-amphetamine with the serotonergic system in the brain do not agree. Amphetamine administration significantly accelerates the turnover rate of brain serotonin, and this effect may be related to the hyperthermia produced by amphetamine (Reid, 1970). Until tolerance to the behavioral actions of d-amphetamine develops, this drug will not release serotonin into perfusing CSF (Sparber and Tilson, 1972). Amphetamine will release ^{3}H-serotonin from brain striatal homogenates at high concentrations. Amphetamine psychosis may be related to the release of serotonin by high concentrations of amphetamine (Azzaro and Rutledge, 1973). Release of brain serotonin can influence certain behavioral actions of amphetamine.

Serotonergic fibers have an inhibitory action on catecholaminergic fibers responsible for amphetamine-induced motor activity. When serotonin is decreased, amphetamine hyperactivity is potentiated (Breese et al., 1974). d-Amphetamine stimulates ^{3}H-serotonin release from synaptosomes (Raiteri et al., 1975). Approximately equal concentrations of d-amphetamine will release and inhibit neuronal uptake of ^{3}H-serotonin (Holmes and Rutledge, 1976).

The preceding two sections indicate that both cocaine and d-amphetamine will interact with the serotonergic system but only at higher concentrations than those needed to show effects on dopaminergic systems. However, the mechanism of interaction for each drug seems to be the same for the serotonergic system as for the dopaminergic system.

Up to this point this literature review has been concerned primarily with in vitro and peripheral studies of cocaine. A CNS neuron cannot be fully studied with a simple in vitro preparation. A neuron in a brain slice is in a resting state, disconnected from nervous influences which normally regulate its functions and metabolism. Under such circumstances the neuronal response to drugs is different from that in an in vivo preparation. The in vitro studies are useful, however, for indicating what actions of a drug may contribute to its pharmacological effects. Until the in vitro biochemical actions of a drug can be shown to be the same in vivo after establishing a reasonable pharmacological concentration, their significance is questionable. For all of these reasons an in vivo model was chosen for the studies described in this thesis.

VII. Detection of Neurotransmitter Release *In Vivo*

The cerebroventricular perfusion technique was developed to provide a quantitative approach for the study of the blood-brain-barrier for pharmacologically active substances (Bhattacharya and Feldberg, 1958b). Since then it has been developed to the point where it is used to study the pharmacology of drugs on structures lining the ventricle.

In the first experiments perfusates were collected from the cisterna. Drugs injected intravenously caused great variations in the concentrations of chemicals measured in outflow from the cisterna, but these changes did not occur when the collection was from the aqueduct. This difference occurred because perfusion collection from the cisterna included relatively large areas of the subarachnoidal spaces since in cats the foramen of Luschka forms the only outlet from the fourth ventricle (Bhattacharya and Feldberg, 1958a). For example, when acetylcholine was added to the perfusion fluid entering the lateral ventricle its recovery from the cisterna was incomplete and irregular. This could be explained by the devious route the perfusion fluid had to take in order to reach the cisterna and the unavoidable mixture with the CSF of the subarochnoidal space. When collection was from the aqueduct 90% or more of the acetylcholine added to the perfusion fluid was recovered. The acetylcholine originated mainly from structures lining the lateral and third ventricle because the amounts in the effluent from the aqueduct were only a little less than those in the cisternal effluent (Bhattacharya and Feldberg, 1958b).

Different parts of the cerebral ventricles may be perfused with a drug by inserting several cannulae into different parts of the

ventricular system. One can be used to deliver the artificial CSF and another to collect the perfusate. Variations in the choice of cannulae for perfusing the artificial CSF as well as for collecting the outflow, make it possible to perfuse separately or in combination the anterior or the posterior part of the lateral ventricle, and ventral or dorsal half of the third ventricle (Carmichael et al., 1964).

VIII. Recent Cerebroventricular Perfusion Studies

Intraventricularly administered ^{3}H-norepinephrine accumulated primarily in those regions lining the ventricular system which contain high endogenous levels of catecholamines including the hypothalamus and caudate nucleus (Carr and Moore, 1969b). After it was established that ^{3}H-norepinephrine is specifically taken up and spontaneously released, a pulse of amphetamine added to the perfusing CSF was shown to increase the efflux of ^{3}H-norepinephrine. When the ventricles were injected with ^{14}C-inulin rather than ^{3}H-norepinephrine prior to amphetamine treatment, no increase in the efflux of ^{14}C-inulin was observed indicating a specific amphetamine action on catecholamine neurons (Carr and Moore, 1969a, 1970b). These experiments with amphetamine were repeated but this time catecholamine stores were loaded with ^{3}H-dopamine. It was found that small concentrations of amphetamine would increase the efflux of ^{3}H-dopamine into the ventricles. A small amount of ^{3}H-norepinephrine endogenously synthesized from the ^{3}H-dopamine was found in the samples, also indicating a specific amphetamine action on catecholamine neurons. When d-amphetamine was given intravenously, the increase in the efflux of ^{3}H-norepinephrine still occurred. Therefore, this technique was shown to be useful for studying the effects of systemically administered drugs on brain catecholamine release (Carr and Moore, 1970b).

Intraventricular perfusions of cocaine, after ^{3}H-norepinephrine was injected intraventricularly, did not significantly alter the efflux of ^{3}H-norepinephrine. It was concluded that cocaine does not act by increasing the synaptic concentration of norepinephrine in structures lining the ventricular system (Carr and Moore, 1970a). By contradicting previous studies, this report added to the confusion surrounding the biochemical actions of cocaine in the brain. Direct stimulation of the caudate nucleus after dopamine stores were labelled with ^{3}H-dopamine increased the efflux of ^{3}H-dopamine. When the substantia nigra was stimulated under the same conditions, the efflux of ^{3}H-dopamine increased, indicating release from dopamine terminals (Von Voigtlander and Moore, 1971b). Because stimulation of the nigrostriatal pathway is capable of releasing ^{3}H-dopamine previously stored in the caudate nucleus, dopamine was shown to be the probable neurotransmitter of this pathway. Therefore, drugs that block the uptake of dopamine might be expected to act in a supra-additive manner with stimulation-evoked release, while drugs which act by directly releasing dopamine would be additive or infra-additive with stimulation (Von Voigtlander and Moore, 1971a).

When added to the perfusing CSF, d-amphetamine and tyramine caused a concentration-related efflux of ^{3}H-dopamine into the ventricular effluent from exogenously labelled stores in the caudate nucleus. When these experiments were repeated using cats with chronic lesions of the nigrostriatal dopaminergic fibers, the drug-evoked efflux was greatly reduced. Two conclusions were drawn from these results. First, ^{3}H-dopamine injected into the lateral ventricle is taken up primarily

by dopaminergic neurons in the caudate nucleus. Secondly, efflux of ^{3}H-catecholamines after a drug treatment originates primarily from dopaminergic nerve terminals. A second type of experiment showed that an acute lesion of the nigrostriatal fibers reduced the increased ^{3}H-dopamine efflux induced by amphetamine but had no affect on tyramine-induced increases in efflux. This last observation indicates that tyramine actively releases dopamine independent of dopaminergic nerve activity whereas amphetamine increases the efflux of dopamine by facilitating neurogenic release or by blocking the uptake of neurogenically released amine (Von Voigtlander and Moore, 1973).

To ensure that observed release of the catecholamines came only from catecholamine nerve terminals, ^{3}H-tyrosine was perfused into the ventricles rather than loading the terminals with exogenous catecholamines. Any ^{3}H-catecholamines measured now would be endogenously synthesized in the catecholaminergic neurons because only these neurons contain tyrosine hydroxylase, the rate limiting enzyme for the synthesis of catecholamines. Electrical stimulation of the nigrostriatal fibers increased the efflux of the endogenously synthesized ^{3}H-dopamine as did a pulse of amphetamine. Because amphetamine released much more ^{3}H-dopamine than ^{3}H-norepinephrine, it would appear that amphetamine acts at the terminals of the dopaminergic nigrostriatal neurons in the caudate nucleus (Chiueh and Moore, 1974a).

Experiments done with amine stores labelled with ^{3}H-dopamine show that α-methyltyrosine will not affect the increase in efflux caused by d-amphetamine. But when ^{3}H-tyrosine is perfused, α-methyltyrosine will block the amphetamine response. When ^{3}H-tyrosine is given before α-methyltyrosine, the amphetamine response is restored. Therefore,

α-methyltyrosine, blocks synthesis of dopamine, not the ability of amphetamine to release amines (Chiueh and Moore, 1974b). Pretreatment with reserpine, while using ^{3}H-tyrosine as a precursor, established that amphetamine initially increases the efflux of ^{3}H-dopamine from a storage pool. Pretreatment with α-methyltyrosine showed that the newly synthesized dopamine pool was required for continued release (Chiueh and Moore, 1975a,b).

When the uptake blocker, benztropine, was added to the perfusing CSF after the nerve terminals had been loaded with ^{3}H-dopamine, an increase in the efflux of this dopamine was observed. After an acute electrolytic nigrostriatal lesion, the benztropine-induced increase in efflux of ^{3}H-dopamine was no longer observed. Therefore, this action of benztropine seems to require ongoing activity of the dopaminergic nigrostriatal pathway. Also, an intravenous injection of benztropine did not increase the efflux of ^{3}H-dopamine (Goodale and Moore, 1975).

Dopaminergic agonists such as d-amphetamine did not alter the efflux of endogenously synthesized ^{3}H-serotonin. A concentration of 10^{-4}M amphetamine caused only a very slight increase in the efflux of the amine (Chiueh and Moore, 1976).

The purpose of this study was to investigate the ability of cocaine to influence the efflux of dopamine and/or serotonin from the brain. Thus, the experiments presented in this thesis are in many ways a continuation of the experiments described above. But there are some important differences. All of the present experiments were done using the amine precursors, ^{3}H-tyrosine or ^{3}H-tryptophan. Therefore, direct comparisons with these previous experiments where amine stores were previously labelled with ^{3}H-dopamine may not agree with results obtained

from continuous perfusion with ^{3}H-tyrosine. In the former case, efflux from a storage pool is being measured and in the latter case efflux from a newly synthesized pool is being measured. The above discussion cited work supporting the idea that these amine pools are differently affected by drugs (Chiueh and Moore, 1975a,b).

METHODS

I. Cerebroventricular Perfusion Technique

The cerebroventricular perfusion technique is an *in vivo* method which allows one to monitor the efflux of dopamine and serotonin from brain structures lining the lateral cerebral ventricles (Richards *et al.*, 1973; Aghajanian and Gallager, 1975), with a minimum of damage to these structures (Figure 2B). The procedure used in the following experiments evolved from the technique described in the past (Carr and Moore, 1969a,b, 1970a,b; Von Voigtlander and Moore, 1971a,b, 1973; Chiueh and Moore, 1974a,b; 1975a,b, 1976).

Mongrel cats of either sex (2.5-3.5 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and a tracheotomy was performed. The femoral artery and vein were cannulated to allow the blood pressure to be continuously monitored with a pressure transducer and for intravenous infusion of drugs. Body temperature was monitored with a rectal probe and maintained at 38°C with a heating pad.

The head was placed in a stereotaxic apparatus and the animal was then prepared for ventricular perfusion by placing an inflow cannula in the anterior horn of the left lateral ventricle (A 16.5, L 3.5, H 7.5; Snider and Niemer, 1961) and an outflow catheter was placed at the cerebral aqueduct (Figure 2A).

Figure 2A. Diagram of cat brain depicting the method used for perfusing the cerebral ventricles with artifical CSF and drugs.

Figure 2B. Diagram of cat brain depicting the brain structures affected by the cerebroventricular perfusion technique.

Figure 2A

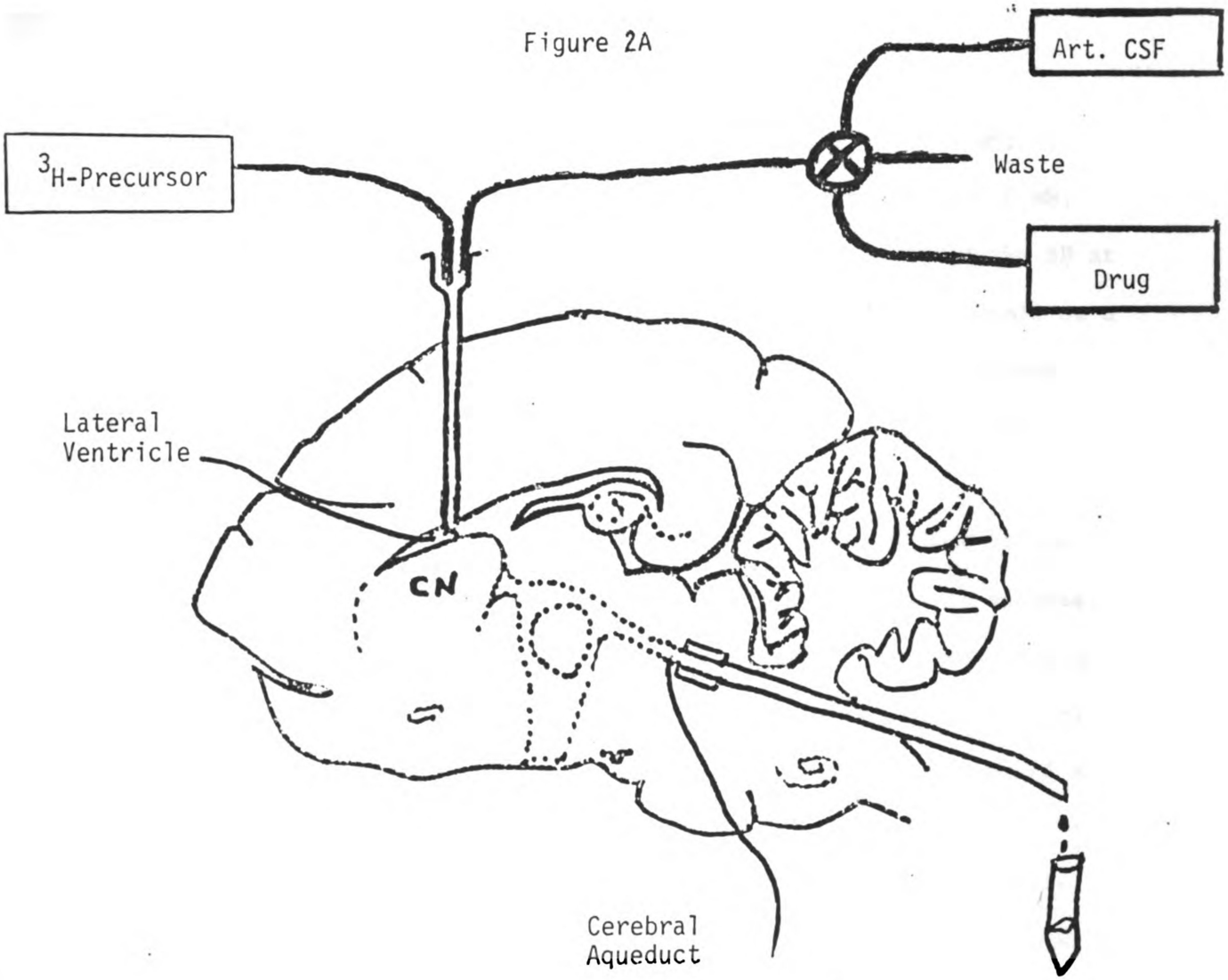
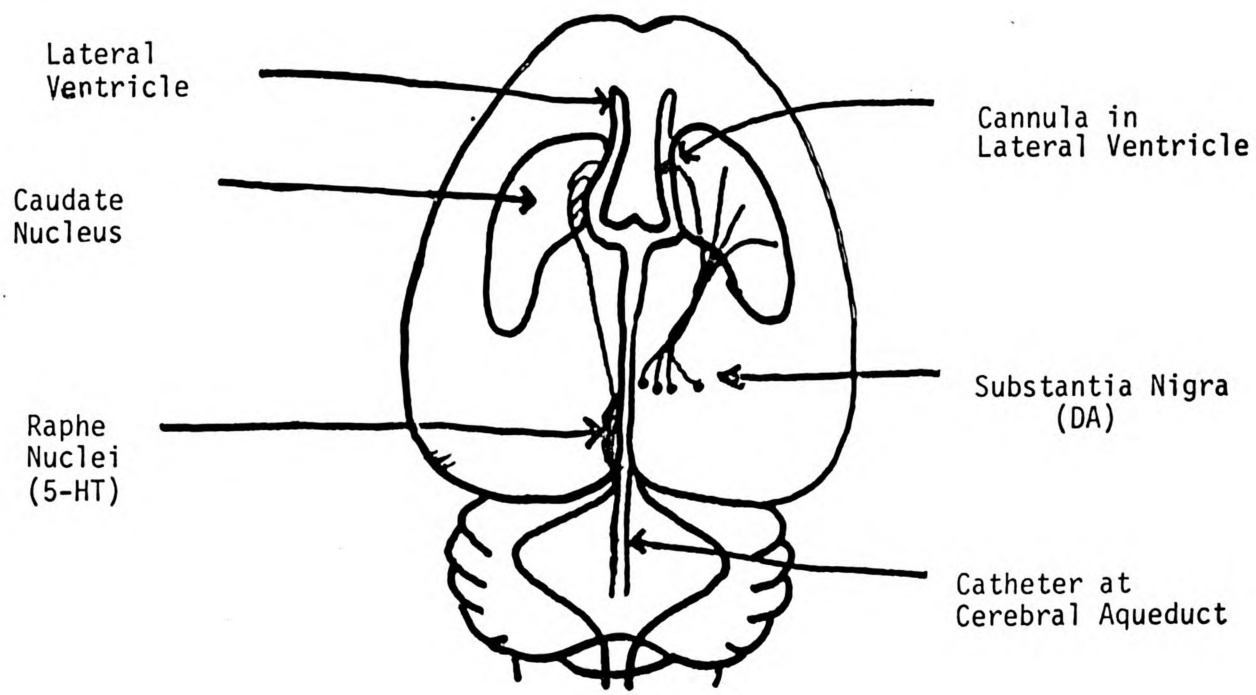


Figure 2B



Artifical cerebrospinal fluid (CSF) containing NaCl, 129 mM; $NaHCO_3$, 24.4 mM; KCl, 2.9 mM: $CaCl_2 \cdot 2H_2O$, 1.3 mM; $MgCl_2 \cdot 6H_2O$, 0.8 mM; Na_2HPO_4, 0.5 mM and saturated with 95% O_2 and 5% CO_2 to buffer the pH at 7.4 (Pappenheimer et al., 1962) was infused into the inflow cannula at a constant rate of 0.15 ml/min with a Harvard infusion pump. A second infusion pump and a stopcock allowed rapid change from CSF to drug (Figure 2A).

The ventricular perfusate was collected from the catheter in the aqueduct in 15 ml centrifuge tubes which were changed every 10 minutes. When the amine precursor used was ^{3}H-tyrosine, these tubes contained a stabilizing solution consisting of 0.1 ml of 1.0 N acetic acid, 0.1 ml of 0.15% disodium ethylenediamine tetra-acetate (Na_2EDTA), 0.1 ml of a solution containing 100 µg/ml of dopamine and 0.05 ml of ethanol. For experiments using ^{3}H-tryptophan as the amine precursor, the perfusate collection tubes were changed every 5 minutes. These tubes contained 0.01 ml of a stabilizing solution containing 100 µg/ml of serotonin, 0.01 ml of a solution containing 100 µg/ml of 5-hydroxyindole acetic acid, 0.08 ml of 4.2 N perchloric acid and 0.2 mg of cysteine.

At the termination of the experiment, the cat was sacrificed by a rapid intravenous injection of air.

II. Administration of Radioactive Precursors

Since all of these experiments were concerned with the effects of drugs on the efflux of newly synthesized dopamine or serotonin, amine stores in tissues lining the cerebroventricular system were labelled by continuous infusion (0.05 ml/min) of the ventricles with purified ^{3}H-tyrosine (66.6 µC/ml) or ^{3}H-tryptophan (2 µC/ml) with a separate

Harvard infusion pump (Figure 2A). The flow rate from the CSF/drug pumps (0.15 ml/min) plus the flow rate from the ^{3}H-precursor pump (0.05 ml/min) resulted in a perfusate delivery rate from the aqueduct of 0.2 ml/min.

III. Purification of Radioactive Tyrosine

In experiments in which radioactive tyrosine was continuously infused into the cerebroventricular system, ^{3}H-tyrosine of higher specific activity (greater than 50 Ci/mmole) was purified before the start of the experiment because ^{3}H-tyrosine is converted spontaneously to ^{3}H-DOPA during storage (Evans, 1966; Waldeck, 1970).

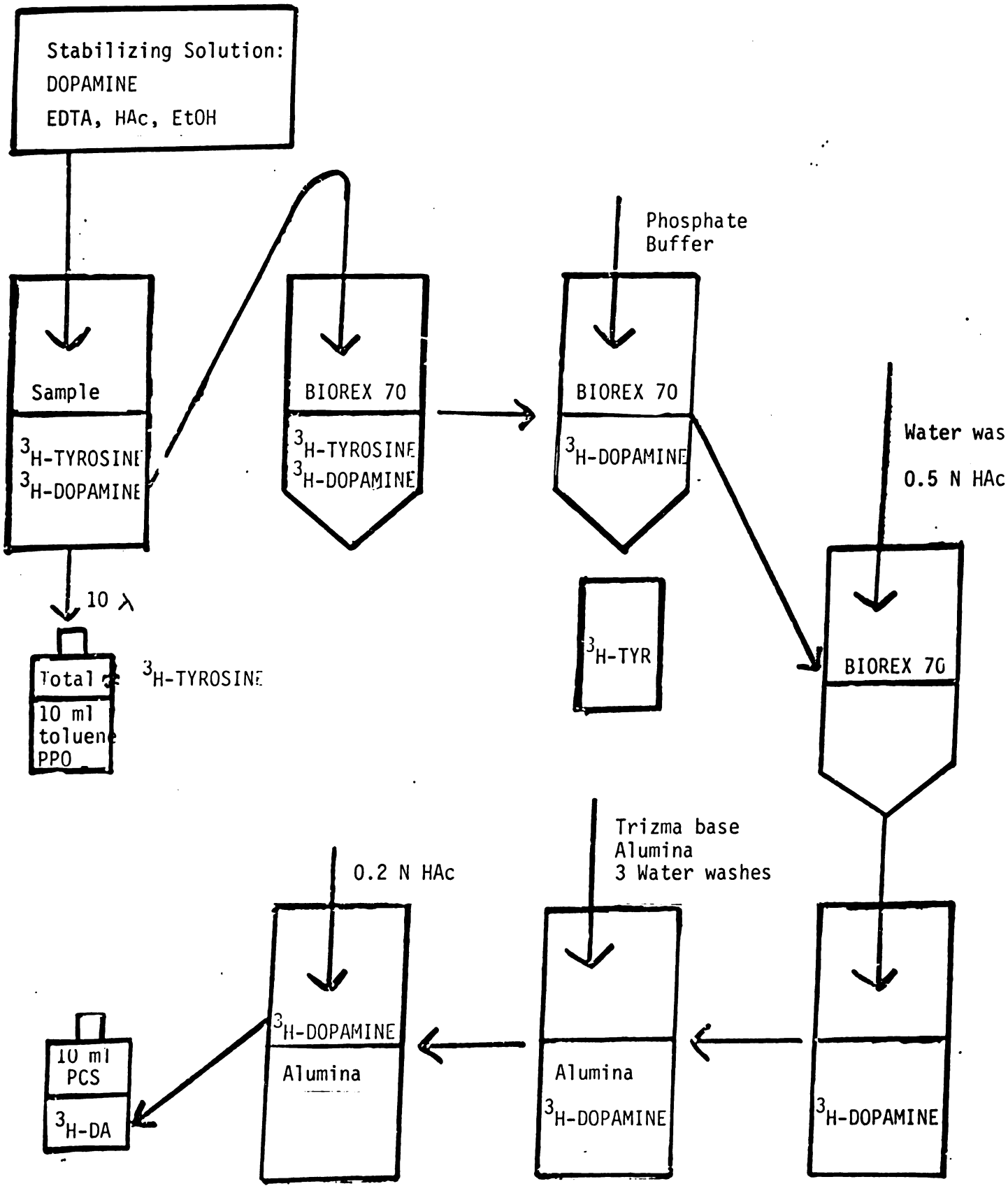
One ml (1 millicurie) of ^{3}H-tyrosine was placed on a column containing BIOREX 70 resin (Na^{+}, 200-400 mesh, 26 mm^{2} x 25 mm, BioRad Labs., Richmond, Calif.) to remove amine impurities (for example, tyramine). The effluent and subsequent 9 ml wash with artificial CSF flowed directly into a second column containing washed aluminum oxide (pH 8.4, 26 mm^{2} x 20 mm, Woelm, Eschwege, Germany) to remove the catechol impurities (mostly DOPA). The effluent from the alumina column was collected directly into a vial as was an additional 5 mls of CSF added only to the alumina column. This 15 mls of ^{3}H-tyrosine (66.6 µCi/ml) was then used for the experiment.

IV. Separation and Analysis of ^{3}H-Dopamine

The perfusate and brain samples were analyzed for dopamine by alumina adsorption and ion-exchange chromatography using modifications of procedures described previously (Bhatnagar and Moore, 1972; Carr and Moore, 1969b; Barchas et al., 1972; Chiueh and Moore, 1974a; Chiueh 1974; Figure 3).

Figure 3. Schematic diagram of the procedure used for the separation and analysis of ^{3}H-dopamine.

Figure 3


Stabilizing Solution:
DOPAMINE
EDTA, HAc, EtOH
Sample
^{3}H-TYROSINE
^{3}H-DOPAMINE
BIOREX 70
^{3}H-TYROSINE
^{3}H-DOPAMINE
Phosphate Buffer
BIOREX 70
^{3}H-DOPAMINE
Water was
0.5 N HAc
10 λ
Total
10 ml toluene PPO
^{3}H-TYROSINE
^{3}H-TYR
BIOREX 70
Trizma base
Alumina
3 Water washes
0.2 N HAc
10 ml PCS
^{3}H-DA
^{3}H-DOPAMINE
Alumina
Alumina
^{3}H-DOPAMINE
^{3}H-DOPAMINE

In experiments with the continuous infusion of ^{3}H-tyrosine, the total radioactivity of perfusate effluent was determined by adding 10 µl of the perfusate to 10 ml of a toluene/PPO scintillation solution.

^{3}H-Dopamine was separated from ^{3}H-tyrosine by a combination of weak cation-exchange and alumina adsorption chromatography. The cerebroventricular perfusate were adjusted to pH 6.5 with 0.25 ml of 1.0 M phosphate buffer (pH 8.0) and placed on the columns containing BIOREX 70 resin (Na^{+}, 200-400 mesh, 26 mm^{2} x 30 mm), which were prepared according to the method of Barchas et al. (1972). The effluent and subsequent 15 ml wash with 0.02 M phosphate buffer (pH 6.5, 0.1% Na_2EDTA) containing ^{3}H-tyrosine and deaminated metabolites were discarded. After washing the column with 3 ml of redistilled water and 0.5 ml of 0.5 N acetic acid, ^{3}H-dopamine was eluted with 3 ml of 0.5 N acetic acid.

The eluates were then adjusted to pH 8.4 with 1.5 ml of 1.0 M Trizma base and shaken with 200 mg of washed alumina for 10 min. The effluent was discarded and the alumina was washed 3 times with 5 ml of redistilled water. ^{3}H-Dopamine was eluted from the alumina with 1 ml of 0.2 N acetic acid and the radioactivity was determined by adding the total eluate to a counting vial containing 10 ml of PCS phosphor solution.

Recovery for ^{3}H-dopamine using this procedure is 70.6±5.1% (Chiueh, 1974). ^{3}H-Tyrosine contamination in the alumina eluate was 0.00011± 0.00002% of the total radioactivity (Chiueh, 1974).

V. Separation and Analysis of ^{3}H-Serotonin

The perfusates were analyzed for serotonin by separation with both an ion exchange resin and a resin which separates by both charge and molecular weight, using modifications of procedures described previously (Barchas et al., 1972; Chiueh and Moore, 1976; Figure 4).

In experiments with the continuous infusion of ^{3}H-tryptophan, the total radioactivity of perfusate effluent was determined after the sample and 7 ml of 0.02N acetic acid (+ 2.0 mg/10 ml cysteine) had been added to the Sephadex G-10 column (26 mm^2 x 50 mm) and collected. One hundred µl of this effluent was added to 10 ml of a toluene/PPO scintillation solution. Serotonin was eluted from these columns with another 7 ml of 0.02 N acetic acid (+2.0 mg/10 ml cysteine).

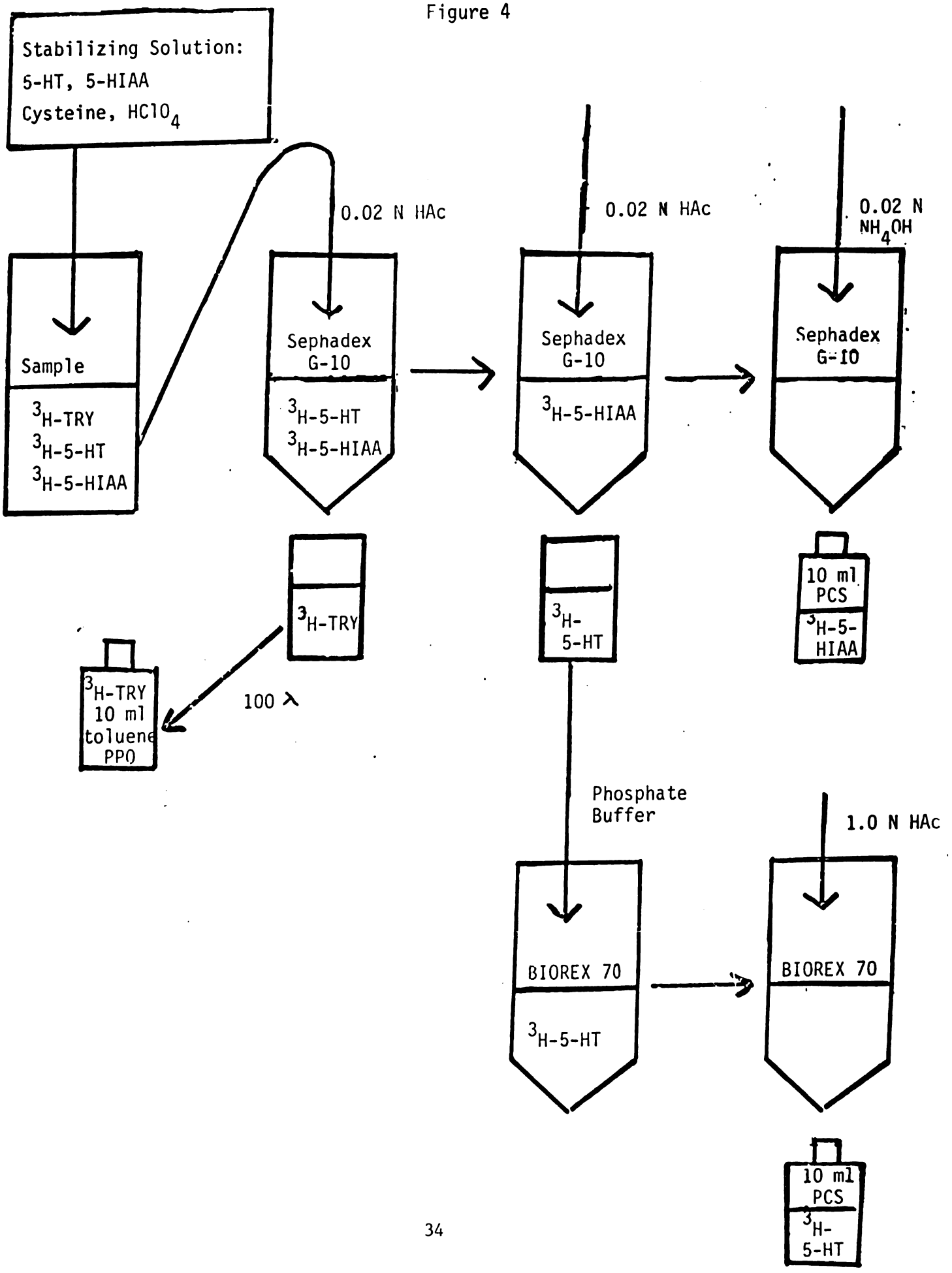
After 1.0 ml of 0.1 M EDTA (pH 8) was added to the serotonin containing eluates, they were placed on columns containing BIOREX 70 resin (Na^+, 200-400 mesh, 26 mm^2 x 30 mm). The effluent was discarded and the columns were further washed with 10 ml of 0.02 M phosphate buffer, 3.0 ml of redistilled water and 1.0 ml of 0.5 N acetic acid. Serotonin was eluted with 2.0 ml of 1.0 N acetic acid; this sample was collected directly into counting vials containing 10 ml of PCS.

VI. Lesion At A 10

In the experiments where a lesion was to be produced, an extra section of bone was removed from the skull during the initial surgical procedures for ventricular perfusion. The lesion was made at A 10 in the left side of the brain just caudal to where the inflow cannula was placed. After the first half of the experiment was completed, the dura mater was cut and an 8 mm spatula, with its medial edge located

Figure 4. Schematic diagram of the procedure used for the separation and analysis of ^{3}H-serotonin.

Figure 4


Stabilizing Solution:
5-HT, 5-HIAA
Cysteine, $HClO_4$
Sample
^{3}H-TRY
^{3}H-5-HT
^{3}H-5-HIAA
0.02 N HAc
Sephadex G-10
^{3}H-5-HT
^{3}H-5-HIAA
0.02 N HAc
Sephadex G-10
^{3}H-5-HIAA
0.02 N NH_4OH
Sephadex G-10
^{3}H-TRY
100 λ
^{3}H-TRY 10 ml toluene PPO
^{3}H-5-HT
10 ml PCS
^{3}H-5-HIAA
Phosphate Buffer
1.0 N HAc
BIOREX 70
^{3}H-5-HT
BIOREX 70
10 ml PCS
^{3}H-5-HT

1.5 mm from the central sinus, was lowered into the brain at A 10, to approximately H -5.0 (Snider and Niemer, 1961; Figure 5).

Although no sinus or major blood vessel was cut during this procedure the lateral ventricle was, and the catheter at the opening of the cerebral aqueduct occasionally filled with blood immediately after the lesion, and had to be removed, cleaned and replaced before starting collections for the second part of the experiment. In most cases, it took no longer than 15 min from time of lesion to the start of collection for the second half of the experiment. The same pattern of intraventricular drug administration was followed before and after the lesion.

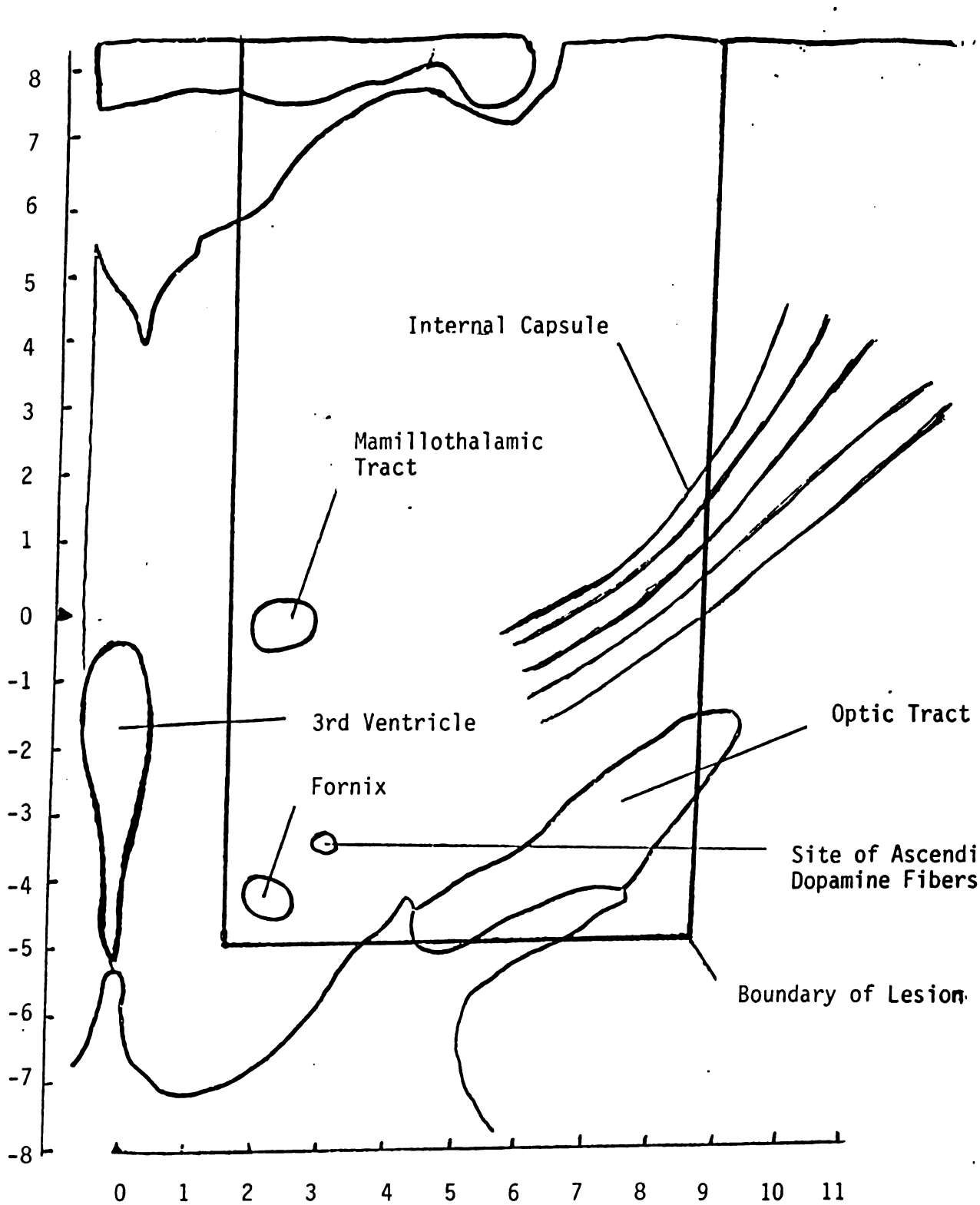
VII. Analysis of Dopamine in the Caudate Nuclei

At the end of the lesion experiments, the brain was quickly removed and a visual inspection was performed at the site of the knife cut to determine the approximate location of the lesion. The caudate nuclei of both the lesioned and unlesioned sides were dissected out, weighed, homogenized in 3.0 ml of 0.4 N perchloric acid, centrifuged and the supernatant were frozen until they could be assayed for dopamine using a modified method of Chang (1964).

After the samples were thawed, 0.5 ml of 0.2 N Na_2EDTA, 10 drops of alumina and 2.5 ml of 1.0 M Trizma base were added and the tubes were shaken for 5 min, centrifuged for 5 min and the effluent aspirated off and discarded. After 2 washes with 5 ml of redistilled H_2O the dopamine was eluted off the alumina with 1.5 ml of 0.2 N acetic acid by shaking for 10 min and centrifuging for 5 min. Two-tenths of a milliliter of this acetic acid effluent was used for the analysis of dopamine.

Figure 5. Diagram of cat brain at Level A 10 (Snider and Niemer, 1961) indicating the area affected by the knife cut.

Figure 5



(Level A 10; Snider and Niemer, 1961)

To the 0.2 ml sample, 0.8 ml of 0.2 N acetic acid and 0.2 ml of 0.1 M Na_2EDTA was added. Then 0.2 ml of 0.1 N iodine was added and mixed well. Exactly 2 min later, 0.2 ml of alkaline sulfite was added and mixed well. Two min later 0.2 ml of 5.0 N acetic acid was added and the samples were boiled for 5 min. Once the samples cooled in ice, the fluorescence was read at activation wavelength = 320 nm, emission wavelength = 380 nm. These values were corrected by subtracting the value of a tissue blank determined for each experiment.

VIII. Method of Presenting Data nand Statistics

The first 4-6 collection periods of each experiment consisted of ventricular perfused CSF with just ^{3}H-precursor added, to establish a baseline efflux of endogenously synthesized amines. An average baseline efflux was calculated for each experiment by determining the average values from collection periods 2, 3 and 4. The values from each subsequent collection period were then divided by this average baseline efflux to give a percent of baseline efflux. All experiments of a given type were then combined so that statistical significance could be evaluated.

An average value for the baseline efflux (set at 100%) in DPM's is given for each figure. This was determined by combining all values used in the calculation of the average baseline for each experiment and determining the mean and S.E.

Although a drug was added to the perfusing CSF for only one collection period, its effect was generally seen during the next several collection periods. For this reason each individual collection period was not analyzed by itself but rather groupings (consisting of samples collected during the periods of drug action, combined and expressed

as a mean and S.E. of the individual values) were made which corresponded to collection periods before addition of the drug, during addition of the drug and after the addition of the drug. These groupings varied with the type of experiment and magnitude of drug effect, and are described on the abscissa of each figure. In most experiments, the drug action lasted for 2 periods. In all cases groupings consisting of at least 4 values were combined for statistical analysis.

A Student t-test ($p<.05$) was used to test for significant differences between treatment and baseline values.

RESULTS

I. Effects of Drugs on the Efflux from the Brain of Endogenously Synthesized ^{3}H-Dopamine.

In all experiments, the brain perfusates were analyzed for ^{3}H-tyrosine and ^{3}H-dopamine. The concentrations of ^{3}H-tyrosine in perfusate samples did not change in response to any of the drugs or surgical manipulations; accordingly, these values are not presented graphically.

The concentration of ^{3}H-dopamine in each 10 min perfusate sample is reported as a percentage of the concentration of ^{3}H-dopamine in samples collected during the control periods at the beginning of each experiment. For the purpose of graphical representation and for the simplification of statistical analyses, the efflux of ^{3}H-amines in two or more consecutive collection periods were combined. For example, in Figure 6, a pulse of cocaine was added to the perfusing CSF for a single 10 min period, but the drug-induced increase in ^{3}H-dopamine efflux persisted into the next period; the efflux of ^{3}H-dopamine in these two periods was combined.

A. Intraventricular and Intravenous Administration of Cocaine

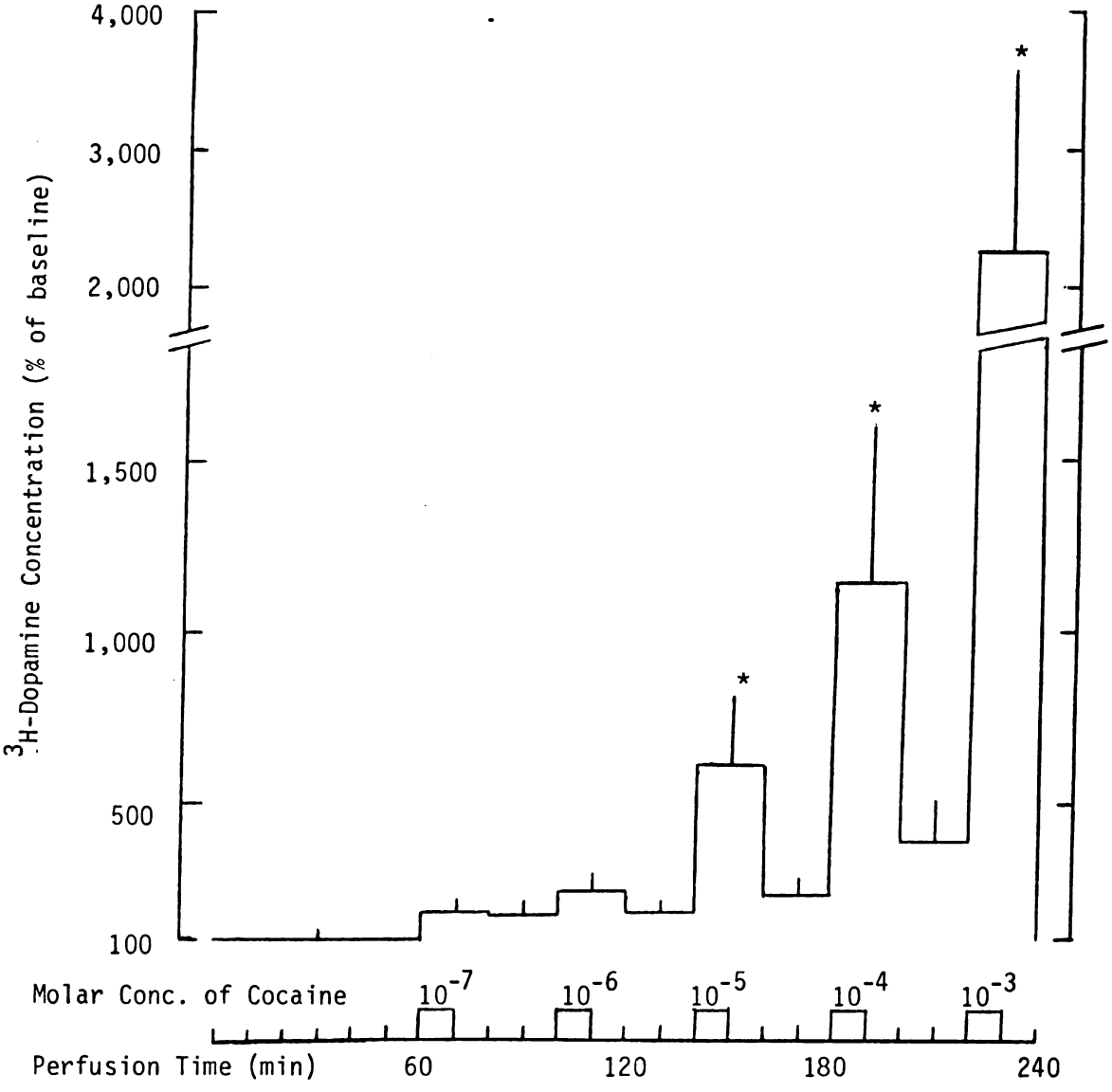
The experiments in this section were designed to determine if intraventricular or intravenous administration of cocaine would affect the efflux of endogenously synthesized ^{3}H-dopamine.

The addition of 10 min pulses of increasing concentrations of cocaine (10^{-7} - 10^{-3}M) at 40 min intervals to the perfusing CSF caused

Figure 6. Efflux of endogenously synthesized ^{3}H-dopamine from the brain in response to intraventricular infusions of increasing concentrations of cocaine.

CSF containing ^{3}H-tyrosine was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 4 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-dopamine in the perfusate samples collected during the indicated times. Values were obtained from 4 separate experiments and 100% represents a baseline efflux of 1,015±100 DPM. Various molar concentrations of cocaine (10^{-7} - 10^{-3}) were added to the perfusing solution during 10 min collection periods as indicated on the abscissa by the open squares. Asterisks indicate cocaine-induced increases of ^{3}H-dopamine concentrations that are significantly greater than the preceding control concentration ($p<0.05$).

Figure 6


4,000
3,000
2,000
1,500
1,000
500
100
^{3}H-Dopamine Concentration (% of baseline)
*
*
*
Molar Conc. of Cocaine
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Perfusion Time (min)
60
120
180
240

concentration-related increases in the efflux of ^{3}H-dopamine (Fig. 6). The minimally effective concentration was 10^{-5}M.

The systemic administration of cocaine also increased the efflux of endogenously synthesized ^{3}H-dopamine from the brain. Following the intravenous injection of cocaine HCl (5 mg/kg) there was a transient (5 min) increase in the blood pressure and a slight but prolonged increase in the efflux of ^{3}H-dopamine. The mean efflux of ^{3}H-dopamine (146±13%) during the 60 min period following the intravenous injection of cocaine was significantly greater than baseline efflux (100±10%; $p<0.05$).

B. Drug-induced Efflux of ^{3}H-Dopamine After Acute Lesions of the Nigrostriatal Pathway

Previous studies have revealed that most ^{3}H-dopamine appearing in the brain perfusate originates from terminals of nigrostriatal neurons in the caudate nucleus (Von Voigtlander and Moore, 1973). Acute lesions of these neurons reduce the ability of amphetamine to increase the efflux of ^{3}H-dopamine suggesting that this drug facilitates the neurogenic release of dopamine or blocks the reuptake of the neurogenically released amine. On the other hand, the increased efflux of dopamine in response to tyramine is not altered by acute lesions of the nigrostriatal neurons suggesting that this drug acts independently of nerve activity (Von Voigtlander and Moore, 1973).

The experiments in this section were designed to determine if the increased efflux of dopamine in response to cocaine is influenced by nerve impulse traffic in the nigrostriatal pathway. Accordingly, the ability of cocaine to increase the efflux of ^{3}H-dopamine was determined before and after acute sectioning of the nigrostriatal neurons.

1. Endogenous Dopamine in the Caudate Nuclei

Inhibition of impulse traffic in nigrostriatal neurons by drugs or axotomy increases the dopamine concentration in the terminals of these neurons (Andén et al., 1971). This increase in the concentration of dopamine in the caudate nucleus has been used as an index to confirm a successful lesion of the nigrostriatal pathway (Von Voigtlander and Moore, 1973; Goodale and Moore, 1975).

In the present experiments the concentration of dopamine in the caudate nucleus on the side of the knife cut at A 10 (14.8±0.9 µg/g) was significantly higher ($p<0.01$) than the dopamine concentration in the caudate nucleus on the contralateral nonlesioned side (9.8±0.3 µg/g, N=8). The caudate nuclei were removed from the brain 2 hours after the knife cut and the values for the endogenous dopamine concentration agree closely with those values obtained after electrolytic lesions of the nigrostriatal pathway (Von Voigtlander and Moore, 1973; Goodale and Moore, 1975).

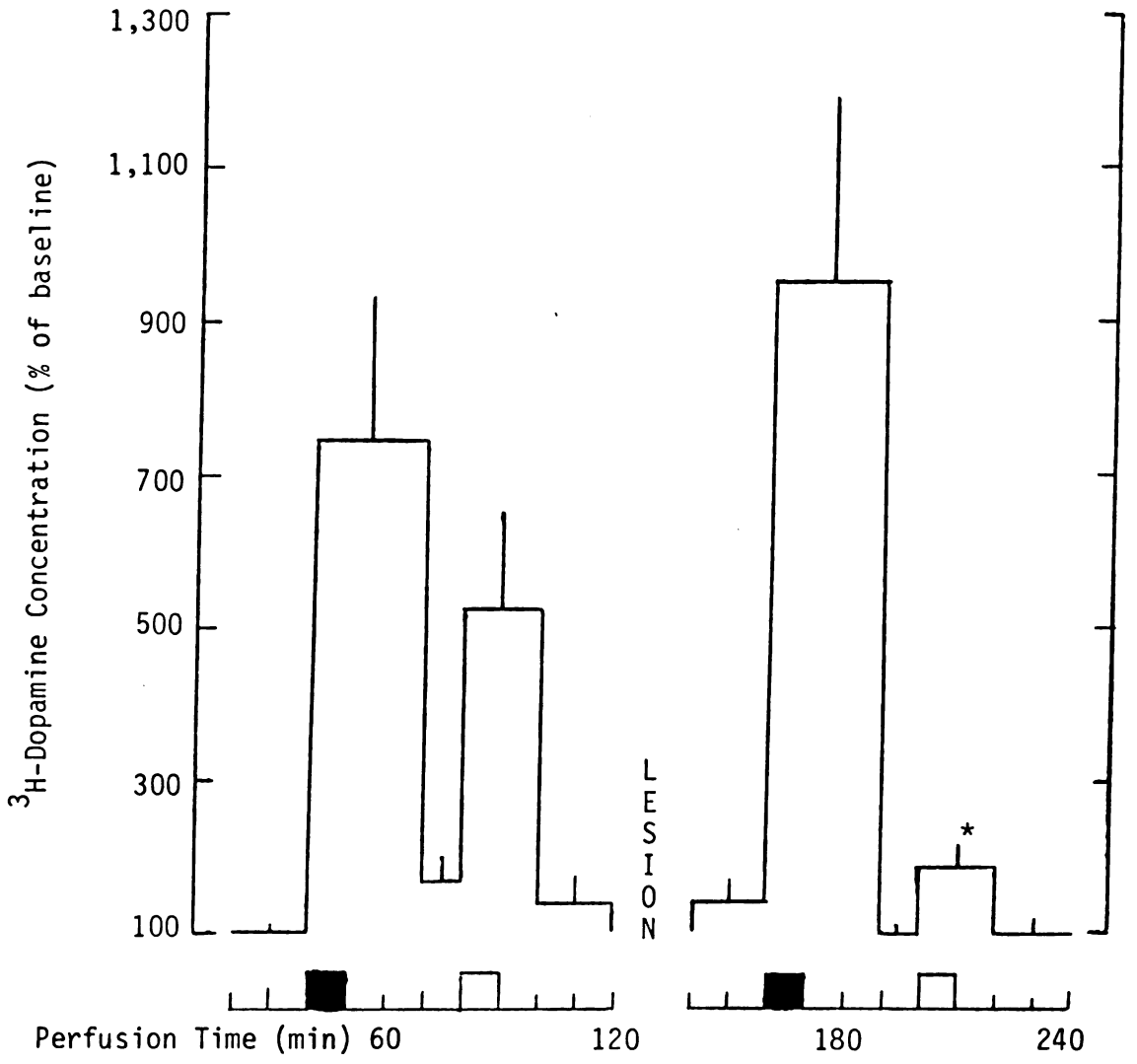
2. Effects of Amphetamine and Cocaine

The efflux of ^{3}H-dopamine in response to intraventricular pulses of d-amphetamine and cocaine, before and after lesioning nigrostriatal neurons is summarized in Fig. 7. A 10 min pulse of d-amphetamine (10^{-5}) followed 30 min later by a 10 min pulse of cocaine (10^{-5}M) were perfused into the left lateral cerebral ventricle. Thirty minutes after stopping the cocaine perfusion, the nigrostriatal neurons were cut by inserting a spatula blade through the left side of the brain at A 10. After removing blood from the aqueduct cannula, the pulses of amphetamine and cocaine were repeated. The increase in the efflux of ^{3}H-dopamine induced by the intraventricular administration of cocaine,

Figure 7. Efflux of endogenously synthesized ^{3}H-dopamine from the brain in response to an intraventricular infusion of amphetamine or cocaine, before and after an acute lesion of the nigrostriatal pathway.

CSF containing ^{3}H-tyrosine was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 4 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-dopamine in the perfusate samples collected during the indicated times. Values were obtained from 4 separate experiments and 100% represents a baseline efflux of 1177±255 DPM. Amphetamine (10^{-5}M) or cocaine (10^{-5}M) was added to the perfusing solution for 10 min collection periods as noted on the abscissa by the solid and open squares, respectively. All drug-induced increases of ^{3}H-dopamine concentrations were significantly greater than the preceding control concentration ($p<0.05$). The asterisk indicates that the cocaine-induced increase of ^{3}H-dopamine concentration after the lesion was significantly less than before the lesion ($p<0.05$).

Figure 7


1,300
1,100
900
700
500
300
100
^{3}H-Dopamine Concentration (% of baseline)
LESION
*
Perfusion Time (min) 60
120
180
240

but not that of d-amphetamine, was significantly reduced by the knife cut ($p<0.05$).

3. Effects of Cocaine and Tyramine

A similar protocol in which the effects of cocaine were compared with tyramine rather than d-amphetamine is summarized in Fig. 8. In these experiments, 10 min pulses of both cocaine (10^{-5}M) and tyramine (10^{-4}M) increased the efflux of ^{3}H-dopamine, but the lesion did not significantly alter the effects of either drug.

Nevertheless, when the results of all 7 experiments with cocaine were combined, the drug caused significantly less efflux of ^{3}H-dopamine after the lesion than before the lesion; in the 10 min period after the start of the cocaine perfusion the increased efflux of ^{3}H-dopamine was 4,095±472 dpm before the lesion and 2385±347 dpm after the lesion.

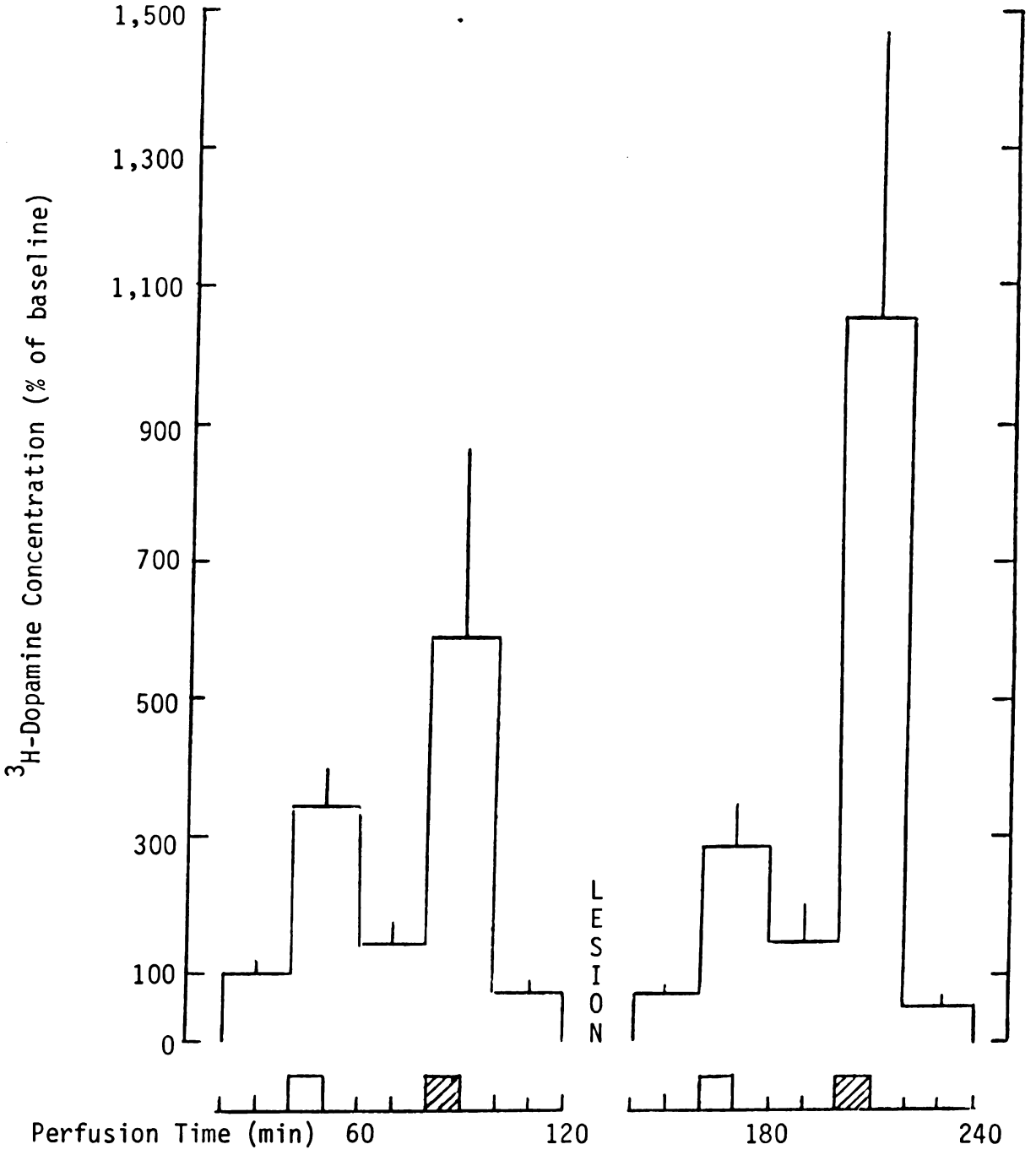
C. Drug Interactions on the Efflux of ^{3}H-Dopamine

Cocaine, amphetamine and tyramine have peripheral sympathomimetic effects due to their ability to increase the effective concentration of norepinephrine at receptor sites. Tyramine appears to act by releasing norepinephrine from the nerve terminal, cocaine by blocking reuptake of released norepinephrine, and amphetamine by a combination of both actions. Cocaine reduces the sympathomimetic actions of tyramine but not amphetamine, presumably because cocaine blocks the uptake of tyramine but not of amphetamine into the noradrenergic nerve terminals. The objective of the experiments in this section was to determine if these drugs have similar actions on dopamine neurons in the brain.

Figure 8. Efflux of endogenously synthesized ^{3}H-dopamine from the brain in response to an intraventricular infusion of cocaine or tyramine, before and after an acute lesion of the nigrostriatal pathway.

CSF containing ^{3}H-tyrosine was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 4 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-dopamine in the perfusate samples collected during the indicated times. Values were obtained from 3 separate experiments and 100% represents a baseline efflux of 1029±203 DPM. Cocaine (10^{-5}M) or tyramine (10^{-5}M) was added to the perfusing solution for 10 min collection periods as noted on the abscissa by the open and hatched squares, respectively. All drug-induced increases of ^{3}H-dopamine concentrations were significantly greater than the preceding control concentrations ($p<0.05$).

Figure 8


1,500
1,300
1,100
900
700
500
300
100
0
^{3}H-Dopamine Concentration (% of baseline)
LESION
Perfusion Time (min)
60
120
180
240

1. Cocaine and Amphetamine

In the experiments summarized in Fig. 9, pulses of amphetamine (10^{-5}M) were added to the perfusing CSF before and during a continuous infusion of cocaine (10^{-5}M). Both amphetamine and cocaine increased the efflux of ^{3}H-dopamine. The amphetamine-induced efflux was not influenced by the concomitant infusion of cocaine. That is, the efflux of ^{3}H-dopamine in response to a 10 min pulse of amphetamine was the same before and during the continuous infusion of cocaine.

2. Cocaine and Tyramine

An experiment of similar design is depicted in Fig. 10, where the effects of tyramine (10^{-5}M) and cocaine (10^{-5}M) are summarized. Both tyramine and cocaine increased the efflux of ^{3}H-dopamine. But as in the previous experiment, the concomitant infusion of cocaine did not influence the tyramine-induced efflux.

II. Effects of Drugs on the Efflux from the Brain of Endogenously Synthesized ^{3}H-Serotonin

In all experiments the brain perfusates were analyzed for ^{3}H-tryptophan and ^{3}H-serotonin. The concentrations of ^{3}H-tryptophan in perfusate samples did not change in response to any of the drugs or surgical manipulations. Accordingly, these values are not presented graphically.

The concentration of ^{3}H-serotonin in each 5 min perfusate sample is reported as a percentage of the concentration of ^{3}H-serotonin in samples collected during the control periods at the beginning of each experiment. Just as for the dopamine experiments, for the purpose of graphical representation and for the simplication of statistical analyses, the

Figure 9. Efflux of endogenously synthesized ^{3}H-dopamine from the brain in response to intraventricular infusions of amphetamine and cocaine.

CSF containing ^{3}H-tyrosine was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 4 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-dopamine in the perfusate samples collected during the indicated times. Values were obtained from 5 separate experiments and 100% represents a baseline efflux of 741±143 DPM. d-Amphetamine (10^{-5}M) was added to the perfusing solution for 10 min collection periods as noted on the abscissa by the solid squares. Cocaine (10^{-5}M) was added to the perfusing solution for 100 min as noted on the abscissa by an open bar. All drug-induced increases of ^{3}H-dopamine concentrations were significantly greater than the preceding control concentrations ($p<0.05$). Comparison of the difference between ^{3}H-dopamine concentrations during amphetamine treatment and the preceding treatment (CSF or cocaine) indicated no significant differences in the magnitude of amphetamine-induced efflux of dopamine ($p<0.05$).

Figure 9

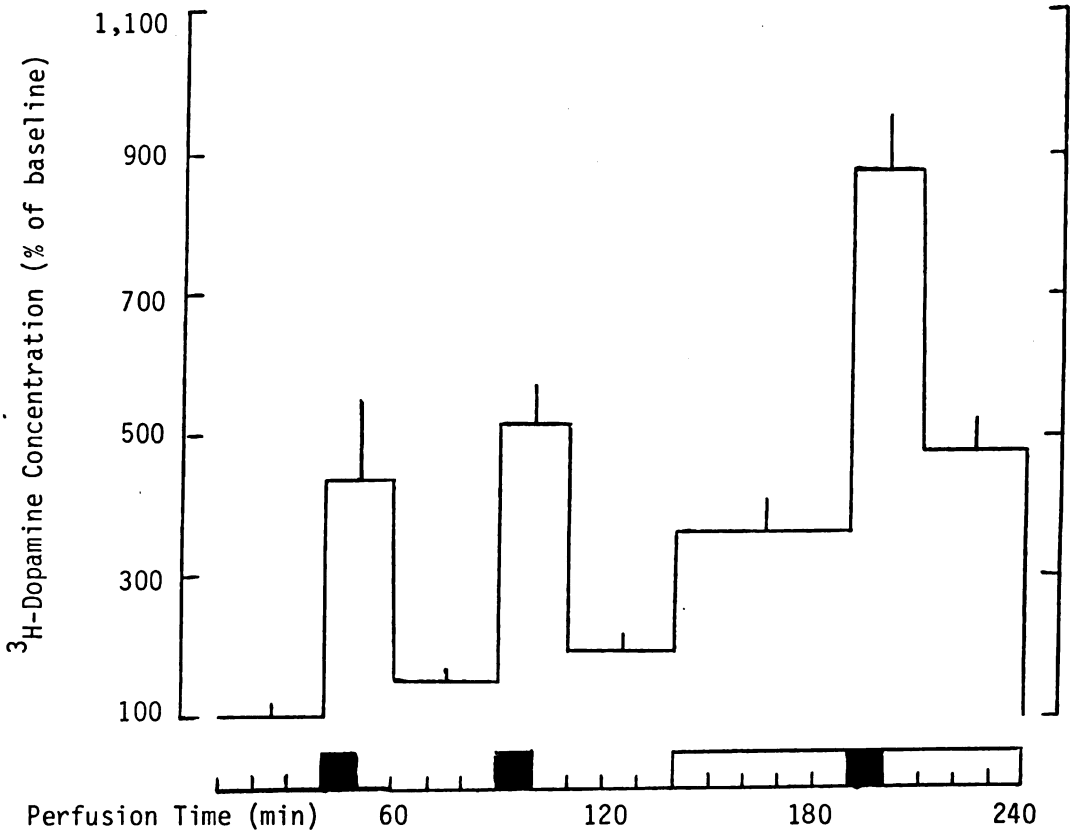
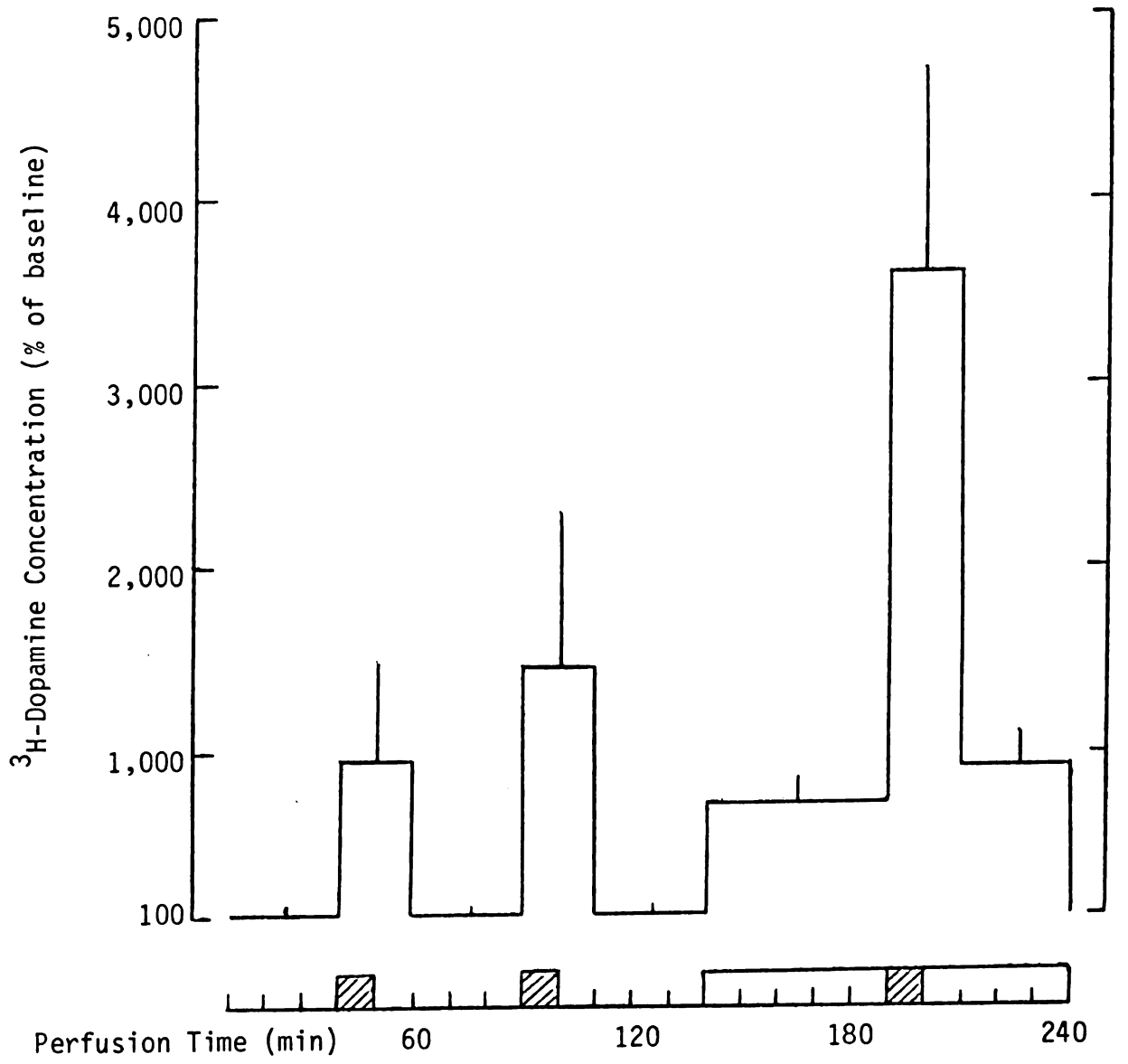

1,100
900
700
500
300
100
^{3}H-Dopamine Concentration (% of baseline)
Perfusion Time (min)
60
120
180
240

Figure 10. Efflux of endogenously synthesized ^{3}H-dopamine from the brain in response to intraventricular infusions of tyramine and cocaine.

CSF containing ^{3}H-tyrosine was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 4 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-dopamine in the perfusate samples collected during the indicated times. Values were obtained from 4 separate experiments and 100% represents a baseline efflux of 454±70 DPM. Tyramine (10^{-4}M) was added to the perfusing solution for 10 min collection periods as noted on the abscissa by the hatched squares. Cocaine (10^{-5}M) was added to the perfusing solution for 100 min as noted on the abscissa by an open bar. All drug-induced increases of ^{3}H-dopamine concentrations were significantly greater than that preceding control concentrations ($p<0.05$). Comparison of the difference between ^{3}H-dopamine concentrations during tyramine treatment and the preceding treatment (CSF or cocaine) indicated no significant differences in the magnitude of tyramine-induced efflux of dopamine ($p<0.05$).

Figure 10


5,000
4,000
3,000
2,000
1,000
100
^{3}H-Dopamine Concentration (% of baseline)
Perfusion Time (min)
60
120
180
240

efflux of ^{3}H-serotonin in two or more consecutive collection periods were combined.

A. Intraventricular and Intravenous Administration of Cocaine

Stimulation of the raphé nuclei, intraventricular administration of K^{+}, and intraventricular administration of high concentrations of amphetamine increase the efflux of ^{3}H-serotonin synthesized endogenously from administered ^{3}H-tryptophan (Chiueh and Moore, 1976). The present experiments, employing similar methods, were designed to determine if cocaine also could increase the efflux of endogenously synthesized ^{3}H-serotonin.

The addition of 5 minute pulses of increasing concentrations of cocaine (10^{-6} - 10^{-3}M)to the perfusing CSF at 20 min intervals caused only small non-concentration-related increases in the efflux of ^{3}H-serotonin (Fig. 11).

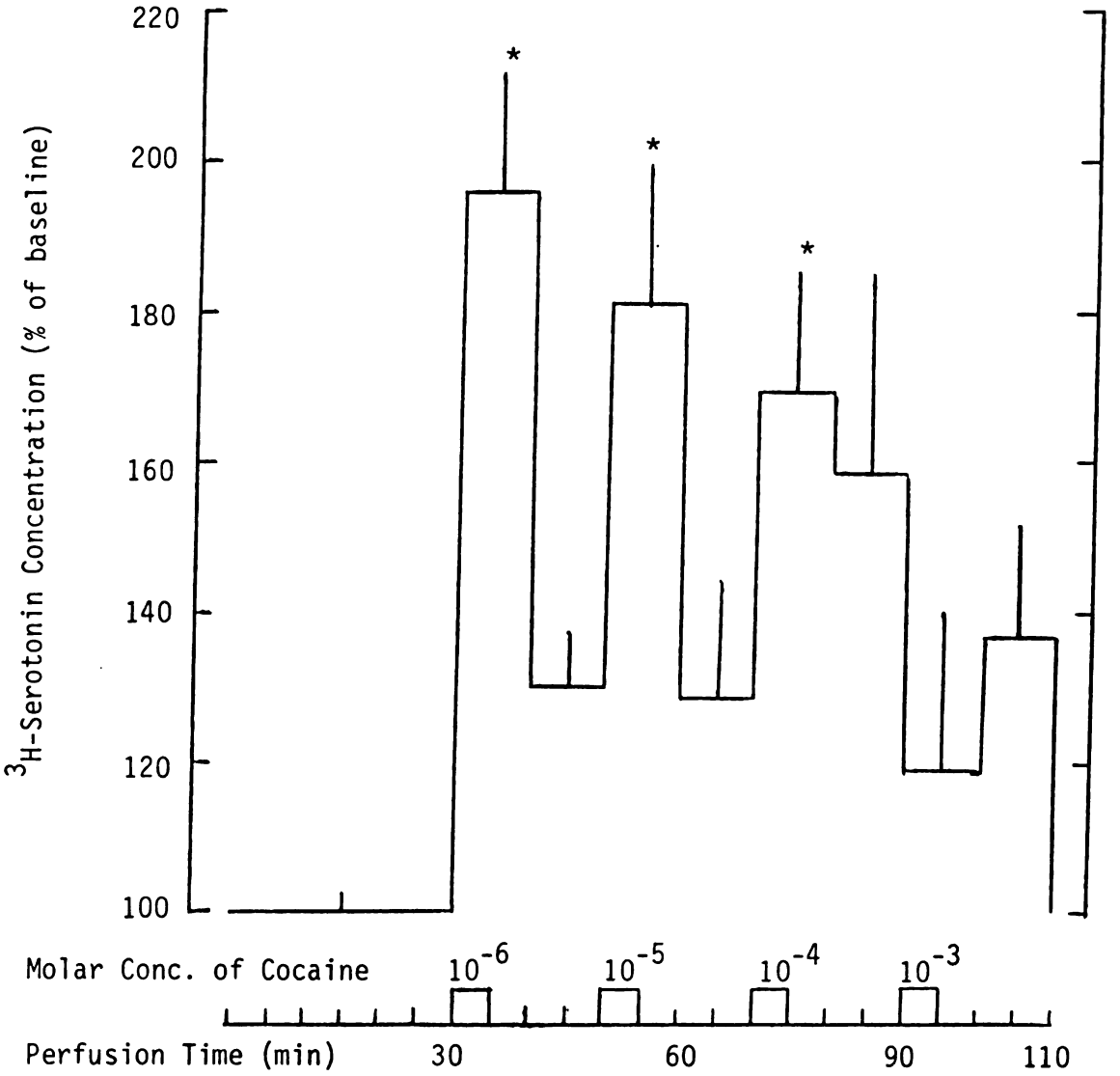
These increases were not of the same magnitude as for the increases observed while measuring ^{3}H-dopamine, where the average increase of efflux was 800% of baseline for 10^{-5}M cocaine. The average increase in efflux of ^{3}H-serotonin was only 200% of baseline for any of the concentrations of cocaine tested.

The systemic administration of cocaine produced an apparent decrease in the efflux of endogenously synthesized ^{3}H-serotonin. Following the intravenous injection of cocaine HCl (5 mg/kg) there was a transient (5 min) increase in the blood pressure and a slight but prolonged decrease in the efflux of ^{3}H-serotonin. The mean efflux of ^{3}H-serotonin (82.5±6%) during the 30 min period following the intravenous injection of cocaine was significantly less than baseline efflux (100±2%; $p<0.05$).

Figure 11. Efflux of endogenously synthesized ^{3}H-serotonin from the brain in response to intraventricular infusions of increasing concentrations of cocaine.

CSF containing ^{3}H-tryptophan was continuously infused into the lateral ventricle at a constant rate of 0.2 ml/min for 2 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-serotonin in the perfusate samples collected during the indicated times. Values were obtained from 5 separate experiments and 100% represents a baseline efflux of 668±49 DPM. Various molar concentrations of cocaine (10^{-6} - 10^{-3}) were added to the perfusing solution during 5 min collection periods as indicated on the abscissa by the open squares. Asterisks indicate cocaine-induced increases of ^{3}H-serotonin concentrations that are significantly greater than the preceding control concentration ($p<0.05$).

Figure 11


220
200
180
160
140
120
100
^{3}H-Serotonin Concentration (% of baseline)
*
*
*
Molar Conc. of Cocaine
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Perfusion Time (min)
30
60
90
110

Because of the discrepancy in effects on efflux of ^{3}H-serotonin observed between the intravenous and intraventricular administration of cocaine, and because of the extremely high concentration of cocaine needed to produce an increase in efflux of endogenously synthesized ^{3}H-serotonin, no experiments were conducted on ^{3}H-serotonin efflux following an acute lesion of the ascending fibers from the raphé nuclei.

B. Drug Interactions on the Efflux of ^{3}H-Serotonin

Because a concentration-effect relationship could not be established, and because of the small increase in efflux measured, further testing was required to select a concentration of cocaine which could produce a continuous increased efflux of serotonin for the next series of experiments. The results of these tests indicated that by continuously perfusing the lateral ventricles with a concentration of 10^{-3}M cocaine, a sustained increase in the efflux of ^{3}H-serotonin of about 200% of baseline could be obtained. This concentration of cocaine was used for further experiments with the knowledge that it was much higher than what would appear in the brain after other routes of cocaine administration and the results were evaluated accordingly.

The objective of the experiments in this section was to determine if cocaine, amphetamine and tyramine have similar actions on serotonergic neurons in the brain as those described previously for these various drugs in peripheral noradrenergic nerve terminals. The concentrations of all of the drugs used were increased because the serotonergic system is not as responsive as the dopaminergic system to these drug treatments.

1. Cocaine and Amphetamine

In the experiments summarized in Fig. 12, pulses of amphetamine (10^{-4}M) were added to the perfusing CSF before and during a continuous infusion of cocaine (10^{-3}M). Both amphetamine and cocaine increased the efflux of ^{3}H-serotonin. The amphetamine-induced efflux was not influenced by the concomitant infusion of cocaine. In other words, the efflux of ^{3}H-serotonin in response to a 5 min pulse of amphetamine was the same before and during the continuous infusion of cocaine.

2. Cocaine and Tyramine

An experiment of similar design is depicted in Fig. 13, where the effects of tyramine (10^{-4}M) and cocaine (10^{-3}M) are summarized. Both tyramine and cocaine increased the efflux of ^{3}H-serotonin. But as in the previous experiment, the concomitant infusion of cocaine did not influence the tyramine-induced efflux.

Figure 12. Efflux of endogenously synthesized ^{3}H-serotonin from the brain in response to intraventricular infusions of amphetamine and cocaine.

CSF containing ^{3}H-tryptophan was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 2 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-serotonin in the perfusate samples collected during the indicated times. Values were obtained from 2 separate experiments and 100% represents a baseline efflux of 321±26 DPM. d-Amphetamine (10^{-4}M) was added to the perfusing solution for 5 min collection periods as noted on the abscissa by the solid squares. Cocaine (10^{-3}M) was added to the perfusing solution for 50 min as noted on the abscissa by an open bar. All drug-induced increases of ^{3}H-serotonin concentrations were significantly greater than the preceding control concentrations ($p<0.05$). The asterisks indicate that the amphetamine-induced and the amphetamine and cocaine-induced increases of ^{3}H-serotonin concentrations were significantly greater than the cocaine-induced increase in ^{3}H-serotonin concentration ($p<0.05$).

Figure 12

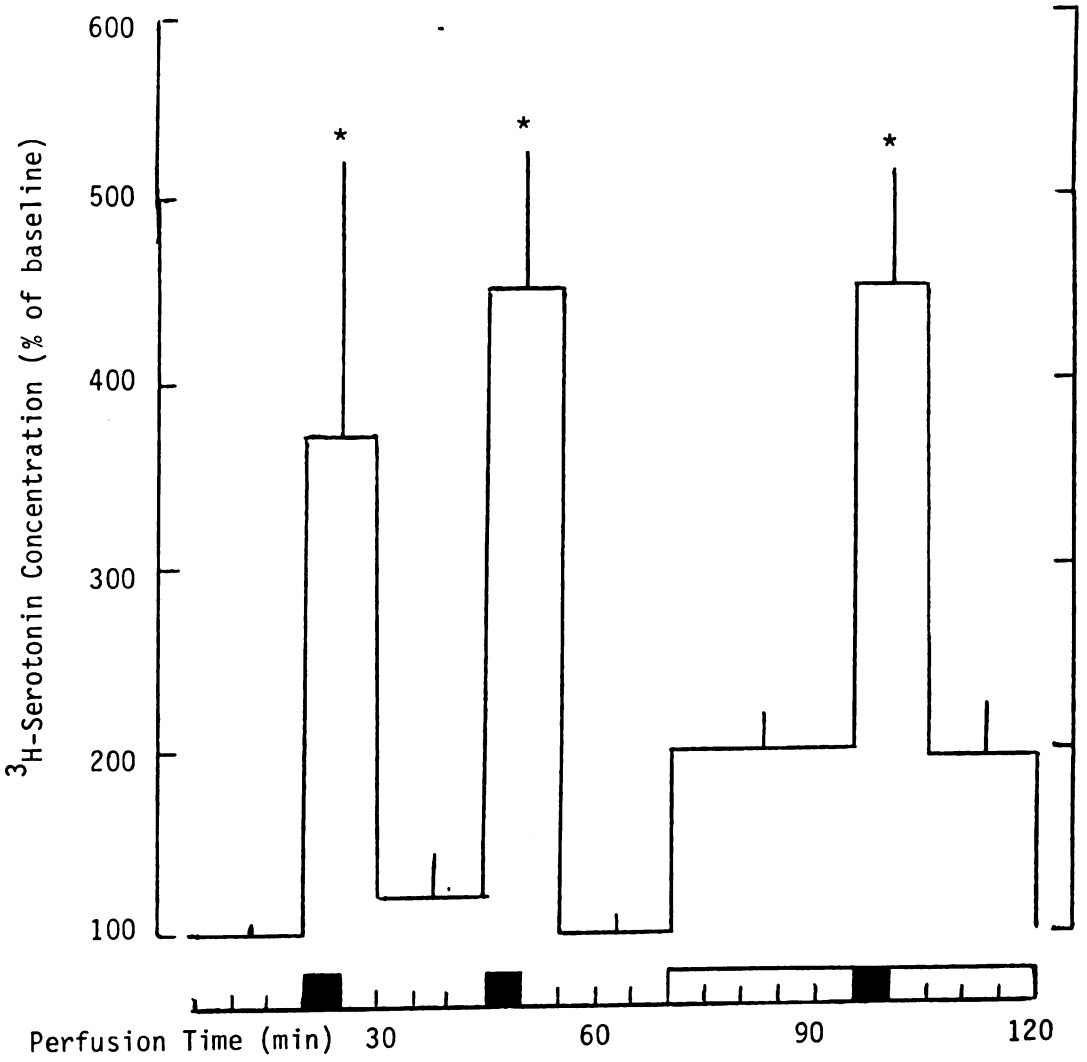
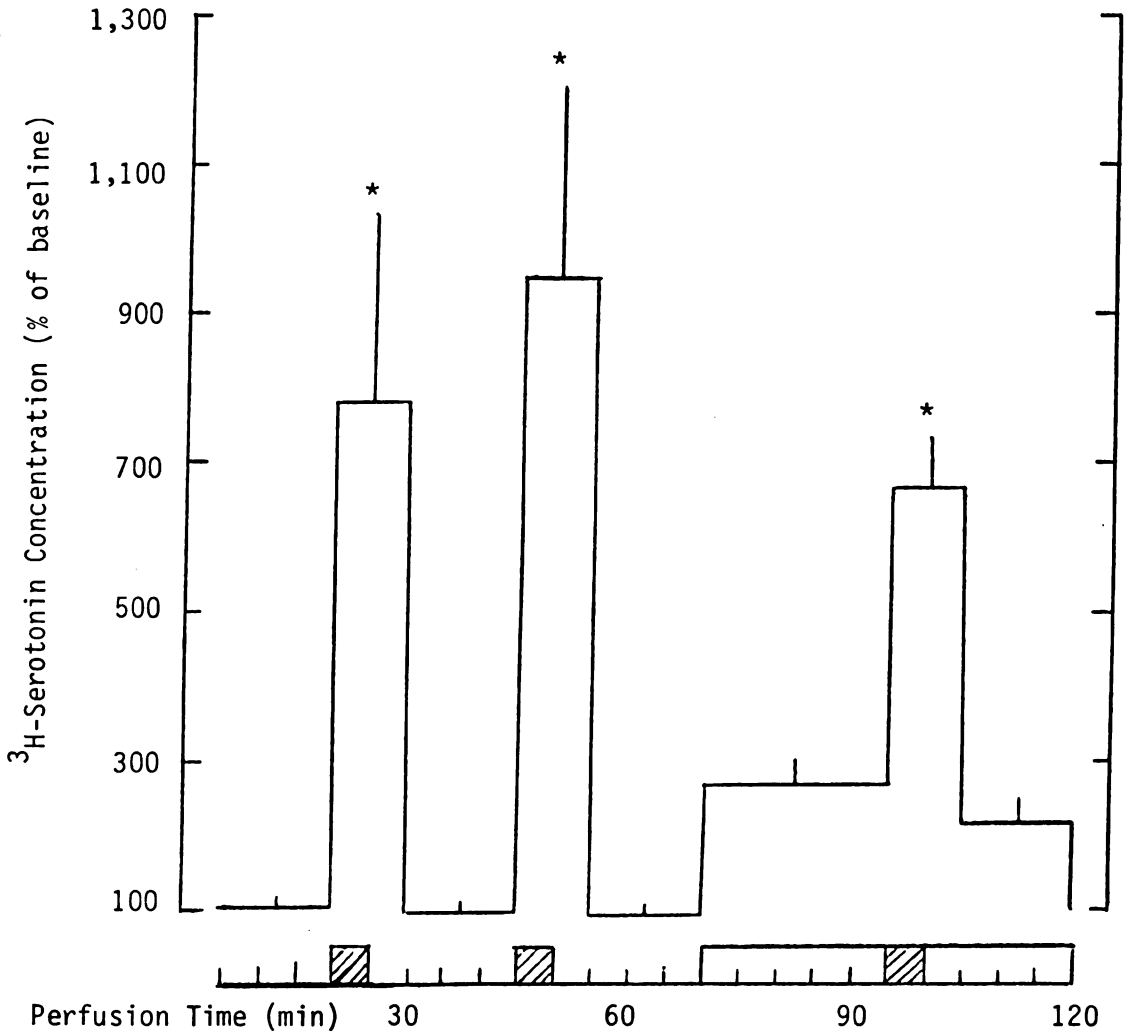

600
500
400
300
200
100
^{3}H-Serotonin Concentration (% of baseline)
*
*
*
Perfusion Time (min)
30
60
90
120

Figure 13. Efflux of endogenously synthesized ^{3}Hserotonin from the brain in response to intraventricular infusions of tyramine and cocaine.

CSF containing ^{3}H-tryptophan was continuously infused into the left lateral ventricle at a constant rate of 0.2 ml/min for 2 hours. The height of each bar represents the mean and the vertical lines one S.E. of the percent of baseline efflux of ^{3}H-serotonin in the perfusate samples collected during the indicated times. Values were obtained from 3 separate experiments and 100% represents a baseline efflux of 216±45 DPM. Tyramine (10^{-4}M) was added to the perfusing solution for 5 min collection periods as noted on the abscissa by the hatched squares. Cocaine (10^{-3}M) was added to the perfusing solution for 50 min as noted on the abscissa by an open bar. All drug-induced increases of ^{3}H-serotonin concentrations were significantly greater than the preceding control concentrations ($p<0.05$). The asterisks indicate that the tyramine-induced and the tyramine and cocaine-induced increases of ^{3}H-serotonin concentrations were significantly greater than the cocaine-induced increase in ^{3}H-serotonin concentration ($p<0.05$).

Figure 13


1,300
1,100
900
700
500
300
100
^{3}H-Serotonin Concentration (% of baseline)
*
*
*
Perfusion Time (min)
30
60
90
120

DISCUSSION

Azzaro et al. (1974) and Heikkila et al. (1975b) reported that cocaine does not increase the efflux of norepinephrine or dopamine from brain tissue in vitro. But there is no coordinated neuronal activity in vitro and therefore no "neurogenic release" of amines from the nerve terminal. Since in vitro studies cannot duplicate ongoing neuronal processes which might contribute to the action of a drug in vivo, data from these two kinds of procedures might lead to different conclusions.

In fact, there is even a difference in data obtained from different in vitro preparations. Orlansky and Heikkila (1974) found that various antiparkinsonian agents had a lower ED_{50} for inhibition of ^{3}H-dopamine accumulation in synaptosomes than in tissue slices. They suggested that the drugs could penetrate more easily into synaptosomes and thus exert a greater effect. Their experiments were therefore carried out in tissue slices, which retain catecholamines much better than synaptosomes and are therefore superior for release studies.

The results of the concentration-effect study indicated an ability of cocaine to increase the efflux of newly synthesized ^{3}H-dopamine into the brain. This contradicts the results of the in vitro studies previously mentioned. There are two reasons for this difference. These in vitro studies measured only the efflux of exogenously stored amines.

Possibly cocaine influences only the newly synthesized pool of amines. Secondly, ongoing neuronal processes are necessary for the cocaine-induced increase in efflux of dopamine observed *in vivo*.

Von Voigtlander and Moore (1973) demonstrated that acute electrolytic lesions of the nigrostriatal pathway blocked the ability of amphetamine, but not of tyramine, to increase the efflux of exogenously labelled ^{3}H-dopamine from cat brain *in vivo*. This method was used to distinguish between drugs which act independently of neuronal activity (*e.g.*, tyramine) from those that are dependent upon ongoing neuronal activity (*e.g.*, amphetamine). The experiments described in this thesis measuredthe efflux of endogenously synthesized ^{3}H-dopamine rather than exogenously stored ^{3}H-dopamine in response to various drug and surgical manipulations. An acute mechanical lesion of the nigrostriatal pathway did not block the ability of either amphetamine or tyramine to increase the efflux of endogenously synthesized ^{3}H-dopamine. In 5 out of 7 experiments, the nigrostriatal lesion reduced but did not completely block the cocaine-induced increase in efflux of endogenously synthesized ^{3}H-dopamine.

These results can be interpreted as further evidence of a differential drug effect on newly synthesized and stored dopamine pools. The increase in efflux of newly synthesized ^{3}H-dopamine induced by amphetamine is not dependent on an intact nigrostriatal pathway but an increase in efflux of exogenously labelled stores of ^{3}H-dopamine by amphetamine is dependent on nigrostriatal pathway activity.

Because the cocaine effect is reduced after a nigrostriatal lesion, it would appear that an intact nigrostriatal pathway is necessary for

the cocaine-induced increase in efflux of endogenously synthesized ^{3}H-dopamine. A previous study reporting the effect of cocaine on exogenously labelled stores of ^{3}H-norepinephrine showed no effect (Carr and Moore, 1970a). Possibly cocaine has an effect only on newly synthesized amines in the following way. Newly synthesized amines form a pool which is preferentially released in response to ongoing neuronal processes (Shore and Dorris, 1975). If cocaine acts by blocking the uptake of neurogenically released amines then these effects would be seen only when an experiment measured the efflux from newly synthesized rather than exogenously labelled stores of ^{3}H-dopamine. A decrease in cocaine-induced efflux after a nigrostriatal lesion would be predicted from this model because less amine would be released and therefore less available to be blocked during uptake. Another possible mechanism of action for cocaine which is suggested by these data is that of facilitation of neurogenic release of neurotransmitter.

In the experiments described in this thesis, cocaine did not block the increase in efflux of dopamine or serotonin caused by amphetamine. This also contradicts the in vitro data, probably because of the same reasons pointed out earlier in regard to in vitro procedures. These experiments suggest that cocaine has no effect on the ability of amphetamine to increase the efflux of amines in the central nervous system.

Studies in the periphery show that cocaine blocks the tyramine-induced increase in blood pressure (Trendelenburg, 1961). Work done in vitro with brain slices shows that cocaine blocks tyramine uptake at the neuronal membrane (Steinberg and Smith, 1970). In the experiments

presented here, cocaine did not block the increase in efflux of amine caused by tyramine. This is not consistent with the effects of these drugs on blood pressure or on tyramine uptake in vitro. These data might indicate that in the central nervous system, either cocaine does not block the uptake of tyramine, or that tyramine acts without first being taken up into the neuron. Another obvious possibility is that there are some differences between peripheral norepinephrine and central dopamine neurons.

Because of the high concentrations of drugs used to investigate the serotonergic system in this study, it is difficult to interpret the results in terms of brain concentrations found after systemic administration of these same drugs. The need for higher concentrations of drugs was not unexpected. The previous reports on the serotonergic system indicated that high concentrations of drugs were necessary to obtain a response (Chiueh and Moore, 1976). In spite of the extremely high concentration of cocaine used in the drug interaction studies, neither the effect of amphetamine nor tyramine was blocked. This would indicate that cocaine does not block the uptake of these two drugs into serotonin neurons or that their uptake is not necessary for their action.

In summary, the experiments presented in this thesis have shown that cocaine administered systemically or intraventricularly can increase the efflux of endogenously synthesized ^{3}H-dopamine from the brain. This action is dependent on neurogenic release of dopamine, as it is reduced when nerve impulse traffic along the nigrostriatal pathway is reduced.

Cocaine does not interfere with the actions of amphetamine or tyramine on central dopaminergic neurons and therefore its mechanism of action is probably not the same as either of these sympathomimetic drugs. Therefore, evidence indicates that either blockade of uptake at the neuronal membrane or facilitation of neurogenic release of transmitter, could be the mechanism of action of cocaine in the central nervous system.

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