Biochemical Pharmacology, Vol. 18, pp 1907–1918. Pergamon Press. 1969. Printed in Great Britain

DISTRIBUTION AND METABOLISM OF NOREPINEPHRINE AFTER ITS ADMINISTRATION INTO THE CEREBROVENTRICULAR SYSTEM OF THE CAT*†

L. A. CARR‡ and K. E. MOORE

Department of Pharmacology, Michigan State University, East Lansing, Mich. 48823, U.S.A.

(*Received* 1 *November* 1968; *accepted* 31 *January* 1969)

Abstract—Five μc of DL-^{3}H-norepinephrine was injected into the cerebroventricular system of cat brain by means of stereotaxically implanted cannulae in the third or lateral ventricles. At various times after its administration various brain regions were examined for content of ^{3}H-norepinephrine, ^{3}H-normetanephrine, ^{3}H-deaminated catechol metabolites and ^{3}H-deaminated *O*-methylated metabolites. After the injection into the left lateral ventricle, ^{3}H-norepinephrine was concentrated in the left caudate nucleus and hypothalamus. Lesser amounts were found in the left hippocampus, the wall of the left lateral ventricle, brain stem and cerebellum. After injection into the third ventricle, ^{3}H-norepinephrine was retained primarily by areas caudal to the third ventricle (hypothalamus and brain stem). Thus, the regional distribution of ^{3}H-norepinephrine depends upon the site of injection and the ability of various areas lining the ventricular system to accumulate norepinephrine. The major metabolite in each area was ^{3}H-normetanephrine. Much smaller amounts of deaminated metabolites were detected. The pattern of metabolites was not affected by various anesthetic agents and remained relatively constant over a 24-hr period after injection of ^{3}H-norepinephrine. Pretreatment with a monoamine oxidase inhibitor did not alter the brain content of endogenous or ^{3}H-norepinephrine but did increase the percentage of ^{3}H-normetanephrine. Pretreatment with reserpine reduced endogenous levels of norepinephrine and ^{3}H-norepinephrine but increased the percentage of ^{3}H-normetanephrine and ^{3}H-deaminated metabolites in all brain regions.

DURING the past 15 yr numerous investigators have demonstrated the presence of norepinephrine and dopamine in discrete anatomical regions of mammalian brain.[1, 2] Current evidence suggests that these amines function as neurotransmitters in the central nervous system. Indeed, many of the criteria that must be satisfied before a substance can be considered a neurotransmitter have been fulfilled by the catecholamines. For example, the amines are located within nerve terminals[3] and enzymatic machinery for their synthesis[4] and mechanisms for terminating their actions[5] have been demonstrated in brain. Although documentation is not extensive it has been shown that norepinephrine and dopamine can be released from the central nervous system upon appropriate stimulation *in vitro*[6, 7] and *in situ*.[8] All of this evidence has

* Supported by U.S. Public Health Service Grant MH 13174.

† Preliminary accounts of this work were reported previously in *Proc. Can. Fedn Biol. Soc.* **2**, 127 (1968) and in *Pharmacologist* **10**, 195 (1968).

‡ U.S. Public Health Service Predoctoral Fellow 1-F1-GM 34020.

been offered in support of a transmitter role for catecholamines within the central nervous system.

Examining the functional significance of brain catecholamines by direct experimentation *in situ* is difficult because of the anatomical inaccessibility of specific noradrenergic and dopaminergic pathways and restrictions placed upon the transfer of catecholamines by the blood-brain barrier. This barrier has been circumvented by injecting small amounts of radioactive dopamine and norepinephrine directly into rat cerebral ventricles.[9, 10] Catecholamines administered in this manner mix with endogenous stores.[11] Studies utilizing intraventricular injections and similar techniques (e.g. cisternal injections[12]) have provided information on the uptake, metabolism and release of brain catecholamines and on the effect of drugs upon these processes.[5]

Most of the previous studies utilizing intraventricular injections of ^{3}H-norepinephrine have been performed in rats. The experiments described in the present report were preliminary to studies designed to explore the utility of cerebroventricular perfusion techniques for detecting the release of transmitter substances from brain. Since cerebroventricular cannulation procedures,[13] cerebrospinal fluid dynamics[14] and cerebroventricular perfusion techniques[15] have been carefully documented for the cat,this species is being used in our current studies. The present report summarizes the results of the distribution and metabolism of intraventricularly administered ^{3}H-norepinephrine.

METHODS

Mongrel cats of either sex weighing 2–4 kg were anesthetized by an intraperitoneal injection of sodium pentobarbital (30 mg/kg) or a urethane–barbiturate mixture (Dial-urethane, Ciba; sodium diallylbarbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg) or with the open-drop administration of metoxyflurane. The cats were then placed in a stereotaxic apparatus (David Kopf, Inc.) and a self-tapping screw-type cannula implanted into either the anterior horn of the left lateral cerebral ventricle or in the ventral portion of the third ventricle as described by McCarthy and Borison.[13] Surgical procedures for the "unanesthetized" and "spinal cord-sectioned" (at C_1) animals were carried out under methoxyflurane anesthesia. The respiration of spinal-sectioned animals was maintained with a respirator pump. Except for the experiments with unanesthetized animals the cats remained in the stereotaxic instrument throughout the experimental period.

Ten to 30 min after the cannulae were fixed in place, 5 μc of DL-norepinephrine-7-^{3}H hydrochloride (7·38 c/mM, New England Nuclear Corp.) were injected intraventricularly in an effective volume of 10 μl of 0·1 N acetic acid. At various times thereafter (usually 1 hr) 5 μl of 1% methylene blue were injected intraventricularly to confirm the cannula tip position. The chest was opened along the midline (unanesthetized animals received sodium pentobarbital, 30 mg/kg) and the whole vascular system was perfused with saline by means of a catheter placed into the aorta through an incision in the left ventricle. Blood was washed out through the incised right atrium. The brain was then quickly removed from the skull and the following brain regions were dissected and weighed: brain stem (medulla and pons), caudate nuclei, hypothalamus, hippocampi, a portion of the roof of the left lateral ventricle consisting of ependyma and callosal white matter, a portion of the ventral surface of the cerebellum and a portion of the frontal lobe of the cortex.

The tissues were homogenized in 2 ml of cold 0·4 N perchloric acid (brain stem in

6 ml). The homogenates were kept on ice for 30 min and then centrifuged at 9500 *g* for 5 min. The supernatant was collected, the pellet rehomogenized in 2 or 6 ml of 0·4 N perchloric acid and the resulting homogenate centrifuged as before. The supernatants were combined and total radioactivity determined by adding 100 μl of the tissue extracts to scintillation vials containing 1 ml of water and 10 ml of modified Bray's solution (6 g of 2,5-diphenyloxazole and 100 g of naphthalene/liter of dioxane). Radioactivity was determined with a Beckman DPM-100 liquid scintillation counter. The counting efficiency for this mixture was 25 per cent.

The remaining portions of the tissue extracts were adjusted to pH 4 with 10 N potassium hydroxide and centrifuged to remove the precipitate of potassium perchlorate. The supernatant was transferred to 50 ml glass-stoppered centrifuged tubes containing 400 mg of washed aluminum oxide (Woelm) and 0·5 ml of 0·2 M disodium ethylenediamine tetraacetate. The alumina–tissue extract mixture was adjusted to pH 8·6–8·7 with 1 and 0·2 M potassium hydroxide and tubes were shaken for 5 min and then centrifuged. The supernatant (alumina effluent) was set aside and subsequently assayed for *O*-methylated metabolites (see below). The alumina was then washed with 5 ml of 0·2 M sodium acetate and with 10 ml of distilled water. The amines and deaminated catechol metabolites were eluted from the alumina with 2 ml of 0·5 N acetic acid. Five min of shaking was used in all wash steps and 10 min in the elution step. One ml of acid eluate was analyzed for endogenous norepinephrine or dopamine as described by Moore and Rech.[16] The radioactivity in 100 μl of the eluate was determined and another 100 μl was spotted on precoated cellulose thin layer plastic sheets (Distillation Products Industries) along with 5 μg of norepinephrine. The sheets were developed in a solvent containing 1 N acetic acid, butanol and ethanol (10:35:10). The norepinephrine spot was identified under ultraviolet light and a 4 cm^2 section containing this spot was cut out and placed in a scintillation vial containing 1 ml of water and 10 ml of modified Bray's solution. The samples were shaken for 15 min and counted. ^{3}H-norepinephrine was corrected for counting efficiency (30 per cent), alumina recovery (69·3 $\pm$ 1·3 per cent) and thin-layer recovery (34·8 $\pm$ 2·5 per cent). The radioactivity at the fluorescent norepinephrine spot, corrected for thin-layer recovery, was subtracted from the total radioactivity in the alumina eluate to obtain the value for deaminated catechols. ^{3}H-norepinephrine used for injection was checked for purity by chromatographing it with 5 μg of cold norepinephrine. A radiochromatogram of the developed sheet indicated that all the radioactivity corresponded with the norepinephrine fluorescent spot.

O-methylated metabolites of ^{3}H-norepinephrine were determined in the following manner. The effluent from the alumina was adjusted to pH 6 with 0·2 N acetic acid and placed on columns of Dowex 50W-$\times$8 (H^+ form, 6 $\times$ 40 mm). The columns were washed with 10 ml of distilled water and this wash was added to the Dowex effluent. The radioactivity in 100 μl of the combined effluent-wash represented the *O*-methylated deaminated metabolites (counting efficiency was 30%). ^{3}H-normetanephrine was eluted from the column with 10 ml of 1:1 mixture of 6 N HCl and ethanol. Radioactivity in this eluate (100 μl) was corrected for counting efficiency (13 per cent) and chromatographic recovery of a ^{3}H-normetanephrine standard (83·4 $\pm$ 3·6 per cent). The acid eluate from the Dowex column was identified as ^{3}H-normetanephrine by thin-layer chromatography using the same solvent system that was used for separating norepinephrine.

RESULTS

The distribution and metabolism of intraventricularly administered ^{3}H-norepinephrine

Cats were sacrificed 1 hr after an injection of 5μc of ^{3}H-norepinephrine into either the left lateral or the third cerebral ventricles. The concentrations of endogenous catecholamines and of ^{3}H-norepinephrine and its metabolites were determined in those brain regions listed in Table 1. The values for endogenous catecholamines are similar to those which have been reported previously.[17]

TABLE 1. DISTRIBUTION OF ^{3}H-NOREPINEPHRINE IN THE BRAIN OF CATS AFTER THE ADMINISTRATION OF THIS AMINE INTO THE LATERAL AND THIRD CEREBRAL VENTRICLES*

Brain region	Tissue wt.	Endogenous catecholamines (μg/g)	Lateral ventricle H^3-norephinephrine (mμc/g)	Third ventricle H^3-norepinephrine (mμc/g)
Brain stem	1·70 ± 0·09	0·42 ± 0·01	62 ± 9	116 ± 55
Hypothalamus	0·08 ± 0·01	2·96 ± 0·19	1251 ± 142	6487 ± 1966
Left caudate nucleus	0·20 ± 0·01	12·27 ± 0·95	3703 ± 404	95 ± 86
Right caudate nucleus	0·20 ± 0·01	11·05 ± 0·90	9 ± 4	6 ± 4
Left hippocampus	0·13 ± 0·01	0·18 ± 0·05	147 ± 89	12 ± 5
Right hippocampus	0·14 ± 0·01	0·18 ± 0·03	14 ± 4	11 ± 1
Cerebellum	0·33 ± 0·02	0·11 ± 0·02	59 ± 5	105 ± 26
Left ventricular wall	0·12 ± 0·01		49 ± 15	3 ± 3
Cortex	0·16 ± 0·01		16 ± 8	1 ± 1

* Cats were anesthetized with sodium pentobarbital (30 mg/kg) and cannulae were placed in either the left lateral (three animals) or third cerebral ventricles (three animals). Five μc of ^{3}H-norepinephrine were injected intraventricularly and the animals sacrificed 1 hr later. Endogenous catecholamines represent norepinephrine except in the caudate nuclei where the values for dopamine are presented. All values represent mean ± 1 standard error.

When injected into the left lateral ventricle the highest concentrations of ^{3}H-norepinephrine were found in the left caudate nucleus and in the hypothalamus. These regions also contain the highest concentrations of endogenous catecholamines. Lesser amounts of ^{3}H-norepinephrine accumulated in the cerebellum, left ventricular wall and left hippocampus and only barely detectable amounts were found in structures lining the right lateral ventricle.

After injection into the third ventricle ^{3}H-norepinephrine was concentrated in the hypothalamus with lesser amounts in the brain stem and cerebellum. Very little radioactivity was found in areas lining the lateral ventricles. Negligible amounts of radioactivity were found in the frontal cortex following either lateral or third ventricular injections, indicating that the injected volume probably did not distribute to the subarachnoid spaces. These results indicate that the distribution of the small volume of intraventricularly administered ^{3}H-norepinephrine is dependent upon the site of injection and the inherent ability of various regions to accumulate catecholamines.

The levels of some metabolites of ^{3}H-norepinephrine in brain 1 hr after the injection of this amine into the left lateral ventricle are shown in Table 2. The major metabolite in all areas was ^{3}H-normetanephrine while deaminated catechol and *O*-methylated-deaminated metabolites were found in much smaller amounts. Since these two groups of metabolites comprised such a small percentage of the total radioactivity, they were listed together as total deaminated metabolites in subsequent tables. There appeared to be some correlation between the distribution of endogenous norepinephrine and the

accumulation of ^{3}H-norepinephrine. That is, in those regions containing the highest endogenous content of catecholamines the percentage of radioactivity represented by ^{3}H-norepinephrine was high (caudate nucleus and hypothalamus, 73 and 63 per cent respectively), whereas areas containing low levels of endogenous catecholamines contained a low percentage of unchanged amines (left ventricular wall and cerebellum, 35 and 23 per cent respectively).

TABLE 2. DISTRIBUTION OF ^{3}H-NOREPINEPHRINE AND ITS METABOLITES IN CAT BRAIN AFTER THE ADMINISTRATION OF THE RADIOACTIVE AMINE INTO THE LATERAL CEREBRAL VENTRICLE*

Brain region	Total radioactivity (mμc/g)	^{3}H-norepinephrine	^{3}H-normetanephrine	^{3}H-deaminated catechol metabolites	^{3}H-deaminated *O*-methyl metabolites
Brain stem	137 ± 18	46 ± 1	28 ± 3	3 ± 3	21 ± 2
Hypothalamus	1972 ± 142	63 ± 8	15 ± 2	3 ± 1	7 ± 1
Left caudate nucleus	4997 ± 830	73 ± 7	14 ± 1	5 ± 5	3 ± 1
Left hippocampus	543 ± 264	23 ± 4	46 ± 6	5 ± 3	15 ± 1
Cerebellum	262 ± 28	23 ± 3	52 ± 4	2 ± 2	15 ± 2
Left ventricular wall	141 ± 42	35 ± 1	32 ± 9	8 ± 5	17 ± 5

* Cats were anesthetized with sodium pentobarbital (30 mg/kg) and cannulae placed in the left lateral ventricle. Five μc of ^{3}H-norepinephrine were injected intraventricularly and the animals sacrificed 1 hr later. ^{3}H-norepinephrine and its metabolites are expressed as the mean percentage of total radioactivity (± standard error) in three animals.

Effect of anesthesia on the endogenous catecholamine levels and the distribution of ^{3}H-norepinephrine and its metabolites in brain

Table 3 summarizes the distribution of ^{3}H-norepinephrine and its metabolites in the brain of cats which were either anesthetized with sodium pentobarbital or with the urethane–barbiturate mixture or had recovered from methoxyflurane anesthesia (unanesthetized and spinal cord-sectioned animals). Although all areas listed in Table 1 were analyzed, only data from those brain regions having the highest concentrations of ^{3}H-norepinephrine (brain stem, hypothalamus and caudate nucleus) are presented in Table 3. However, the pattern of distribution of ^{3}H-norepinephrine and its metabolites in all regions of the brain was essentially the same for all treatment groups. That is, most of the radioactivity was represented by ^{3}H-norepinephrine, while ^{3}H-normetanephrine represented the major metabolite. The only obvious alteration in the pattern was a significantly lower level of total radioactivity in the brain stem and hypothalamus of cord-sectioned animals.

Also included in Table 3 are the data from a group of animals, anesthetized with pentobarbital, in which ^{3}H-norepinephrine was administered into the third rather than the lateral ventricle. Although the total amount of radioactivity in the hypothalamus and caudate nucleus was markedly different the percentages of radioactivity represented by ^{3}H-norepinephrine and its metabolites were essentially the same for both the pentobarbital-anesthetized groups. A similar picture is seen in the two urethane–barbiturate groups. While the amount of total radioactivity in the hypothalamus and brain stem was less after 5 hr than 1 hr, the percentages of ^{3}H-norepinephrine and its metabolites were essentially the same.

TABLE 3. ^{3}H-NOREPINEPHRINE AND METABOLITES IN THE BRAINS OF ANESTHETIZED AND UNANESTHETIZED CATS*

Brain region	Treatment	N†	Total radioactivity	^{3}H-norepinephrine	^{3}H-normetanephrine	^{3}H-deaminated metabolites
Brain stem	Sodium pentobarbital	3	137 ± 18	46 ± 1	28 ± 3	24 ± 5
	Sodium pentobarbital‡	3	221 ± 91	51 ± 5	27 ± 4	17 ± 4
	Urethane-barbiturate	4	194 ± 14	41 ± 4	12 ± 5	15 ± 5
	Urethane-barbiturate§	3	52 ± 1	37 ± 8	25 ± 6	28 ± 6
	Unanesthetized	3	80 ± 29	67 ± 6	25 ± 4	12 ± 2
	Spinal cord section	3	11 ± 3	46 ± 5	13 ± 5	29 ± 14
Hypothalamus	Sodium pentobarbital	3	1972 ± 142	63 ± 8	15 ± 2	9 ± 2
	Sodium pentobarbital‡	3	9127 ± 2563	70 ± 7	18 ± 5	8 ± 4
	Urethane-barbiturate	4	2086 ± 196	56 ± 9	16 ± 1	21 ± 5
	Urethane-barbiturate§	3	518 ± 317	64 ± 7	11 ± 2	19 ± 2
	Unanesthetized	3	1069 ± 351	72 ± 1	28 ± 5	11 ± 2
	Spinal cord section	3	654 ± 259	59 ± 6	23 ± 4	16 ± 4
Caudate nucleus	Sodium pentobarbital	3	4997 ± 830	73 ± 7	14 ± 1	7 ± 5
	Sodium pentobarbital‡	3	121 ± 105	68 ± 6	14 ± 8	6 ± 3
	Urethane-barbiturate	4	3391 ± 383	79 ± 8	11 ± 1	7 ± 5
	Urethane-barbiturate§	3	3873 ± 676	74 ± 5	12 ± 1	2 ± 1
	Unanesthetized	3	3506 ± 1676	83 ± 6	25 ± 6	4 ± 2
	Spinal cord section	3	3380 ± 791	81 ± 3	21 ± 5	3 ± 1

* Cats were anesthetized with sodium pentobarbital, urethane–barbiturate mixture or methoxyflurane and a cannula was placed in the left lateral ventricle (except for ‡). Animals anesthetized with methoxyflurane had their spinal cord sectioned at C_1 (spinal-cord section) or were allowed to recover consciousness (unanesthetized). Five μc ^{3}H-norepinephrine were then injected intraventricularly and the animals were sacrificed 1 hr later (except for §). Total radioactivity is expressed as mμc/g (mean ± 1 standard error) and ^{3}H-norepinephrine, ^{3}H-normetanephrine and total deaminated metabolites are expressed as percentages of total radioactivity (± 1 standard error).

† N = Number of animals.

‡ ^{3}H-norepinephrine was injected into the third ventricle.

§ Sacrificed 5 hr after the ^{3}H-norepinephrine injection.

TABLE 4. ENDOGENOUS CATECHOLAMINE LEVELS IN VARIOUS BRAIN AREAS OF ANESTHETIZED AND UNANESTHETIZED CATS*

Treatment	N†	Brain stem norepinephrine	Hypothalamus norepinephrine	Caudate nucleus dopamine
Pentobarbital	6	0·42 ± 0·01	2·96 ± 0·19	11·7 ± 0·7
Urethane–bartiturate	6	0·41 ± 0·02	3·40 ± 0·37	11·2 ± 1·0
Urethane–barbiturate‡	3	0·33 ± 0·04	2·69 ± 0·33	10·0 ± 0·5
Unanesthetized	3	0·35 ± 0·03	2·00 ± 0·53	10·4 ± 1·7
Cord section	3	0·31 ± 0·02	3·03 ± 0·52	12·1 ± 1·8

* Cats were anesthetized as described in Table 3 for cannula placement. One or 5 hr after an injection of ^{3}H-norepinephrine the animals were sacrificed and various areas were analyzed for endogenous norepinephrine or dopamine. Values are expressed as μg/g (mean ± 1 standard error).

† N = Number of animals.

‡ Sacrificed 5 hr after ^{3}H-norepinephrine injection.

The endogenous norepinephrine content in brain stem and hypothalamus and the dopamine content of the caudate nucleus of the anesthetized and unanesthetized groups of animals is summarized in Table 4. Except for a low level of norepinephrine in the brain stem of spinal-sectioned animals there were no significant differences in the catecholamine contents among any of these groups.

The brain content of ^{3}H-norepinephrine and its metabolites at various times after the intraventricular injection of ^{3}H-norepinephrine

Total radioactivity fell progressively in all brain regions examined during the 24-hr period after the intraventricular injection of ^{3}H-norepinephrine in unanesthetized cats. Only data from the hypothalamus and caudate nucleus are shown in Table 5. Despite the marked reduction in total counts the percentage of total radioactivity represented by norepinephrine and its metabolites remained remarkably constant.

TABLE 5. ^{3}H-NOREPINEPHRINE AND ITS METABOLITES IN THE HYPOTHALAMUS AND CAUDATE NUCLEUS AT VARIOUS TIMES AFTER THE INTRAVENTRICULAR INJECTION OF ^{3}H-NOREPINEPHRINE*

Brain region		Hours after injection 1	6	24
Hypothalamus	Total radioactivity	1069 ± 352	380 ± 43	147 ± 23
	Norepinephrine	72 ± 1	70 ± 8	78 ± 9
	Normetanephrine	28 ± 5	21 ± 1	13 ± 4
	Deamin. catechol met.	1 ± 1	2 ± 2	1 ± 1
	Deamin. *O*-methyl met.	11 ± 2	8 ± 2	14 ± 2
Left caudate nucleus	Total radioactivity	3951 ± 1743	1442 ± 437	354 ± 66
	Norepinephrine	83 ± 6	64 ± 10	56 ± 8
	Normetanephrine	25 ± 6	19 ± 2	17 ± 4
	Deamin. catechol met.	1 ± 1	5 ± 2	2 ± 2
	Deamin. *O*-methyl met.	3 ± 1	6 ± 1	20 ± 2

* A cannula was placed in the left lateral ventricle under methoxyflurane anesthesia. Following recovery from the anesthesia 5 μc of ^{3}H-norepinephrine were injected intraventricularly and the animals sacrificed at 1, 6 or 24 hr after the injection. Total radioactivity is expressed as mμc/g and the norepinephrine and metabolites as percentages of total radioactivity (mean ± 1 standard error) as determined from three cats at each of the time periods

Effects of JB516 and reserpine pretreatment on the brain contents of ^{3}H-norepinephrine and its metabolites

Monoamine oxidase inhibitors and reserpine alter both the steady state levels of endogenous catecholamines and the patterns of norepinephrine metabolites in rat brain.[18] Figure 1 illustrates the effects of these same agents in cat brain. Although all regions listed in Table 1 were analyzed, only results obtained in hypothalamus and caudate nucleus are reported in Fig. 1.

Cats were pretreated with 10 mg/kg of the monoamine oxidase inhibitor JB516 (pheniprazine, Catron) for 12 hr. They were then given an intraventricular injection of 5 μc of ^{3}H-norepinephrine and sacrificed after 1 hr. Pretreatment with JB516 did not significantly alter the endogenous catecholamine levels, total radioactivity or the percentage of total radioactivity represented by ^{3}H-norepinephrine in any brain region. In the hypothalamus, brain stem, hippocampus and cerebellum, JB516 markedly increased the percentage of ^{3}H-normetanephrine and decreased the percentage of total ^{3}H-deaminated metabolites. Pretreatment with the drug, however, did not alter the percentage of labeled metabolites in the caudate nucleus.

Reserpine (0·5 mg/kg) was administered to another group of cats. Twenty-four hr later they received 5 μc of ^{3}H-norepinephrine intraventricularly and were sacrificed after 1 hr. Endogenous levels of catecholamines were markedly reduced in caudate

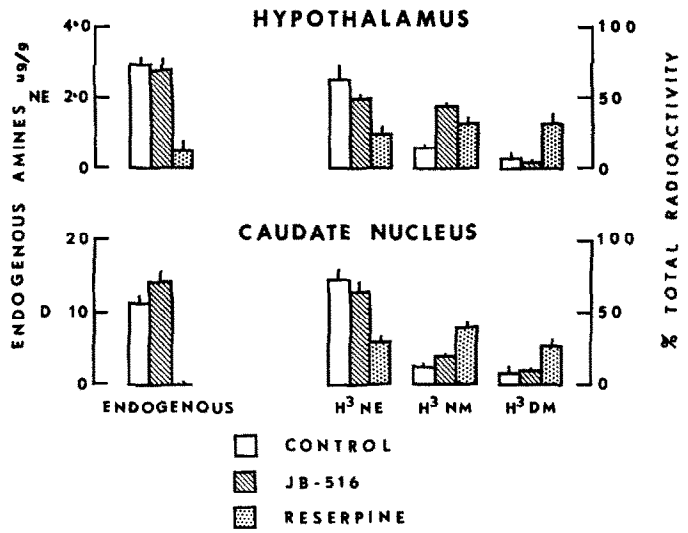

FIG. 1. Effects of JB516 and reserpine on endogenous catecholamines levels and metabolites of ^{3}H-norepinephrine in the cat brain after intraventricular injection of ^{3}H-norepinephrine. Cats were pretreated for 12 hr with JB516 (10 mg/kg i.p.) or 24 hr with reserpine (0·5 mg/kg i.p.). Each cat was then anesthetized with sodium pentobarbital for cannula implantation and then injected with 5 μc ^{3}H-norepinephrine intraventricularly and sacrificed 1 hr later. The bars on the left represent the mean concentration (vertical lines above the bars represent one standard error) of endogenous norepinephrine in the hypothalamus and dopamine in the caudate nucleus of six control, six JB516 and six reserpine pretreated cats. The bars on the right represent the mean percentage of total radioactivity of norepinephrine, normetanephrine and total deaminated metabolites.

nucleus, hypothalamus, brain stem and hippocampus. Total radioactivity in all brain areas was reduced to approximately 30–50 per cent of control values. Reserpine also reduced the percentage of total radioactivity represented by ^{3}H-norepinephrine from 60–70 down to 30–40 per cent in the hypothalamus and caudate nucleus, from 46 down to 7 per cent in the brain stem and from 23 down to 6–7 per cent in hippocampus and cerebellum. Pretreatment with this drug also caused a 2 to 3-fold increase in the percentage of ^{3}H-normetanephrine in the brain stem, hypothalamus and caudate nucleus and a similar increase in total ^{3}H-deaminated metabolites in all brain areas.

DISCUSSION

Development of the intraventricular injection technique for labeling brain catecholamine stores[9] has greatly facilitated the study of these proposed neurotransmitter substances. Using this technique Glowinski *et al.*[11, 18, 20] have described the regional distribution of the injected amine, the pattern of metabolites formed and the effects of centrally acting drugs on these patterns. They also showed that when administered by this route, radioactive catecholamines mix with the endogenous amine stores.[11] It would seem logical then to compare the results of the present report with the pioneering studies of Glowinski *et al.*[11, 18–20]

The most obvious differences between the studies are the species of animal used and the volumes of ^{3}H-norepinephrine injected. Glowinski *et al.* injected 20–30 μl

into the lateral ventricle of the rat brain. This is two to three times the absolute volume used in the present study. The difference in volumes injected into the cat and rat ventricular systems would become more evident if it was expressed as a percentage of the ventricular volumes of the two species. That is, the ventricular volume in rat brain is only a small fraction of that in cat brain. Differences in relative injection volumes could account for some of the more obvious differences between the studies. For example, Glowinski *et al.* found that 60 per cent of injected radioactivity disappeared from the brain into the circulation within 6 min after injection.[20] In the present study relatively small amounts of radioactivity (represented by noncatechol metabolites) were detected in venous blood draining the brain during the 1-hr period after the intraventricular injection. Thus, a large proportion of the administered ^{3}H-norepinephrine must have remained within the ventricular system, at least during the 1-hr period after its injection. This might have been predicted from the volume–distribution studies of McCarthy and Borison.[13] When 10 μl of radiopaque media (the volume used in the present study) was injected into the anterior horn of the lateral ventricle it was immediately distributed to the olfactory recess, the intraventricular foramen and the anterior and ventral portion of the third ventricle. Injection of a similar volume into the hypothalamic cleft of the third ventricle was distributed to the hypothalamic cleft and the infundibular recess. These investigators showed that injection volumes of 100 μl administered into the anterior horn of the lateral ventricle were immediately distributed to at least as far as the lateral aperatures of the fourth ventricle. We have found that when this same volume of dye is administered into the lateral ventricle it rapidly stains the dorsal and ventral surface of the brain indicating a rapid transfer of dye into the dorsal and ventral subarachnoid spaces. After 100 μl of ^{14}C-norepinephrine were injected into a lateral ventricle of cat brain, radioactivity rapidly appeared in the jugular vein, the consequence of the injected volume overflowing from the cerebroventricular system.[21]

The distribution of the injected volume, subsequent to the immediate distribution, depends upon the dynamics of cerebrospinal fluid formation and flow. It would appear that relatively small amounts of ^{3}H-norepinephrine reached the subarachnoid spaces since, unlike in the rat,[19] little radioactivity was detected in the cerebral cortex. A small portion of the radioactivity in various areas of rat brain might represent ^{3}H-norepinephrine or metabolites in blood within the cerebral vasculature. In the present study this possible source of contamination was avoided by perfusing the cerebral blood vessels with saline prior to dissecting the brain.

In addition to the problem of large amounts of radioactivity escaping into the blood, large injection volumes may abruptly enlarge ventricular spaces and thereby deform the cerebroventricular system and adjacent brain tissue. For example, Noble *et al.*[22] reported that when 30 μl of ^{3}H-norepinephrine was injected into the lateral ventricle of the rat it was uniformly distributed throughout the brain. These workers also reported that the injected ventricle appeared to be dilated. This was certainly not the case in the present study. After injection into the left lateral ventricle, most radioactivity was found in areas lining the left lateral and third ventricles; only small amounts of label appeared in structures lining the right or contralateral ventricle. That is, the injected volume moved with, not against, the normal flow of cerebrospinal fluid. Similarly, when injected into the third ventricle, little radioactivity was found in structures lining the lateral ventricles. Rather, the radioactivity was distributed

caudal to the injection site. These patterns of distribution were similar to those reported by McCarthy and Borison using radiographic techniques.[13] Thus, the injection volume used in the present study did not appear to cause abnormal volume displacement.

Intraventricularly administered ^{3}H-norepinephrine accumulated primarily in those regions lining the ventricular system of the cat brain which contain high endogenous levels of catecholamines. As in rat brain,[19] the hypothalamus and caudate nucleus (or striatum), which contain the highest concentrations of endogenous norepinephrine and dopamine, respectively, also contained the highest concentrations of ^{3}H-norepinephrine. Lower concentrations of radioactivity were observed in the brain stem, hippocampus and cerebellum. This pattern of distribution is consistent with studies *in vitro* on the regional uptake of ^{3}H-norepinephrine.[23] Even though the caudate nucleus contains low levels of endogenous norepinephrine it must possess an uptake mechanism for this amine. The high concentration of radioactivity in the caudate nucleus is not the result of this region's proximity to the lateral ventricle injection site but rather is due to a concentrating mechanism since the ventricular wall immediately adjacent to the caudate nucleus retained little ^{3}H-norepinephrine. The brain stem, which contains relatively high amounts of endogenous norepinephrine, retained considerably less ^{3}H-norepinephrine than the caudate nucleus and hypothalamus. This could be the result of at least two factors. First, the mass of brain stem is not in immediate contact with the cerebrospinal fluid and it has been demonstrated that catecholamines introduced into the cerebroventricular system distribute to only a narrow zone of tissue (approximately 300 μ) lining this system.[24] Secondly, the brain stem is some distance from the site of injection and the immediate distribution of ^{3}H-norepinephrine. In this regard, as would be expected, more radioactivity was found in the brain stem after third than after lateral ventricular injections.

Although the percentages of norepinephrine and its metabolites were not altered by the anesthetics, levels of total radioactivity in brain stem and hypothalamus were lowered in unanesthetized and spinal-sectioned animals. These differences may have resulted from any one of a number of factors (e.g. reduced resistance in the ventricular system of spinal-sectioned animals, increased turnover of norepinephrine in catecholamine containing neurons[25] in unanesthetized animals). However, the significance of the apparent differences in total radioactivity should be tempered by the fact that they comprise only a small number of determinations.

^{3}H-normetanephrine was the major metabolite of injected ^{3}H-norepinephrine in all brain areas of the cat brain. This confirms an earlier report describing the metabolism of intraventricularly administered ^{14}C-norepinephrine.[21] This finding points out an important species difference. In rat brain deaminated products constituted the major metabolites of intraventricularly administered ^{3}H-norepinephrine while normetanephrine usually accounted for less than 10 per cent of total radioactivity.

Neither the site of injection nor the anesthetics affected the pattern or distribution of ^{3}H-norepinephrine metabolites. Even though there were marked differences in total radioactivity, depending on whether the injection was made in the lateral or third ventricle, the percentages of ^{3}H-norepinephrine, ^{3}H-normetanephrine and ^{3}H-deaminated metabolites remained essentially the same. In addition, during the 24-hr period following intraventricular injection, the percentages of total radioactivity represented by ^{3}H-norepinephrine and its metabolites did not change. Similar results

were reported by Glowinski *et al.* in the rat brain.[20] However, in a later study[19] these same investigators reported that the percentage of norepinephrine increased while that of the metabolites decreased with time.

The effects of monoamine oxidase inhibitors on catecholamine metabolism in the cat are difficult to interpret. Tranylcypromine and iproniazid have been reported to increase the concentration of dopamine in the caudate nucleus.[26, 27] Iproniazid had little effect on the metabolism of intraventricularly administered ^{14}C-norepinephrine[21] and lowered brain levels of norepinephrine.[28] Pheniprazine, the monoamine oxidase inhibitor used in the present study, is reported to have no effect on the level of endogenous norepinephrine in cat hypothalamus[29] or brain stem,[30] whereas it markedly increases the rat brain content of both norepinephrine and dopamine.[31] In the present study pheniprazine did not alter brain levels of endogenous norepinephrine or dopamine. In the rat this drug markedly increased the brain levels of ^{3}H-norepinephrine and ^{3}H-normetanephrine and decreased ^{3}H-deaminated products;[32] similar changes were not obvious in cat brain. These results confirm the previous reports that indicate monoamine oxidase plays a minor role in the metabolism of norepinephrine in cat brain.[21, 29]

The results of the present study indicate that reserpine reduces the ability of several brain regions to retain endogenous and ^{3}H-norepinephrine; the same is true in rat brain.[32] However, there are some differences between the percentages of norepinephrine in the cat and rat brains. In the rat, reserpine decreased the percentage of radioactivity represented by normetanephrine but increased that represented by deaminated metabolites. In the cat, reserpine increased the percentages of both normetanephrine and deaminated metabolites.

Brain levels of ^{3}H-norepinephrine and its metabolites reported in the present study represent values at fixed points in time. Such data offer no information on the rates or relative importance of various pathways of norepinephrine metabolism. For example, the low levels of deaminated catechols are offered as evidence for the minor role of deamination in cat brain. However, the lack of deaminated products may merely reflect the rapid loss of these metabolites into the circulation. Although the lack of effect of monoamine oxidase inhibitors on endogenous and exogenously administered ^{3}H-norepinephrine support the proposal that little deamination is occuring, time studies on the dynamics of the disposition of ^{3}H-norepinephrine are needed. Studies involving the continuous monitoring of ^{3}H-norepinephrine and its metabolites in cerebroventricular perfusions are currently in progress and should help to clarify this question. In addition, the results of these studies on the metabolism of exogenously administered ^{3}H-norepinephrine may not be representative of the metabolism of endogenous catecholamines. To test this possibility studies on the metabolism of norepinephrine in cat brain, formed endogenously from ^{3}H-dopamine or ^{14}C-tyrosine, have been initiated. It is encouraging that in rat brain exogenously administered and endogenously formed norepinephrine are altered in a similar manner by stressful procedures.[25]

Acknowledgement—The authors would like to thank Miss Donna Moltzau for her technical assistance.

REFERENCES

1. M. Vogt, *J. Physiol., Lond.* **123**, 451 (1954).
2. A. Carlsson, *Pharmac. Rev.* **11**, 490 (1959).

3. A. DAHLSTROM and K. FUXE, *Acta physiol. scand.* **62**, suppl., 232 (1964).
4. S. UDENFRIEND and P. ZALTZMAN-NIRENBERG, *Science, N.Y.* **142**, 394 (1963).
5. J. GLOWINSKI and R. J. BALDESSARINI, *Pharmac. Rev.* **18**, 1201 (1966).
6. N. E. ANDEN, A. CARLSSON, N-A. HILLARP and T. MAGNUSSON, *Life Sci.* **4**, 129 (1965).
7. R. J. BALDESSARINI and I. J. KOPIN, *Science, N.Y.* **152**, 1630 (1966).
8. H. MCLENNAN, *J. Physiol., Lond.* **174**, 152 (1965)
9. G. MILHAUD and J. GLOWINSKI, *C. r. Lebd. Seanc. Acad. Sci., Paris* **255**, 203 (1962).
10. G. MILHAUD and J. GLOWINSKI, *C. r. Lebd. Seanc. Acad. Sci., Paris* **256**, 1033 (1963).
11. J. GLOWINSKI, S. H. SNYDER and J. AXELROD, *J. Pharmac. exp. Ther.* **152**, 282 (1966).
12. S. M. SHANBERG, J. J. SCHILDKRAUT and I. J. KOPIN, *J. Pharmac. exp. Ther.* **157**, 311 (1967).
13. L. E. MCCARTHY and H. L. BORISON, *Anat. Rec.* **155**, 305 (1966).
14. L. E. MCCARTHY and H. L. BORISON, *Expl. Neurol.* **17**, 57 (1967).
15. E. A. CARMICHAEL, W. FELDBURG and K. FLEISCHAUER, *J. Physiol., Lond.* **177**, 354 (1964).
16. K. E. MOORE and R. H. RECH, *J. Pharmac. exp. Ther.* **156**, 70 (1967).
17. K. E. MOORE, L. E. MCCARTHY and H. L. BORISON, *J. Pharmac. exp. Ther.* **148**, 169 (1965).
18. J. GLOWINSKI, J. AXELROD and L. L. IVERSON, *J. Pharmac. exp. Ther.* **153**, 30 (1966).
19. J. GLOWINSKI and L. IVERSON, *J. Neurochem.* **13**, 655 (1966).
20. J. GLOWINSKI, I. J. KOPIN and J. AXELROD, *J. Neurochem.* **12**, 25 (1965).
21. E. MANNARINO, N. KIRSHNER and B. S. NOSHOLD, *J. Neurochem.* **10**, 373 (1963).
22. E. P. NOBLE, R. J. WURTMAN and J. AXELROD, *Life Sci.* **6**, 281 (1967).
23. S. H. SNYDER, A. GREEN, E. D. HENDLEY and E. GFELLER, *Nature, Lond.* **218**, 174 (1968).
24. K. FUXE and U. UNGERSTEDT, *Histochemie* **13**, 16 (1968).
25. A. THIERRY, F. JAVOY, J. GLOWINSKI and S. S. KETY, *J. Pharmac. exp. Ther.* **163**, 163 (1968).
26. P. SINGH, L. J. POIRIER and R. BOUCHER, *Can. J. Physiol. Pharmac.* **45**, 897 (1963).
27. P. L. MCGEER, E. G. MCGEER and J. A. WADA, *Archs Neurol., Chicago* **9**, 81 (1963).
28. M. VOGT, *Physiol. Rev.* **11**, 483 (1959).
29. S. SANAN and M. VOGT, *Br. J. Pharmac. Chemother.* **18**, 109 (1962).
30. S. SPECTOR, P. A. SHORE and B. B. BRODIE, *J. Pharmac. exp. Ther.* **128**, 15 (1960).
31. J. R. CROUT, C. R. CREVELING and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **132**, 269 (1961).
32. J. GLOWINSKI and J. AXELROD, *J. Pharmac. exp. Ther.* **149**, 43 (1965).