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DISPLACEMENT OF NOREPINEPHRINE FROM THE RAT HEART BY ^{14}C-METARAMINOL

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Abstract—Rats were injected with submaximal quantities (0·24 mg/kg) of ^{14}C-metaraminol, i.e. enough to lower the concentration of norepinephrine in the heart by about 60 per cent, measured 18 hr after drug administration. In the ventricles at 18, 42, and 66 hr after metaraminol injection and in the atria at the two latter times, radioactivity was present in amounts sufficient to account exactly for the norepinephrine missing, on a mole-for-mole displacement basis. Atrial radioactivity 18 hr after metaraminol administration was insufficient to account for the whole of the missing catecholamine. Possibly at 18 hr a steady state had not been reached in the atria, an interpretation which is strengthened by the fact that other catecholamines (epinephrine and dopamine) were present only in trace amounts. Chemically determined metaraminol was consistently less than indicated by radiocounting. Even though no evidence was gained for the presence of ^{14}C-α-methylnorepinephrine in the tissue, it is possible that metabolites of metaraminol account for part of the norepinephrine displacement observed.

The administration of larger amounts of ^{14}C-metaraminol (3 mg/kg) resulted in catecholamine depletion greater than that accounted for by radio-activity in the tissue, and this was particularly striking in the atria. Thus, the relationship between tissue radioactivity and norepinephrine concentration depends upon several factors, and only under certain conditions, as described above, is a one-for-one displacement of the catecholamine clearly demonstrated.

IN ADDITION to being a direct-acting sympathomimetic agent,[1, 2] metaraminol depletes sympathetically innervated tissue of norepinephrine.[3, 4] However, whether the agent, injected as such or derived from the metabolism of α-methyl-*m*-tyrosine or α-methyl-*m*-tyramine, displaces stoichiometric amounts of the catecholamine from its binding sites has been the subject of conflicting reports.[4–9] Results obtained with either ventricles or whole hearts of various animal species and with differing analytical technics and experimental conditions have been interpreted to demonstrate a one-for-one displacement of norepinephrine by metaraminol, or a greater than mole-for-mole displacement. The present work, with ^{14}C-labeled metaraminol, shows that the stoichiometry between depleting agent and norepinephrine in the heart varies with the amount of metaraminol administered, the time elapsed, and the part of the heart examined. One-for-one displacement of norepinephrine is evident only under certain well-defined conditions.

EXPERIMENTAL

Materials. 3-^{14}C-metaraminol bitartrate, sp. act. 6·5 μc/mg, was made available by Drs. C. Rosenblum and H. T. Meriwether, Merck, Sharp & Dohme Research

Laboratories, Rahway, N. J. By fluorescence assay,[11] it matched exactly the unlabeled product marketed as Aramine bitartrate,* which was used as a standard in chemical assay of the tissue. This product is 99·5 per cent pure or better, as judged from non-aqueous titration, optical rotation, u.v. and infrared spectra, and melting range data. The norepinephrine used as a primary standard was a sample of racemic dihydrochloride (Winthrop), which exactly matched, in the trihydroxyindole fluorescence assay, two other samples (Mann Research Laboratories), one the racemic hydrochloride (assay 98·7 per cent), the other the levorotatory bitartrate monohydrate (assay 99·4 per cent).

Methods. Female albino rats weighing 450–500 g were used. The metaraminol salt was injected intraperitoneally as an aqueous solution in amounts calculated as weight of base per kg of body weight. After appropriate times, the animals were killed by a blow to the head, hearts were removed rapidly and trimmed, and atria (right and left) were separated from the ventricles. The tissue was frozen immediately and stored for up to 3 days until analyzed.

Pooled atria or ventricles from (usually) 6 rats were homogenized with 9 vol. of 0·4 N perchloric acid, in an all glass homogenizer for the atria or in a steel-blade blendor for the ventricles.† Aliquots of the extracts were used in all subsequent measurements.

For radioactivity determinations, 1 ml of the perchloric acid filtrate plus 0·4 ml of 3 N KOH was added to 20 ml of phosphor solution (100 g naphthalene, 7 g PPO,* 0·3 g POPOP,* 1000 ml *p*-dioxane[10]) and counted by liquid scintillation. Counting efficiencies were calculated from internal standard data, and were from 40 to 50 per cent. The sp. act. of the ^{14}C-metaraminol, determined by counting replicate samples of standard solutions, was used to convert dpm to micromoles of amine in the extracts.

Metaraminol was determined chemically by the method of Shore and Alpers.[11] In both this and the catecholamine assay, known quantities of the appropriate amine were carried through the entire procedure, and amine concentrations in the tissue were calculated from the results so obtained.

Catecholamines were purified by adsorption on alumina and elution with 0·1 N HCl,[12] and determined by the trihydroxyindole method with iodine oxidation.[13]

Data are reported as micromoles of amine per gram of tissue divided by micromoles of norepinephrine per gram of tissue from untreated rats, as determined in each experiment. Thus, the numbers represent the fraction of the normal, total catecholamine space occupied by the amine.

RESULTS

Eighteen hr after the administration of metaraminol (0·026, 0·079, 0·238 mg/kg), norepinephrine concentrations in atria and ventricles were decreased proportionately (Table 1). Therefore, data from the two heart parts were pooled for examination by the method of least squares. A linear relationship was revealed between norepinephrine concentration and log dose of metaraminol, and the ED_{50} of the administered amine was found to be 0·142 mg/kg with 95 per cent confidence limits of 0·117 and 0·169 mg/kg.

* Registered trade name of Merck & Co., Inc., Rahway, N. J.

† Extraction of radioactivity from the tissue was essentially complete: 99·1 per cent from the atria, 98·4 per cent from the ventricles.

* PPO, 2,5-diphenyloxazole; POPOP, 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene.

TABLE 1. NOREPINEPHRINE AND CHEMICALLY DETERMINED METARAMINOL IN ATRIA AND VENTRICLES 18 HR AFTER VARIOUS DOSES OF METARAMINOL

Metaraminol dose (mg/kg)	Tissue amine (fraction of total norepi. space)			
	Atria		Ventricles	
	Metaram.	Norepi.	Metaram.	Norepi.
0	0	1·000	0	1·000
0·026	0·058	0·829	0·094	0·821
0·079	0·165	0·639	0·327	0·605
0·238	0·263	0·384	0·486	0·401

Analysis of data: (1) X, log dose metaraminol; Y, norepinephrine in tissue (18 groups of 6 rats each)

	Atria	Ventricles	Pooled data*
Slope	−0·458	−0·436	− 0·447
95 per cent confidence limits			− 0·361, − 0·533
Regression equation			$Y_x = 0·121 - 0·447\ X$
ED_{50} of metaraminol	0·143	0·139	0·142
95 per cent confidence limits			0·117, 0·169

Analysis of data: (2) *X*, metaraminol in tissue (chemical); *Y*, norepinephrine in tissue (12 groups of 6 rats each)

	Atria	Ventricles
Slope	− 2·239	− 1·130
95 per cent confidence limits	− 1·990, − 2·488	− 0·990, − 1·270
Regression equation	$Y_x = 0·989 - 2·239\ X$	$Y_x = 0·967 - 1·130X$
X intercept	0·442	0·856
Y intercept	0·989	0·967
S.D. (*Y*)	0·055	0·058

* P for difference between atria and ventricles >0·25.

A plot of norepinephrine vs. chemically determined metaraminol in the atria, although linear (P for deviation from linearity > 0·10), did not suggest a 1:1 relationship between displaced norepinephrine and metaraminol in the tissue: the slope of the line was − 2·24 (95 per cent confidence limits, − 1·99, − 2·49) instead of − 1·00, which would be the case for 1:1 displacement. A similar plot of data for the ventricles yielded a line, the slope of which was close to 1 (− 1·13), but which deviated significantly from linearity (P < 0·01). Its intercept on the metaraminol axis was 0·86 rather than 1. Thus, in neither the atria nor the ventricles was a simple 1:1 relationship found between norepinephrine and the displacing amine.

Metaraminol concentrations, based upon radiocounting, in both atria and ventricles were greater than concentrations of the compound found by the chemical assay (Table 2). Also, these data show that at 18 hr after drug administration the ventricles contained significantly more metaraminol (and radioactivity) than the atria, when compared to the norepinephrine space in the respective parts of the heart.

Radiocounts of ventricles from rats taken 18, 42, and 66 hr after the administration of ^{14}C-metaraminol (0·238 mg/kg) showed a linear relationship to norepinephrine concentrations (Table 3). The slope of the regression line (P for deviation from linearity > 0·10) was − 1·024 (95 per cent confidence limits, − 0·89, − 1·16) and

TABLE 2. METARAMINOL IN ARTIA AND VENTRICLES BY CHEMICAL ANALYSIS AND BY ^{14}C COUNTING, 18 HR AFTER ADMINISTRATION OF SINGLE DOSE (0·238 MG/KG) OF ^{14}C-METARAMINOL

Part of heart	Metaraminol (fraction of total norepi. space)	
	Chemical	^{14}C
Atria	0·263	0·408
Ventricles	0·486	0·628

Analysis of variance* (6 groups of 6 rats each; one missing value)

Source	df	ss	ms	F	P
Total	22	0·447313			
Part	1	0·293267	0·293267	183·64	< 0·001
Method	1	0·123697	0·123697	77·46	< 0·001
Part x method	1	0·000010	0·000010	< 1	>0·25
Error	19	0·030339	0·001597		

* Snedecor.[17]

TABLE 3. NOREPINEPHRINE AND RADIOACTIVITY IN ATRIA AND VENTRICLES 18, 42, AND 66 HR AFTER ADMINISTRATION OF A DOSE (0·238 MG/KG) OF ^{14}C-METARAMINOL

Time after metaraminol (hr)	Tissue amine (fraction of total norepi. space)			
	Atria		Ventricles	
	^{14}C	Norepi.	^{14}C	Norepi.
18	0·408	0·394	0·628	0·341
42	0·320	0·667	0·444	0·558
66	0·167	0·773	0·204	0·800

Analysis of data: X, radioactivity; Y, norepinephrine (18 groups of 6 rats each)

	Atria	Ventricles
Slope	− 1·350	− 1·024
95 per cent confidence limits	− 1·104, − 1·596	− 0·888, − 1·160
Regression equation	$Y_x = 0·998 - 1·350\ x$	$Y_x = 0·998 - 1·024\ X$
X intercept	0·739	0·975
Y intercep:	0·998	0·998
S.D. (Y)	0·084	0·071

the intercepts on the radiocount and norepinephrine axes were 0·98 and 1·00 respectively. Therefore, in the ventricles, a 1:1 displacement of norepinephrine by radioactive substances is established.

Similar treatment of the atria data yielded a regression line with a slope of − 1·35 (95 per cent confidence limits, − 1·10, − 1·60); this line deviated significantly from linearity ($P < 0·05$). However, examination of the 42- and 66-hr data only shows that the mathematical requirements are fulfilled to reveal a 1:1 relationship between radioactivity and displaced norepinephrine. The slope of the regression line is − 1·04, and the intercepts on the radioactivity and norepinephrine axes are 0·95 and 0·99

respectively. Also, it may be seen (Table 3) that, in the atria at 42 and 66 hr, the sum of amine concentrations is very nearly one, *i.e.* 0·99 and 0·94.

Since more 'metaraminol' was found in the tissue by radiocounting than by chemical assay, the presence of metaraminol metabolites would be expected and they were sought for. Paper chromatography (butanol–acetic acid–water, 4:1:1) of extracts from the ventricles of ^{14}C-metaraminol-treated rats gave a single radioactive spot with an R_f identical to that of metaraminol, 0·66. Furthermore, no significant amount of radioactivity was detected in the extracts which could be adsorbed onto alumina and eluted with dilute HCl.

Tissue was assayed also for epinephrine and dopamine in order to explain, if possible, the apparent deficit of norepinephrine in the hearts observed 18 hr after the administration of metaraminol. Very little epinephrine was detected, and the amount of dopamine found and its change with drug administration was entirely insufficient to account for the observed norepinephrine deficit.

In the foregoing experiments, the amount of metaraminol administered was considerably less than that ordinarily employed in balance studies of this type. It was considered of interest, therefore, to examine the hearts of rats after the administration of an excessive quantity of the compound, *i.e.* 3 mg/kg. The tissue was assayed 42 hr after the injection of metaraminol, at a time when, as shown above, a 1:1 relationship obtained between radioactivity and displaced norepinephrine when a smaller amount of metaraminol had been given. The ventricles contained enough radioactivity to account for 80 per cent to the norepinephrine missing (Table 4); however, radioactivity in the atria was quite low, and accounted for only 38 per cent of the norepinephrine deficit.

TABLE 4. NOREPINEPHRINE AND RADIOACTIVITY IN ATRIA AND VENTRICLES 42 HR AFTER ADMINISTRATION OF A LARGE DOSE (3 MG/KG) OF ^{14}C-METARAMINOL

Part of heart	Tissue amines (fract. of total norepi. space)*		
	Norepi.	^{14}C	Sum
Atria	0·178	0·298	0·476
	0·128	0·339	0·467
	0·128	0·335	0·463
Average	0·145	0·324	0·469
Ventricles	0·145	0·714	0·859
	0·130	0·684	0·814
	0·145	0·675	0·820
Average	0·140	0·691	0·831

* Values given for each of 3 groups of 6 rats.

These findings were confirmed by data from another experiment (Table 5), but the ventricles were somewhat more affected than was found at first. Here, it was found that the intravenous administration of norepinephrine to rats which had received 3 mg/kg of ^{14}C-metaraminol 18 hr previously did not alter significantly the concentration of norepinephrine, and only slightly changed the concentration of radioactive compounds in the hearts.

TABLE 5. NOREPINEPHRINE AND RADIOACTIVITY IN ATRIA AND VENTRICLES 42 HR AFTER ADMINISTRATION OF ^{14}C-METARAMINOL (3 MG/KG), 24 HR AFTER INJECTION OF NOREPINEPHRINE (125 μG/KG)

Part of heart and dose given	Tissue amines (fract. of total norepi. space)*		
	Norepi.†	^{14}C‡	Sum
Atria			
Control	1·000		
Metaram.	0·281	0·210	0·491
Norepi.	0·835		
Metaram. + norepi.	0·228	0·186	0·414
S. D.	0·097	0·030	
Ventricles			
Control	1·000		
Metaram.	0·203	0·472	0·675
Norepi.	0·947		
Metaram. + norepi.	0·256	0·398	0·654
S. D.	0·069	0·040	

* Three groups of 6 rats per treatment.
† P for effect of norepinephrine, $> 0{\cdot}10$.
‡ P for effect of norepinephrine, pooled data from atria and ventricles, $< 0{\cdot}05$.

DISCUSSION

The data show that 18, 42, and 66 hr after the administration of a submaximal amount (0·24 mg/kg) of radioactive metaraminol to rats, the heart ventricles contained radioactivity which exactly accounted for the missing norepinephrine. In the atria, a similar equivalence was observed 42 and 66 hr after metaraminol administration, but not at 18 hr. When a large amount (3 mg/kg) of ^{14}C-metaraminol was injected, radioactivity in the hearts 42 hr later was insufficient to account for the norepinephrine deficit on a mole-for-mole basis. Furthermore, these hearts were incapable of binding administered norepinephrine. Thus, it is clear that the stoichiometry of norepinephrine and metaraminol occupancy of norepinephrine binding sites depends upon the amount of metaraminol injected, the time elapsed between injection and tissue assay, and the part of the heart chosen for examination. It has been shown also that in the hearts of rats which received an intermediate amount of metaraminol (1 mg/kg) cold stress caused a rapid loss of the depletor from the tissue without a compensatory gain of norepinephrine.[9]

The sequence of events in the heart after the administration of metaraminol probably is initiated by a molecule-for-molecule displacement of norepinephrine from its binding sites by metaraminol. Excess drug, initially present in the tissue, rapidly disappears, leaving all catecholamine binding sites occupied by either norepinephrine or metaraminol. As the latter amine slowly vacates the binding sites, its place is taken by norepinephrine, but only if a moderate quantity of the depleting agent is employed; otherwise, the binding sites are temporarily incapable of holding norepinephrine, even after the displacing agent has left the tissue. Although other catecholamines, i.e. epinephrine and dopamine, are not present in the hearts in which a catecholamine deficit was noted in concentrations sufficient to account for the deficit, the

possiblity remains that some other naturally occurring material may block re-establishment of normal norepinephrine concentrations in the tissue. Octopamine is one possibility in this respect, since it has been found in animal tissue.[14] Presumably, however, this particular compound would have been displaced by administered norepinephrine so that its presence, although possible, is not probable. Obviously the data do not prove that those binding sites which hold neither radioactive materials nor norepinephrine are indeed vacant.

However, it is known that metaraminol, as well as other agents, causes myocardial damage when administered in relatively large amounts. Jasmin and Bajusz[15] reported on the cardiac lesions in rats after injection of metaraminol (about 4 mg/kg as free base) and attributed the observed changes to pulmonary and peripheral hemodynamic alteration attributable to the drug. It was observed, also, that the deleterious action of vascoconstrictors was independent of the catecholamine content of the heart. Thus, although there is no direct evidence to support the suggestion, it is possible that certain sympathomimetic agents directly or indirectly alter neuronal catecholamine binding sites, rendering them at least temporarily incapable of binding norepinephrine.

It is apparent that the bulk of the radioactivity in the hearts of ^{14}C-metaraminol-treated rats is present as unchanged metaraminol. However, although every effort was made to obtain strictly quantitative data, chemically determined metaraminol consistently was lower in the tissue than that determined by radiocounts. The suggestion is that small amounts of unidentified metabolites of metaraminol may occur in the tissue, presumbly also occupying catecholamine binding sites. α-Methylnorepinephrine has been detected in the hearts of guinea pigs which received large amounts of metaraminol.[16] In the present experiments, however, extracts of heart tissue from ^{14}C-metaraminol-treated rats contained no radioactivity which was adsorbed onto alumina, and since α-methylnorepinephrine is, like norepinephrine, adsorbable, it is concluded that the hearts contained no significant amount of radioactive catecholamines.

Finally, it should be pointed out that the metaraminol used in this work bears the label at position 3, i.e. it is the α-methyl-^{14}C compound. Since no radioactivity has been detected in CO_2 expired by ^{14}C-drug-treated rats,* it is unlikely that the metaraminol side chain is degraded extensively in this species. Therefore, it is reasonable to conclude that any radioactivity held in the tissue must represent either metaraminol or the compound which has been metabolized only moderately. In those instances where radioactivity plus norepinephrine does not account for the total, normal catecholamine space, the binding sites must be either vacant or occupied by substances other than those derived from metaraminol.

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