Chemical Evaluation of the Distribution of Catecholamines in the Heart

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THE catecholamine content has been studied in various organs by several investigators. A close relationship between norepinephrine content and the distribution of sympathetic nerves has been well known¹⁻⁴. According to VON EULER⁵, SCHÜMAN⁶, and others, more than 94 per cent of catecholamines consists of dopamine in such a poorly innervated organ as the mucous membrane of the digestive tract, while about 50 per cent of catecholamines was made up of norepinephrine in an organ innervated by the sympathetic nerve. On the other hand, VOGT⁷, CARLSON⁸, SANO⁹ and others¹⁰ described that dopamine constituted a most part of catecholamines in the corpus striatum, and that norepinephrine was present in a high concentration in the brain stem, especially in the hypothalamus. It is therefore of interest to evaluate the distribution of catecholamines in the heart by means of chemical determination.

MATERIALS AND METHODS

In all determinations, the hearts were removed rapidly from 6 dogs, weighing 4 to 15 kg, under thiopental sodium anesthesia (0.03–0.05 g/kg of body weight). Five specimens were collected from each heart immediately after the removal, so that the catecholamine content in both atria, ventricular septum, and both ventricles might be determined. Each specimen was weighed, ground in 3 volumes of 0.4N perchloric acid at 0°C and then centrifuged. The resulting sediment was resuspended in 2 volumes of 0.4N perchloric acid and centrifuged. In this manner were obtained two supernatant fluids, which were pooled for a chemical determination.

Preparation for absorption

Catecholamines were absorbed on to aluminum hydroxide by the method of BURN¹¹. The reaction

TABLE I DETERMINATION OF EPINEPHRINE AND NOREPINEPHRINE

Eluate		1M NaAc Buffer (pH 6.0)	0.5% ZnSO ₄ Solution	Standard Solution (1 µg/ml)	0.25% K ₃ Fe (CN) ₆	2% Ascorbic Acid Solution*	5N NaOH Solution	2% Ascorbic Acid Solution	Water
(ml)	(ml)	(ml)	(ml)	(µg/ml)	(ml)	(ml)	(ml)	(ml)	(ml)
Sample	1.0	2.0	0.1	—	0.1	left for 20 min. at 0°C	0.9	—	to 10
Standard (Ep)	1.0	2.0	0.1	Epinephrine 0.1	0.1	for 20 min. at 0°C	0.9	—	to 10
Standard (NE)	1.0	2.0	0.1	Norepinephrine 0.2	0.1	20 min. at 0°C	0.9	—	to 10
Blank	1.0	2.0	0.1	—	0.1	at 0°C	0.9	0.1	to 10
Ep : Epinephrine NE : Norepinephrine NaAc : Sodium acetate * Freshly prepared									

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TABLE II DISTRIBUTION OF CATECHOLAMINES IN THE HEART OF THE NORMAL DOG

Portion	No. of Dogs	Epinephrine ($\mu\text{g/g}$)	Norepinephrine ($\mu\text{g/g}$)	Dopamine ($\mu\text{g/g}$)	Dopa ($\mu\text{g/g}$)
Right Atrium	6	0.047 ± 0.012	0.814 ± 0.045	0.417 ± 0.030	0.125 ± 0.025
Left Atrium	6	0.045 ± 0.010	0.806 ± 0.052	0.405 ± 0.035	0.145 ± 0.017
Right Ventricle	6	0.047 ± 0.014	0.631 ± 0.031	0.385 ± 0.026	0.133 ± 0.018
Left Ventricle	6	0.046 ± 0.011	0.626 ± 0.028	0.385 ± 0.021	0.128 ± 0.011
(Epicard. layer)	6	0.049 ± 0.018	0.630 ± 0.035	0.380 ± 0.045	0.135 ± 0.012
(Endocard. layer)	6	0.044 ± 0.017	0.622 ± 0.041	0.392 ± 0.046	0.125 ± 0.014
Ventricular Septum	6	0.042 ± 0.013	0.625 ± 0.035	0.391 ± 0.017	0.100 ± 0.012

of the supernatant fluid, to which 0.5 ml of 20 per cent aluminum sulfate had been added, was carefully adjusted to pH 7.8 with sodium hydroxide. Precipitation was allowed to take place in this fluid for 30 minutes and was followed by centrifugation. The resulting sediment was dissolved in 2 ml of 1M phosphoric acid. The solution was centrifuged after its reaction had been adjusted to pH 4.0 with 4N ammonium acetate. These procedures were performed at 0°C.

Recoveries of the added catecholamines were as follows: epinephrine $84 \pm 6\%$, norepinephrine $80 \pm 8\%$, dopamine $79 \pm 7\%$, and dopa $78 \pm 6\%$.

Preparation for separation

Catecholamines were fractionated by cation exchange resin chromatography with Duolite C-25 (300-400 meshes, H-type) at 4-10°C according to the procedure of NAKAJIMA¹². The supernatant fluid, adjusted to pH 4.0, was charged on a column (8 × 80 mm), and the absorbed catecholamines were eluted with the following solutions: (1) effluent and 10 ml of water (acid and neutral catecholamines), (2) 30 ml of 0.35N sodium acetate buffer, pH 5.0 (dopa), (3) 18 ml of 3N sodium acetate buffer, pH 6.0, containing 7% of ethyl alcohol (norepinephrine and epinephrine) and (4) 15 ml of 3N sodium acetate buffer, pH 6.0, containing 25% of ethyl alcohol (dopamine). Recovery was $97 \pm 3\%$ for epinephrine, $96 \pm 3\%$ for norepinephrine, $91 \pm 6\%$ for dopamine, and $89 \pm 7\%$ for dopa.

Determination of catecholamines

Norepinephrine, epinephrine, and dopa were determined fluorimetrically by the modified method of EULER and FOLDING¹³, while dopamine was determined by the method of WEIL-MALHERBE and BONE¹⁴.

The procedure described by EULER and FOLDING was modified as indicated in Table I¹⁵. Oxidation was performed with potassium ferricyanide at 0°C. The amounts of norepinephrine and epinephrine were calculated from the fluorimetric readings at

436/535 and 400/485 m μ .

RESULTS

The data for norepinephrine are given in Table II. It is clear that in all cases the norepinephrine concentration was significantly higher in the atria than in the ventricles ($P < 0.01$), although no significant difference was demonstrated between the right atrium and the left, or between the right ventricle and the left. The norepinephrine concentration was 0.814 and 0.806 $\mu\text{g/g}$ in the right and left atrium, 0.631 and 0.626 $\mu\text{g/g}$ in the right and left ventricle respectively, and 0.610 $\mu\text{g/g}$ in the ventricular septum. Further examination indicated that the epicardial layer of the left ventricle contained nearly the same amount of norepinephrine as its endocardial layer. As is evident from the tabulated values, the concentration of norepinephrine was not higher at the root of the cranial vena cava.

DISCUSSION

It is clear from the results obtained that in the dog heart the atria contain more norepinephrine than the ventricles, but that neither the right atrium nor the right ventricle contains a higher concentration of norepinephrine than its counterpart on the left side¹⁶. These results agree with those reported by SHORE¹⁷, SERRANO¹⁸, MIYAHARA¹⁹, and others²⁰.

On the other hand, it has been found that epinephrine, dopamine, and dopa distributed almost equally throughout the heart. This finding is not in agreement with those made by MIYAHARA¹⁹ and ANGELAKOS²¹, who mention-

ed that dopamine was present in higher concentrations in the atria than in the ventricles and that the right atrium contained more dopamine than the left.

Moreover, such a particular part of the heart as the cranial root of the vena cava contains as large an amount of norepinephrine as any other part of the atrium. This agrees with the results reported by SHORE¹⁷⁾, but does not agree with those reported by some other authors, who stated that the concentration of epinephrine (SERRANO)¹⁸⁾ or that of dopamine (ANGELAKOS)²¹⁾ was higher in the region of sinus nodes than in the rest of the atrium.

The present author has reported that only norepinephrine is contained in higher concentration in the atria, and the other catecholamines are almost equally distributed in the heart. It is suspected that the presence of the cardiac plexuses in the atria may induce a higher concentration of norepinephrine in this part. Consequently, it could not be found that there was any particular part in the heart which contained one of the catecholamines almost exclusively.

SUMMARY

1. The contents of norepinephrine, epinephrine, dopamine, and dopa were determined in various portions of the heart in 6 intact dogs.

Catecholamines were separated with aluminum hydroxide and cation exchange resin using Duolite C-25. Norepinephrine, epinephrine, and dopa were determined fluorimetrically by the modified method of EULER and FOLDING and dopamine was by the method of WEIL-MALHERBE and BONE.

2. Although epinephrine, dopamine, and dopa were distributed almost equally throughout the heart, the norepinephrine concentration was significantly higher in the atria than in the ventricles.

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