CONCENTRATION OF DOPAMINE ANALOGS IN THE ADRENAL MEDULLA

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Seven labeled sulfonanilide analogs of dopamine were synthesized and their tissue distribution in rats were determined as a function of time. The methanesulfonanilide derivative, NP-27, showed significant uptake and retention in the rat adrenal, with 0.66% dose/gm at 5 min and 0.27% dose/gm at 24 hr. The 24-hr target-to-nontarget concentration ratios for the adrenal versus liver, blood, kidney, and heart were 13, 27, 30, and 60, respectively. These results compared reasonably with the corresponding target-to-nontarget ratios of 23, 45, 8, and 15 obtained for ^{14}C-dopamine. The six other analogs showed considerably less uptake and retention. Further evaluation of NP-27 in dogs indicated selective uptake in the adrenal medulla.

Previous work from this laboratory has demonstrated that ^{14}C-labeled dopamine shows a striking concentration in the dog adrenal medulla, reaching concentrations greater than any other ^{14}C-labeled precursor of epinephrine (*1*). A similar uptake of ^{14}C-dopamine has been demonstrated in the human adrenal medulla and neuroblastoma (*2*) and in pheochromocytoma (*3*). The recent syntheses of ^{11}C-dopamine and ^{18}F-dopamine have given further impetus to the search for a scanning agent for the human adrenal medulla (*4,5*). However, no gamma-emitting dopamine analogs utilizing long-lived isotopes ($T_{1/2} > 6$ hr) have yet been synthesized.

In a continuing effort aimed at understanding structure–distribution relationships, which would then lead to agents suitable for visualization of the adrenal medulla, we synthesized seven labeled sulfonanilide analogs of dopamine and determined their tissue distribution in rats as a function of time (Fig. 1). The use of the $NHSO_2R$ moiety as a bioisosteric substitute for the 3-OH group of dopamine was prompted by the observation that the sulfonanilide analogs of phenylethanolamines are in some cases pharmacologically similar to their unmodified counterparts (*6*). We report here that the methanesulfonanilide analog of dopamine (NP-27, where R = $^{35}SO_2CH_3$) shows specific uptake and retention in the rat adrenal and the dog adrenal medulla, the first known case in which a radiolabeled noncatecholic compound has shown specific uptake in the adrenal medulla.

MATERIALS AND METHODS

Labeled compounds. The labeled compounds were synthesized in the Nuclear Pharmacy Laboratory of

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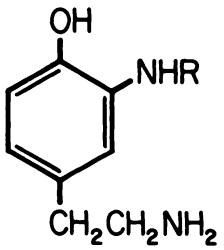

FIG. 1. Structural formula of dopamine analogs.

TABLE 1. SPECIFIC ACTIVITIES OF SEVEN DOPAMINE ANALOGS

Compound	R*	Specific activity
NP-27	$^{35}SO_2CH_3$	5.1 μCi/mg
NP-11	$^{35}SO_2Ph$-I-p	370 μCi/mg
NP-19	SO_2Ph-^{3}H-p	1.67 mCi/mg
NP-44	SO_2CH_2Ph-^{3}H-p	8.50 mCi/mg
NP-42	$SO_2(CH_2)_2Ph$-^{3}H-p	9.55 mCi/mg
NP-46	$SO_2(CH_2)_3Ph$-^{3}H-p	14.0 mCi/mg
NP-52	$SO_2CH_2{}^{125}I$	52.0 μCi/mg

* See Fig. 1 for structural formula.

TABLE 2. RELATIVE TISSUE DISTRIBUTION OF RADIOACTIVITY (% DOSE/GM)

	Adrenal	Liver	Spleen	Pancreas	Renal cortex	Lung	Heart	Blood	Muscle
5 min after dosage									
^{14}C-Dopamine	0.97	1.14	0.43	0.21	1.39	0.55	1.01	0.46	0.14
	±0.05	±0.32	±0.05	±0.01	±0.12	±0.04	±0.08	±0.09	±0.01
NP-27	0.66	0.47	0.05	0.21	0.40	2.14	0.93	0.64	0.09
	±0.10	±0.13	±0.01	±0.11	±0.12	±0.55	±0.26	±0.24	±0.02
NP-11	0.42	3.93	0.36	0.34	3.39	0.57	0.33	0.30	0.12
	±0.02	±0.33	±0.04	±0.03	±0.40	±0.05	±0.02	±0.03	±0.01
NP-19	0.46	1.12	0.45	0.37	1.13	0.59	0.33	0.54	0.19
	±0.05	±0.40	±0.03	±0.11	±0.18	±0.13	±0.10	±0.14	±0.16
NP-44	1.12	2.26	0.99	1.20	4.07	1.54	1.15	0.40	0.21
	±0.02	±0.20	±0.10	±0.14	±0.04	±0.16	±0.11	±0.05	±0.04
NP-42	0.91	3.48	3.82	0.63	3.23	3.42	0.66	1.45	0.21
	±0.74	±0.51	±0.07	±0.10	±0.57	±1.50	±0.14	±0.39	±0.03
NP-46	1.63	1.52	1.16	1.23	3.03	1.44	1.37	0.34	0.45
	±0.09	±0.44	±0.15	±0.06	±0.35	±0.12	±0.06	±0.03	±0.19
at 30 min									
^{14}C-Dopamine	0.83	0.50	0.23	0.15	0.11	0.22	0.43	0.17	0.06
	±0.05	±0.02	±0.04	±0.05	±0.17	±0.09	±0.11	±0.02	±0.01
NP-27	0.32	1.04	0.08	0.15	1.57	0.75	0.45	0.22	0.09
	±0.17	±0.90	±0.06	±0.13	±1.40	±0.70	±0.42	±0.14	±0.08
NP-11	0.29	0.52	0.10	0.18	0.66	0.33	0.15	0.15	0.07
	±0.02	±0.05	<±0.01	±0.00	±0.10	±0.06	<±0.01	±0.01	±0.03
NP-19	0.31	1.15	0.20	0.25	0.10	0.33	0.23	0.37	0.17
	±0.03	±0.20	±0.01	±0.02	±0.07	±0.04	±0.02	±0.09	±0.01
NP-44	0.53	1.59	0.83	1.45	4.41	1.32	0.83	0.39	0.31
	±0.04	±0.08	±0.04	±0.07	±0.66	±0.11	±0.09	±0.07	±0.03
NP-42	0.33	1.70	1.55	0.40	0.91	1.04	0.25	0.26	0.20
	±0.01	±0.09	±0.21	±0.01	±0.09	±0.08	±0.03	±0.07	±0.03
NP-46	1.11	0.92	0.59	0.97	2.41	1.23	1.37	0.30	0.28
	±0.32	±0.09	±0.03	±0.03	±0.26	±0.07	±0.13	±0.13	±0.02
at 1 hr									
^{14}C-Dopamine	1.33	0.58	0.30	0.11	0.59	0.20	0.26	0.16	0.09
	±0.22	±0.01	±0.02	±0.01	±0.26	±0.01	±0.02	±0.03	±0.02
NP-27	0.53	1.77	0.17	0.14	2.88	1.17	0.50	0.32	0.12
	—	—	—	—	—	—	—	—	—
NP-11	0.32	0.33	0.06	0.11	0.61	0.20	0.12	0.19	0.07
	±0.02	±0.02	<±0.01	±0.01	±0.15	±0.04	±0.01	±0.03	±0.01

* Other tissues studied and not listed were renal medulla, intestine, testes, fat, thyroid, and urine. A 4-hr interval was also de-

the Phoenix Memorial Building, University of Michigan. Details of the synthesis will be published elsewhere. The ^{14}C-labeled dopamine (specific activity, 67 μCi/mg) used as a standard in this study was purchased from New England Nuclear Corp. Table 1 shows the resultant specific activities of the compounds evaluated. All compounds were formulated as the hydrobromide salts. The NP-27 and ^{14}C-dopamine were administered in 0.9% saline solution; all other compounds were administered with 20% ethanol in 0.9% saline solutions.

All compounds were characterized by infrared and nuclear magnetic spectroscopy and correct carbon, hydrogen, and nitrogen analysis. Radiochemical purity was determined by thin-layer chromatography on silica gel-G, with all compounds giving single radiopeaks coincident with their respective cold spots.

Animals and tissue specimen. Sprague–Dawley male rats weighing 150 ± 20 gm were anesthetized with sodium pentobarbital (5 mg/100 gm body wt) and injected via the femoral vein with 20 μCi of the compound in 0.2–0.5 ml solution, allowing 3 min to complete the injection. Three or more rats were killed by decapitation at each time interval shown in Table 2.

The organs and tissues were removed and cleaned of fat and connective tissue, and representative 20–40 mg samples were placed in counting vials containing 0.3 ml 10% NaOH. These were left to digest overnight, then dissolved by warming to 70°C, agitated for a few seconds, and left to cool. Next, two drops of glacial acetic acid and three drops of 30% H_2O_2 were added and mixed thoroughly. Then 10 ml PCS solubilizer (Amersham/Searle) and 2.5 ml H_2O were added and vortexed until the solution became clear. The vials were then wiped clean with a soft cloth and placed in the liquid scintillation counter (Searle Radiographics, Unilux IIA) for cooling and

FROM ^{14}C-DOPAMINE AND DOPAMINE ANALOGS IN THE RAT*

	Adrenal	Liver	Spleen	Pancreas	Renal cortex	Lung	Heart	Blood	Muscle
(at 1 hr)									
NP-19	0.36	0.52	0.40	0.25	1.60	0.53	0.18	0.17	0.13
	±0.09	±0.14	±0.23	±0.04	±0.35	±0.24	±0.04	±0.05	±0.04
NP-44	0.37	1.15	0.41	0.94	1.61	1.00	0.65	0.30	0.30
	±0.03	±0.22	±0.18	±0.04	±0.26	±0.15	±0.12	±0.05	±0.05
NP-42	0.25	1.57	1.01	0.18	0.91	0.81	0.15	0.20	0.22
	±0.03	±0.69	±0.05	±0.03	±0.07	±0.26	±0.02	±0.09	±0.08
NP-46	—	—	—	—	—	—	—	—	—
at 6 hr									
^{14}C-Dopamine	0.54	0.03	0.11	0.05	0.13	0.03	0.13	0.01	0.02
	±0.12	±0.01	±0.03	<±0.01	±0.01	±0.01	±0.03	<±0.01	<±0.01
NP-27	0.31	0.06	0.04	0.01	0.04	0.04	0.01	0.03	0.01
	—	—	—	—	—	—,	—	—	—
NP-11	0.03	0.02	0.01	0.01	0.04	0.02	0.01	0.05	0.01
	±0.01	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01	±0.01	<±0.01
NP-19	0.22	0.30	0.14	0.10	0.43	0.20	0.06	0.10	—
	±0.07	±0.06	±0.08	±0.01	±0.20	±0.04	±0.00	±0.07	—
NP-44	0.13	0.36	0.32	0.22	0.41	0.25	0.12	0.45	0.06
	±0.01	±0.13	±0.09	±0.04	±0.11	±0.05	±0.01	±0.25	<±0.01
NP-42	0.22	1.02	1.52	0.28	1.66	2.69	0.40	1.07	0.35
	±0.01	±0.64	±0.17	±0.18	±1.60	±2.55	±0.30	±1.04	±0.28
NP-46	0.16	0.32	0.40	0.27	0.89	0.36	0.17	0.32	0.12
	±0.02	±0.01	±0.10	±0.02	±0.29	±0.05	±0.03	±0.11	±0.03
at 24 hr									
^{14}C-Dopamine	0.91	0.04	0.08	0.03	0.11	0.03	0.06	0.02	0.01
	±0.18	<±0.01	±0.01	<±0.01	±0.03	±0.01	±0.01	±0.02	<±0.01
NP-27	0.27	0.02	0.02	<0.01	0.01	0.02	0.00	0.01	<0.01
	±0.02	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01	<±0.01
NP-11	0.02	0.02	0.02	<0.01	0.05	0.01	0.01	0.03	0.01
	<±0.01	<±0.01	<±0.01	<±0.01	±0.01	<±0.01	<±0.01	<±0.01	<±0.01
NP-19	0.06	0.22	0.08	0.02	0.12	0.13	0.03	0.11	0.04
	±0.01	±0.04	±0.02	<±0.01	±0.04	±0.06	<±0.01	±0.02	±0.01
NP-44	0.10	0.35	0.15	0.08	0.34	0.09	0.06	0.14	0.05
	±0.01	±0.05	±0.02	±0.02	±0.07	±0.01	±0.01	±0.01	±0.01
NP-42	0.15	0.68	0.92	0.24	0.26	0.38	0.20	0.71	0.54
	±0.03	±0.21	±0.22	±0.07	±0.02	±0.09	±0.08	±0.06	±0.17
NP-46	0.06	0.19	0.25	0.04	0.50	0.10	0.06	0.07	0.03
	±0.01	±0.02	±0.09	±0.01	±0.14	±0.04	±0.02	±0.01	±0.01

termined but not included in this table. A minimum of three rats were killed each time interval. Results in mean ±1 s.d.

dark adaptation. Samples were counted for 10 min each and quenching was corrected using the two-channel-ratio method. Data are expressed as percentage of given dose per gram of fresh tissue.

The procedure used to determine the tissue distribution in dogs has been outlined in a previous publication (*1*).

RESULTS

Table 2 presents the relative tissue concentration of the synthesized compounds in % dose/gm in nine selected rat tissues. The other tissues analyzed included the renal medulla, intestine, testes, fat, and thyroid, and all had concentrations less than 0.1% dose/gm. The distribution data on ^{14}C-dopamine are included for comparison. The highest radioactivity concentration in the adrenal was evident with ^{14}C-dopamine. However, NP-27 showed a marked uptake and retention in the adrenal, with 0.66% dose/gm at 5 min and 0.27% dose/gm at 24 hr. The 24-hr distribution pattern for NP-27 is presented in Fig. 2. Figure 3 shows the relative concentration ratios of dopamine against its methanesulfonanilide analog, NP-27. Carbon-14-dopamine showed an adrenal uptake one and a half to three times greater than NP-27 at all time intervals. The 24-hr target-to-nontarget concentration ratios of NP-27 for the adrenal versus liver, blood, kidney, and heart were 13, 27, 30, and 60, respectively. These results compare reasonably with the corresponding ratios of 23, 45, 8, and 15 obtained for ^{14}C-dopamine.

The arylsulfonanilide analogs NP-11 and NP-19 gave moderate uptake in the adrenal at short intervals but the radioactivity was rapidly released. At no time interval were the adrenal-to-liver ratios greater than 2.

Concentration of the tritiated chain-extended analogs NP-44, NP-46, and NP-42 in the rat adrenal,

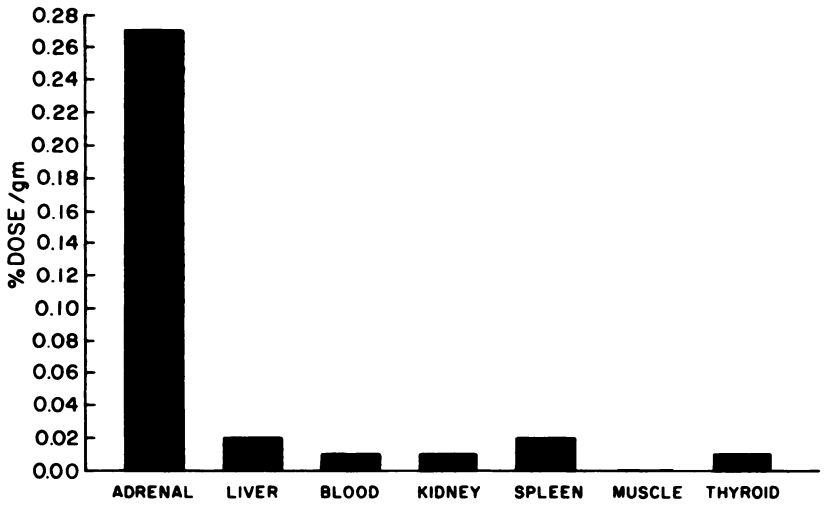

FIG. 2. Tissue ^{35}S concentration of methanesulfonanilide NP-27 at 24 hr after injection.

although again high for short time intervals, diminished quickly after 1 hr. The adrenal-to-liver ratios never rose above 1. These more lipophilic compounds showed greater retention in the major organs at longer intervals than the other analogs tested.

The ^{125}I derivative NP-52 showed low adrenal uptake (0.06% dose/gm at 30 min). At 4 hr, 96% of the radioactivity counted appeared in the thyroid, suggesting in vivo deiodination.

The results of the NP-27 tissue distribution in dogs are presented in Table 3. Long-term retention of NP-27 was evident in the adrenal medulla. All other organs, except the spleen, indicated rapid elimination of NP-27. The spleen concentration decreased by a factor of 6 whereas the adrenal medulla only decreased by a factor of 2. At 48 hr the concentration ratio of adrenal medulla to adrenal cortex was 8.5.

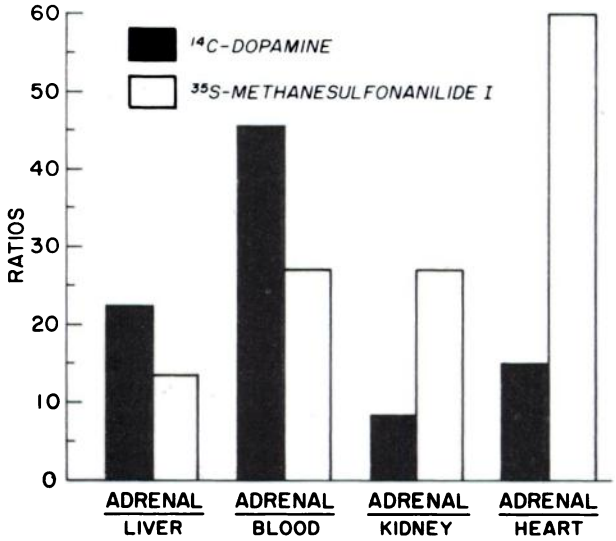

FIG. 3. Tissue radioactivity ratios for ^{14}C-dopamine and methanesulfonanilide NP-27 at 24 hr.

DISCUSSION

In light of the in vivo instability of radioiodinated tyramine derivatives (*7*), it is apparent that if dopamine is to be successfully labeled with radioiodine, the iodine label cannot be substituted directly on the aromatic ring. Larsen, et al (*6,8,9*) found that certain alkylsulfonamide moieties can be substituted for the 3-hydroxy group of phenylethanolamines without loss of adrenergic potency. Thus, the possibility arises that radioiodine might show greater in vivo stability if it was substituted on the 3-arylsulfonamide group of a dopamine analog as in NP-11.

To verify the feasibility of the overall approach, the ^{35}S-methanesulfonanilide analog NP-27 was synthesized and its tissue distribution determined. The specific localization of NP-27 in the rat adrenal,

TABLE 3. TISSUE DISTRIBUTION OF RADIOACTIVITY (% DOSE/GM) FROM NP-27 IN THE DOG

Time (hr)	Adrenal medulla	Adrenal cortex	Liver	Spleen	Pancreas	Renal cortex	Lung	Heart	Blood	Muscle
6	0.0048	0.0044	0.0200	0.0053	0.0074	0.0043	0.0044	0.0066	0.0022	0.0004
24	0.0017	0.0002	0.0011	0.0024	0.0006	0.0007	0.0003	0.0002	0.0010	0.0002
48	0.0021	0.0003	0.0005	0.0009	0.0003	0.0004	0.0002	0.0002	0.0004	0.0002

presented in Fig. 2, was indeed encouraging. The high uptake in the rat adrenal is even more notable in view of the low specific activity of the compound, i.e., the 20 μCi or 4 mg dose per rat represents approximately 15% of the total weight (25 mg) of the average rat adrenal. Wolf, et al found a significant dependence of adrenal uptake on the specific activity of ^{11}C-dopamine (*4*). Thus the adrenal uptake and target-to-nontarget ratios obtained for NP-27 may be greater with higher specific activities.

The logical extension of these initial findings was evaluation of the p-iodophenyl analog NP-11. Since selective adrenal localization did not occur with NP-11, the phenyl analog NP-19 was studied to determine if the bulky iodine atom itself caused the lack of retention of NP-11 in the adrenal. However, essentially the same adrenal uptake and distribution pattern was observed for the two compounds. The added failure of the chain-extended analogs NP-44, NP-46, and NP-42 to show retention in the adrenal emphasized the strict structural constraints placed on potential adrenal-scanning agents.

Phenylethanolamine N-methyl transferase (PNMT) which transfers a methyl group from S-adenosylmethionine to a phenylethanolamine or a phenylethyldiamine, is known to exist in high concentrations in the adrenal medulla (*10,11*). PNMT may be inhibited by phenylethylamines or indolamines and this specificity is due in part to a lack of a β-hydroxy group (*12*). Thus dopamine and its sulfonanilide analog NP-27 may possibly localize because of their specific inhibition of PNMT, as well as by virtue of their intermediacy in the norepinephrine pathway.

An important consideration is the possibly greater in vivo stability of NP-27 over dopamine itself due to the failure of catechol-O-methyl transferase to initiate metabolic breakdown of NP-27. In any case, NP-27 with a gamma emitter like ^{75}Se must be considered a potentially useful agent for visualization of the adrenals.

Although visualization of the human adrenal medulla is not yet a reality, we hope the present work will widen the possible approaches to solving the problem.

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