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REGIONAL STUDIES OF CATECHOLAMINES IN THE RAT BRAIN—III:

SUBCELLULLAR DISTRIBUTION OF ENDOGENOUS AND EXOGENOUS CATECHOLAMINES IN VARIOUS BRAIN REGIONS

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Abstract—The subcellular distribution of ^{3}H-dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine in various regions of the rat brain was examined after the injection of labeled norepinephrine or its precursors into the lateral ventricle. After the injection of ^{3}H-norepinephrine there were differences in the distribution of the labeled catecholamine between particulate and supernatant fractions among the various brain regions. These differences in the subcellular distribution of ^{3}H-norepinephrine correlated with similar differences in the subcellular distribution of endogenous norepinephrine among the regions—the cerebellum having the lowest particulate/supernatant (P/S) ratio for both endogenous and exogenous norepinephrine and the hypothalamus having the highest P/S ratio. In the cerebellum, cortex, hypothalamus, and medulla oblongata, the P/S ratio for ^{3}H-norepinephrine 4 hr after injection was lower than the P/S ratio for endogenous norepinephrine. The P/S ratio for ^{3}H-norepinephrine increased significantly between 1 and 4 hr after injection in the medulla oblongata but was unchanged in the cerebellum and hypothalamus. Similar regional differences in the subcellular distribution of labeled dopamine and norepinephrine were observed after the injection of ^{3}H-dopamine.

In the striatum, endogenous dopamine was predominantly recovered in the supernatant fraction, but exogenous ^{3}H-dopamine or ^{3}H-norepinephrine was found mainly in the particulate fraction. After the injection of ^{3}H-norepinephrine, the labeled amine was localized in a synaptosomal layer, after density gradient centrifugation, in both striatum and hypothalamus. After the injection of ^{3}H-dopa, the labeled amino acid and synthesized dopamine and norepinephrine were also recovered in synaptosomal fractions after density gradient centrifugation of striatum or hypothalamus homogenates.

NOREPINEPHRINE in homogenates of peripheral sympathetically innervated tissues is localized in particles of microsomal dimensions.[1–3] These particles probably correspond to synaptic vesicles liberated from sympathetic nerve terminals by homogenization. Norepinephrine is present in high concentrations in the terminals of specific catecholamine-containing neurons in the brain.[4, 5] In this tissue, however, most of the nerve terminals are not disrupted on homogenization but pinch off to form discrete particles.[6] A considerable proportion of the endogenous norepinephrine in brain homogenates is consequently recovered in a fraction of pinched-off nerve endings or synaptosomes after density gradient centrifugation.[7–10]

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Previous studies have indicated that radioactive norepinephrine injected into the lateral ventricle of the rat brain is taken up into catecholamine-containing neurons.[11, 12] In keeping with this view, ^{3}H-norepinephrine was found to be specifically localized in a synaptosomal layer after density gradient centrifugation of brain homogenates, indicating a parallel subcellular distribution for the labeled and endogenous catecholamine.[10, 13]

Recently we have shown[14, 15] that there are regional differences in the accumulation, metabolism, and turnover of ^{3}H-norepinephrine in various areas of the rat brain. In the present study the subcellular distribution of ^{3}H-norepinephrine and endogenous norepinephrine in various regions of the rat brain will be described. Preliminary results revealed differences in the subcellular distribution of ^{3}H-norepinephrine in different brain areas.[10] The subcellular distribution of labeled norepinephrine and its precursors has also been examined in different brain regions after the injection of tritiated dopamine or dopa.

METHODS

Radioactive materials. DL-7-^{3}H-Norepinephrine hydrochloride (specific activity 5 c/m-mole), ^{3}H-dopamine hydrochloride (specific activity 10 c/m-mole generally labeled), DL-^{3}H-dihydroxyphenylalanine (dopa) acetate (specific activity 10 c/m-mole generally labeled), and DL-7-^{14}C-norepinephrine hydrochloride (29 mc/m-mole), were obtained from New England Nuclear Corp., Boston, Mass. Tritiated dopa was purified by adsorption on alumina and elution with 0·2 N hydrochloric acid prior to use.

Subcellular distribution studies. Sprague-Dawley male rats (280–320 g) were lightly anesthetized with pentobarbital (Nembutal) (35 mg/kg), and the radioactive substances were injected into the lateral ventricle of the brain by a stereotaxic technique.[11] The radioactive substances were diluted in Merle's solution and a volume of 20 to 30 μl. was injected. Rats were stunned and killed by decapitation at various times after the intraventricular injection of the radioactive substances. The brains were carefully removed, blotted, chilled, and dissected into seven regions.[14] The seven areas, described in detail elsewhere,* were: (1) cerebellum, (2) medulla oblongata (medulla oblongata and pons), (3) hypothalamus, (4) midbrain (midbrain, thalamus, and subthalamus), (5) striatum (caudate, putamen, and globus pallidus nuclei), (6) hippocampus and (7) cortex (telencephalon without the striatum, includes white and gray matter of the cerebral cortex).

In most experiments, tissue samples were homogenized for 45 sec in 5 vol. of ice-cold 0·25 M sucrose in a small glass homogenizer with a loosely fitting Teflon pestle. Homogenates were centrifuged for 1 hr at 70,000 *g* to obtain total particulate and soluble supernatant fractions. Radioactive amines and metabolites were extracted from particulate fractions with chilled 0·4 N perchloric acid, and the supernatant fraction was treated with 0·1 vol. of 4 N perchloric acid. After the injection of ^{3}H-norepinephrine, the labeled catecholamine was estimated by an alumina adsorption technique.[16] When ^{3}H-dopamine was injected, ^{3}H-dopamine and ^{3}H-norepinephrine were separated by ion-exchange chromatography as previously described[17]; the data were corrected for recoveries. Endogenous norepinephrine and endogenous dopamine were isolated by alumina adsorption[18] and assayed fluorometrically.[19, 20]

* The dissection procedure is described in detail in: J. Glowinski and L. L. Iversen, Regional studies of catecholamines in the rat brain—I. To be published in *J. Neurochem.*

In some experiments subcellular fractionation was performed with a continuous sucrose gradient similar to that of Potter and Axelrod,[1] by the technique previously described for similar experiments.[10]

In an experiment in which DL-^{3}H-dopa was injected, brain samples were homogenized for 45 sec in 10 vol. of cold sucrose (0·3 M) and submitted to differential centrifugation; four fractions were obtained. P_1 = unbroken cells (800 *g*, 10 min); P_2 = mitochondria and synaptosomes (12,000 *g*, 10 min); P_3 = microsomes (85,000 *g*, 60 min). The P_2 fraction was resuspended in 0·5 ml of 0·3 M sucrose and carefully layered onto a discontinuous gradient similar to that described by de Robertis *et al.*[21] and centrifuged at 50,000 *g* for 2 hr. Calcium chloride (10^{-5} M) was added to all sucrose solutions used in the preparation of the discontinuous gradients[22] (C. Klee, personal communication). ^{3}H-dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine were assayed in all the fractions obtained by an ion-exchange chromatographic method previously described.[17]

RESULTS

1. *Subcellular distribution of endogenous and exogenous norepinephrine in various brain regions after administration of* ^{3}H-*norepinephrine.* Groups of rats received intraventricular injections of DL-^{3}H-norepinephrine (0·21 μg) and were killed 1 or 4 hr later. Brains were removed and dissected into seven regions; tissue samples were then homogenized in isotonic sucrose, and the homogenates were centrifuged for 1 hr at 70,000 *g*. ^{3}H-Norepinephrine was estimated in the resulting particulate and supernatant fractions. In another experiment endogenous norepinephrine was estimated in the particulate and supernatant fractions of four brain regions.

Four hours after the injection of ^{3}H-norepinephrine, there were marked differences in the subcellular distribution of the radioactive catecholamine among the different brain regions (Fig. 1). Significant differences were found when the particulate to supernatant (P/S) ratio for ^{3}H-norepinephrine in the cerebellum or cortex was compared with that in the hypothalamus or medulla oblongata. Similar differences in the P/S ratio for endogenous norepinephrine were found between the different brain regions (Table 1). In the cerebellum, cortex, medulla oblongata, and hypothalamus the P/S ratio for endogenous norepinephrine was greater than that for ^{3}H-norepinephrine at 4 hr after injection, indicating that at this time in these regions the specific activity of ^{3}H-norepinephrine was higher in the supernatant fraction than in the particulate.

In the striatum, a region containing high concentrations of dopamine, endogenous dopamine was predominantly recovered in the supernatant fraction. In contrast, radioactive norepinephrine in this structure was found mainly in the particulate fraction.

The subcellular distribution of ^{3}H-norepinephrine was also examined in the cerebellum, medulla oblongata, and hypothalamus 1 hr after the intraventricular injection of ^{3}H-norepinephrine. In the medulla oblongata the P/S ratio for ^{3}H-norepinephrine was significantly lower at this time than that found 4 hr after injection. Almost no changes in the P/S ratio for ^{3}H-norepinephrine could be detected between 1 and 4 hr after injection in the hypothalamus, and a slight decrease in the P/S ratio was found during this period in the cerebellum (Table 2).

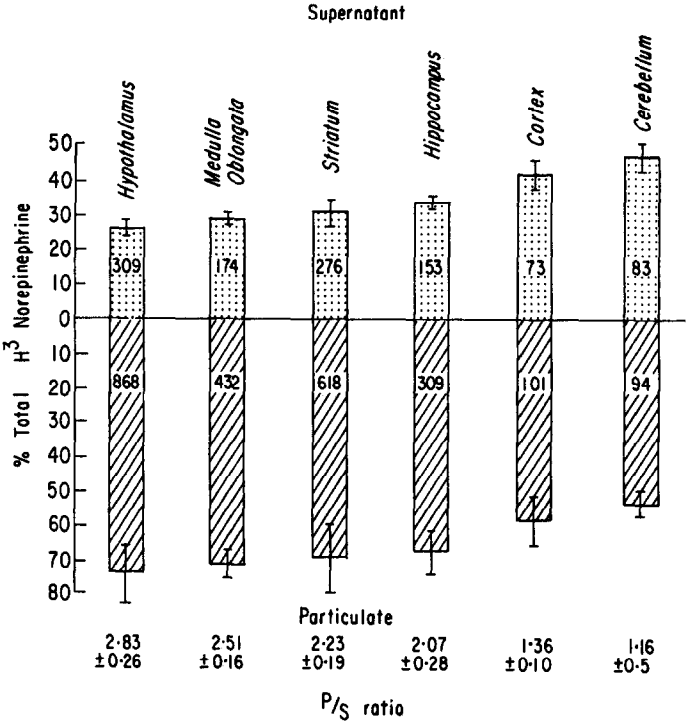


FIG. 1. Distribution of ^{3}H-norepinephrine between particulate and supernatant fractions in different brain regions 4 hr after the intraventricular injection of DL-^{3}H-norepinephrine (6·3 μc). Values are expressed as percentages of total ^{3}H-norepinephrine recovered; absolute values in mμc are indicated by figures inside vertical bars. Each value is the mean for 5 to 6 determinations; vertical lines indicate standard errors of the mean. Particulate/supernatant (P/S) ratios for ^{3}H-norepinephrine are indicated by figures under each region, P/S ratios were estimated individually for each determination, and values are means ± S.E.M.

TABLE 1. SUBCELLULAR DISTRIBUTION OF ENDOGENOUS CATECHOLAMINES IN RAT BRAIN

Region	Endogenous catecholamine content (mμg/g)		P/S ratio endogenous catecholamine	P/S ratio ^{3}H-NE at 4 hr
	Particulate	Supernatant		
Cerebellum	86 ± 4	57 ± 3	1·54 ± 0·15	1·16
Cortex	207 ± 15	82 ± 5	2·52 ± 0·14	1·36
Medulla oblongata	433 ± 37	131 ± 15	3·35 ± 0 10	2 51
Hypothalamus	1430 ± 117	344 ± 41	4 27 ± 0·30	2·83
Striatum (dopamine)	3080 ± 200	2730 ± 300	0·83 ± 0·03	2·23

Subcellular distribution of endogenous norepinephrine (NE) was measured in all areas except striatum where, dopamine was measured. Pooled brain structures from two animals were homogenized in isotonic sucrose and separated into supernatant and particulate fractions by ultracentrifugation. Each value is the mean for five such determinations, ± standard error of the mean. Pellet/supernatant (P/S) ratios were calculated for individual determinations and results are mean values for the five determinations ± S.E.M. P/S ratios for ^{3}H-norepinephrine 4 hr after injection (Fig. 1) are included for comparison.

2. *Distribution of* ^{3}H-*catecholamine between particulate and supernatant fractions of different parts of the brain after* ^{3}H-*dopamine administration.* Groups of rats received ^{3}H-dopamine (0·24 μg) into the lateral ventricle and were killed 2 hr later. The cerebellum, medulla oblongata, and striatum were dissected and homogenized. ^{3}H-Norepinephrine and ^{3}H-dopamine were estimated in the particulate and supernatant

TABLE 2. SUBCELLULAR DISTRIBUTION OF ^{3}H-NOREPINEPHRINE IN RAT BRAIN REGIONS AT VARIOUS TIMES AFTER INTRAVENTRICULAR INJECTION

Region	Time (hr)	^{3}H-Norepinephrine content (mμc/g)		P/S ratio ^{3}H-NE	PS/ratio, endogenous NE
		Particulate	Supernatant		
Cerebellum	1	338 ± 35	245 + 31	1·40 ± 0·05	1·54
	4	94 ± 6	83 + 7	1·16 ± 0·05	
Medulla oblongata	1	783 ± 49	431 + 15	1·83 ± 0·08	3·35
	4	432 ± 25	174 + 17	2·51 ± 0·16*	
Hypothalamus	1	1513 ± 240	578 + 100	2·64 ± 0·11	4·27
	4	868 ± 92	309 + 29	2·83 ± 0·26	

^{3}H-Norepinephrine (NE) was determined in particulate and supernatant fractions of brain homogenates at various times after the intraventricular injection of ^{3}H-norepinephrine (6·3 μc). P/S ratios for ^{3}H-norepinephrine were determined in individual samples, and value is mean ± S.E.M. Values for P/S ratio and for ^{3}H-norepinephrine are means for groups of 5–6 animals. P/S ratios for endogenous NE are included for comparison (Table 1).

* $P < 0·01$ when compared with 1-hr value.

TABLE 3. SUBCELLULAR DISTRIBUTION OF ^{3}H-NOREPINEPHRINE AND ^{3}H-DOPAMINE IN RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF ^{3}H-DOPAMINE

Region	^{3}H-Norepinephrine		^{3}H-Dopamine	
	Total content (mμc/g)	P/S ratio	Total content (mμc/g)	P/S ratio
Cerebellum	129 ± 4	1·50 ± 0 14	62 ± 15	2 40 ± 0·21
Medulla oblongata	220 ± 22	2·26 ± 0 20	125 ± 18	4·36 ± 0·61
Striatum	51 ± 15	1·13 ± 0 16	468 ± 108	1·93 ± 0·30

^{3}H-Norepinephrine and ^{3}H-dopamine were determined in particulate and supernatant fractions of three areas of rat brain 2 hr after the intraventricular injection of ^{3}H-dopamine (15·5 μc). Each value is the mean for 5–6 determinations; P/S ratios were determined on individual samples, and results are mean values ± S.E.M.

fractions obtained after centrifugation of the homogenates for 1 hr at 70,000 *g*. Differences in the P/S ratio for ^{3}H-dopamine and ^{3}H-norepinephrine were found among the three different structures. The medulla oblongata had a higher P/S ratio for both amines than the cerebellum or striatum. In all three structures the P/S ratio for ^{3}H-dopamine was greater than that for ^{3}H-norepinephrine. ^{3}H-Norepinephrine was almost equally distributed between particulate and supernatant fraction in the striatum, but ^{3}H-dopamine was mainly in the particulate fraction in this structure (Table 3).

3. *Subcellular distribution of labeled dopa and catecholamines in the hypothalamus and striatum after the intraventricular injection of* ^{3}H-*dopa.* Rats received an intraventricular injection of DL-^{3}H-dopa (1·55 μg) and were killed 3 hr later. Brain samples from two animals were pooled and homogenized in isotonic sucrose. Homogenates of hypothalamus and striatum were submitted to differential centrifugation, and ^{3}H-dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine were assayed in each of the four fractions obtained (Table 4). In the hypothalamus, ^{3}H-norepinephrine was almost equally distributed between crude pellet (P_1), synaptosomal–mitochondrial fraction (P_2), microsomal fraction $P(_3)$, and soluble supernatant (S). There was relatively more ^{3}H-dopamine and ^{3}H-dopa in the crude pellet and supernatant fractions than ^{3}H-norepinephrine. However, there was only half as much ^{3}H-dopamine as ^{3}H-norepinephrine in the microsomal fraction, and almost no ^{3}H-dopa was found in this fraction. ^{3}H-dopa and ^{3}H-dopamine were similarly distributed in the striatum; ^{3}H-norepinephrine levels in the various fractions from this region were too low to be accurately determined.

TABLE 4. SUBCELLULAR DISTRIBUTION OF ^{3}H-DOPA AND ^{3}H-CATECHOLAMINES IN HYPOTHALAMUS AND STRIATUM AFTER INTRAVENTRICULAR INJECTION OF ^{3}H-DOPA

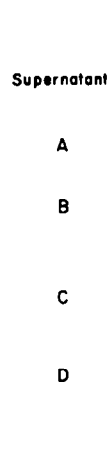
	Hypothalamus			Striatum	
Fraction	Dopa	Dopamine	Norepinephrine	Dopa	Dopamine
P_1	42 1	36·4	28·4	24·7	34·0
P_2	18·6	23·0	26·9	30·0	30·2
P_3	2·3	12·6	22·4	7·4	9·2
S	36·8	27·9	22·1	37·7	26·5

^{3}H-Dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine were determined in various fractions obtained after differential centrifugation of tissue homogenates as described in detail in text. P_1 = coarse pellet; P_2 = synaptosome/mitochondrial pellet; P_3 = microsomal pellet; S = supernatant fraction. DL-^{3}H-dopa (78 μc) was injected intraventricularly 3 hr earlier. Values are expressed as percentages of total amounts of each substance recovered in all fractions. P_2 fractions were further resolved by centrifugation on discontinuous sucrose gradients (Fig. 2). Levels of ^{3}H-norepinephrine in striatum were too low to allow accurate measurement.

P_2 fractions (synaptosomes and mitochondria) of the hypothalamus and striatum were resuspended and layered onto discontinuous sucrose density gradients and centrifuged for 2 hr at 50,000 *g*. The distribution of ^{3}H-dopa and ^{3}H-catecholamines in the various fractions obtained from these gradients is illustrated in Fig. 2 (hypothalamus). ^{3}H-dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine were highly localized in the C band and to some extent in the D band, which are reported to be mainly synaptosomal fractions containing the highest concentrations of endogenous norepinephrine.[21] There was again relatively less ^{3}H-norepinephrine than ^{3}H-dopa or ^{3}H-dopamine in the supernatant layer. The distribution of ^{3}H-dopa and ^{3}H-dopamine after a similar fractionation of the P_2 fraction obtained from the striatum was very similar to that in the hypothalamus, the highest concentrations of both substances being found in the C band.

4. *Subcellular localization of* ^{3}H-*norepinephrine in hypothalamus and striatum.* ^{3}H-norepinephrine (0·67 μg) was injected intraventricularly and the rats were killed 4 hr later. Aliquots of hypothalamus and striatum homogenates were layered onto

continuous sucrose density gradients and centrifuged; ^{3}H-norepinephrine was assayed in alternate five-drop fractions collected by puncturing the bottom of the centrifuge tubes. The labeled catecholamine was localized in a synaptosomal layer in both the hypothalamus and striatum (Fig. 3). A second peak of ^{3}H-norepinephrine was present in the microsomal layer immediately below the clear supernatant which contained the



Fraction No.	% Total		
	Dopa	Dopamine	NE
1	13·4	18·3	9·8
2	3·4	7·6	7·5
3	–	3·8	5·8
4	4·4	9·9	12·8
5	4·4	13·0	15·3
6	67·0	32·6	34·6
7	–	3·1	3·9
8	7·4	11·7	10·3
9	–	–	–

FIG. 2. Distribution of ^{3}H-dopa, ^{3}H-dopamine, and ^{3}H-norepinephrine on discontinuous sucrose density gradient after centrifugation of an aliquot of a synaptosomal/mitochondrial fraction (P_2) from rat hypothalamus. Animals received an intraventricular injection of DL-^{3}H-dopa (78 μc) 3 hr earlier. Appearance of the gradient is indicated on the left; bands are lettered according to de Robertis;[21] fractions were collected by aspiration and analyzed for labeled dopa and catecholamines. Values are from a single experiment and are expressed as percentages of the total amount of each substance recovered in all gradient fractions.

remainder of the ^{3}H-norepinephrine. In similar experiments, the subcellular distribution of labeled dopamine and norepinephrine in striatum homogenates was examined 2 hr after the intraventricular injection of a combined dose of ^{3}H-dopamine (0·29 μg) and ^{14}C-norepinephrine (12·8 μg). The distribution of the two amines in this gradient was almost identical, except that relatively more dopamine than norepinephrine was present in the supernatant layer.

DISCUSSION

The distribution of endogenous and radioactive norepinephrine between total particulate and soluble supernatant fractions in tissue homogenates can give only an overall picture of the intracellular distribution of these substances. Nevertheless, there were marked differences in this distribution among the different brain regions.

Endogenous norepinephrine was almost equally distributed between particulate and supernatant fractions in the cerebellum, whereas about 80 per cent of the endogenous norepinephrine in the hypothalamus or medulla oblongata was recovered in a particulate form. The latter results are in good agreement with those of other workers, who found approximately 70 per cent of the endogenous norepinephrine in rabbit hypothalamus, rat brain stem, or dog hypthalamus in a particulate fraction.[8, 9, 23]

Norepinephrine recovered in the particulate fraction will include amine present in fragments of incompletely homogenized tissue, in synaptosomes, and in submicroscopic vesicles liberated from disrupted nerve terminals. The catecholamine recovered in the soluble supernatant fraction may arise from material liberated from the perikarya and preterminal axons of disrupted neurons and may also include amine liberated from disrupted synaptosomes. Four hours after the injection of radioactive norepinephrine into the lateral ventricle of the brain, the labeled catecholamine had a subcellular distribution similar to that of the endogenous norepinephrine in the various regions. In the regions examined, however, there was relatively more exogenous than endogenous norepinephrine in the supernatant fraction.

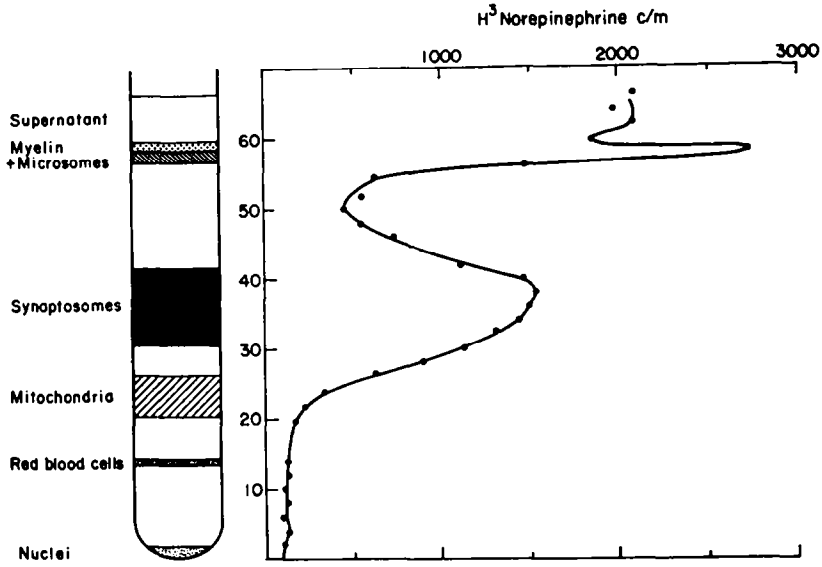


FIG. 3. Distribution of ^{3}H-norepinephrine on a sucrose density gradient after centrigufation of a sample of striatum homogenate obtained 4 hr after the intraventricular injection of DL-^{3}H-norepinephrine (20 μc). Appearance of the gradient is illustrated diagrammatically on the left. Five-drop samples were collected after puncturing the bottom of the tube; ^{3}H-norepinephrine was assayed in alternate samples.

This result differs from that obtained in previous studies of the subcellular distribution of ^{3}H-norepinephrine in the whole brain.[10] Four hours after the injection of ^{3}H-norepinephrine, the specific activity of norepinephrine was higher in the particulate fraction of whole brain homogenates than in the supernatant. This discrepancy between the results obtained in the whole brain and in the cerebellum, cortex, medulla oblongata, and hypothalamus may possibly be related to differences in the distribution of ^{3}H-norepinephrine and endogenous norepinephrine among various brain regions.[14] The P/S ratio for ^{3}H-norepinephrine was relatively high in the hippocampus and striatum, regions which contain only low concentrations of endogenous norepinephrine but which accumulate relatively large amounts of ^{3}H-norepinephrine (Fig. 1). The contribution of these two regions to the whole-brain homogenates may thus increase the specific activity of the particulate fraction of whole-brain homogenates.

In the previous study[10] it was also found that the P/S ratio for ^{3}H-norepinephrine in whole-brain homogenates increased with time after the injection of labeled amine, reaching a maximal value after approximately 4 hr. In the present study, however, the P/S ratio for ^{3}H-norepinephrine in cerebellum and hypothalamus did not increase with time, although in the medulla oblongata an increase in this ratio was found between 1 and 4 hr after the injection of ^{3}H-norepinephrine. This result can possibly be explained in the light of other findings which indicate that ^{3}H-norepinephrine disappears from various brain regions at different rates. ^{3}H-Norepinephrine disappears most rapidly from the cerebellum and cortex and least rapidly from the medulla oblongata and hypothalamus;[15] the rapid disappearance of ^{3}H-norepinephrine from regions in which the P/S ration is low and the slower disappearance from regions with a high P/S ratio may account for the increased P/S ratio for the whole brain at later times.

In the rat striatum, in agreement with previous findings in the dog[8] and rabbit,[22] only 40 per cent of the endogenous dopamine content was recovered in a particulate form; however, about 70 per cent of the ^{3}H-norepinephrine accumulated in this region was found in a particulate fraction. Also, after the intraventricular injection of ^{3}H-dopamine, about 65 per cent of the labeled dopamine in the striatum was particulate bound. The finding that exogenous dopamine in the striatum was predominantly in a particulate form, whereas the endogenous catecholamine was recovered largely in the supernatant fraction, is difficult to interpret. It may be that the dopamine-containing neurons in this region have the catecholamine in high concentrations throughout their length; much of the dopamine will thus be liberated from such preterminal axons after homogenization. The ability to accumulate endogenous dopamine (and possibly norepinephrine) may be localized only in terminal regions which give rise to the particulate synaptosomal fractions. After injections of ^{3}H-dopamina, the distribution of labeled dopamine and norepinephrine in cerebellum and medulla oblongata was similar to that found after ^{3}H-norepinephrine, the P/S ratios for both amines being lower in the cerebellum than in the medulla oblongata, indicating that labeled norepinephrine formed endogenously or introduced exogenously has a similar subcellular distribution. Somewhat surprisingly, the P/S ratios for ^{3}H-dopamine were higher than those for ^{3}H-norepinephrine synthesized from this precursor.

Previous evidence suggests that the relatively large amounts of labeled norepinephrine accumulated in the striatum after the intraventricular injection of ^{3}H-norepinephrine may be due to a nonselective uptake of norepinephrine into the abundant dopamine-containing terminals[24] in this region.[14, 25] In the present study it was possible to demonstrate that the ^{3}H-norepinephrine in the striatum was localized in a synaptosomal fraction after density gradient centrifugation (Fig. 2), and the results obtained from the striatum in this respect were essentially similar to those obtained in the present study in the hypothalamus or in previous studies in the whole brain.[13] In addition to the ^{3}H-norepinephrine recovered in the synaptosomal fractions of both striatum and hypothalamus, a smaller but definite peak of particle-bound ^{3}H-norepinephrine was found in the microsomal layer immediately under the supernatant (Fig. 3). This material may be present in synaptic vesicles liberated from nerve terminals during homogenization. When the striatum was labeled with ^{3}H-dopamine and ^{14}C-norepinephrine by the simultaneous injection of these two amines, there were no

obvious differences in the distribution of the two catecholamines in the particulate fractions of a continuous density gradient, although relatively more labeled dopamine than norepinephrine was found in the supernatant layer. These results are thus consistent with the hypothesis that exogenous norepinephrine is taken up into both norepinephrine and dopamine-containing neurons in the brain.

After the intraventricular injection of ^{3}H-dopa, the subcellular distribution of the amino acid and synthesized dopamine and norepinephrine were examined in the hypothalamus and striatum (Table 4 and Fig. 2). Relatively large amounts of all three substances were recovered in the low-speed P_1 fraction, indicating that homogenization was incomplete in these experiments. Approximately 20–30 per cent of each substance was recovered in the mitochondrial–synaptosomal fraction P_2. Almost no labeled dopa was recovered in fraction P_3, a high-speed pellet of microsomal particles—though appreciable amounts of dopamine and particularly norepinephrine were found in this fraction. On the other hand, relatively large amounts of labeled dopa were found in the soluble supernatant fraction while considerably less dopamine or norepinephrine was present in this fraction. The microsomal fraction P_3 possibly contains synaptic vesicles liberated from disrupted nerve terminals.[26, 27] The present results would therefore indicate that norepinephrine was avidly bound, dopamine was less firmly bound, and dopa was not stored at all in such particles. This is in agreement with the findings that synaptic vesicles isolated from peripheral sympathetic nerves have a higher affinity for binding norepinephrine than dopamine.[28]

When the P_2 fraction was further examined by centrifugation on a discontinuous density gradient, the labeled dopa, dopamine, and norepinephrine were found to have similar subcellular distributions, all three substances being principally localized in the synaptosomal fractions corresponding to layers C and D of de Robertis.[21] Again more dopa and dopamine than norepinephrine were found in the supernatant layer. These results indicate that exogenous dopa is specifically accumulated in nerve terminals in which it is then converted into dopamine and norepinephrine. The terminals of central catecholamine-containing neurons may thus have all the enzymes required for the synthesis of norepinephrine from dopa.

The present experiments represent a preliminary investigation of the complex regional differences in the intracellular distribution of endogenous and exogenous catecholamines in the brain. It is clear that major differences in the subcellular localization of norepinephrine exist between different brain structures. Radioactive norepinephrine and its precursors can be valuable tools for labeling the brain stores of catecholamines; although it seems that in some cases, as in the striatum, the injected norepinephrine enters cells which normally contain dopamine rather than norepinephrine. Previous studies have suggested that dopamine in the striatum is not bound in intraneuronal storage vesicles;[8] the present results suggest, however, that this is not due to the absence of such storage vesicles from striatal neurons but may merely reflect the low affinity of dopamine for storage sites in such particles.

It is interesting that the regions of the brain which had the lowest P/S ratios for endogenous and exogenous catecholamines (cerebellum and cortex) are also those regions which contain the lowest amounts of endogenous norepinephrine and which have previously been found to accumulate the lowest concentrations of ^{3}H-norepinephrine. The turnover of norepinephrine in the cortex and cerebellum is, however, faster than that in the hypothalamus and medulla oblongata which contain higher

amounts of endogenous or exogenous norepinephrine and in which the P/S ratios for endogenous and exogenous norepinephrine were highest. It is possible that these phenomena are interrelated in some ways; for example a poor intracellular binding of norepinephrine in the cerebellum might explain the more rapid turnover of norepinephrine in this region.

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