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Distributions of Catecholamines in the Heart of Mammalian Laboratory Species

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Abstract The regional distribution of heart catecholamines of rats, guinea pigs, rabbits and mice was measured by high-performance liquid chromatography (HPLC) with electrochemical detection (ED). The catecholamines were adsorbed onto alumina and then eluted with 0.5 M hydrochloric acid and assayed by HPLC-ED. The lower limit of detection was about 0.03 -0.05 pmol. The predominant catecholamine was norepinephrine (NE), and dopamine (DA) was second. Epinephrine was not always detected. NE and DA were found in higher concentrations in the atria than in the ventricles. In the mouse NE was found in higher concentrations in the left heart region than in the right heart region, and DA was found in higher concentrations in the left atrium than in the right atrium. In other animals NE and DA were found in higher concentrations in the right heart region than in the left heart region. Thus, in studies of myocardial catecholamines in addition to catecholamine metabolism it may be useful to consider catecholamine concentrations by heart regions and by species.

Key Words: Catecholamine, Heart Tissue, High-Performance Liquid Chromatography with Electrochemical Detection

Introduction

The adrenergic nervous system may play an important role in the mammalian cardiovascular regulation of heart tissues. Recently human myocardial catecholamines have been studied clinically during sympathetic nervous activity in the pathophysiology of heart failure[1,2]. However, the catecholamine distribution in the heart appears to be nonuniform and no general agreement exists on its exact pattern. For example, Angelakos et al[3,4]. demonstrated in the rabbit and dog that the predominant catecholamine was norepinephrine (NE). NE concentration was higher in right heart regions than in left heart regions in both the atria and ventricles. On the other hand, Shore et al[5]. reported no significant difference in NE content of the right and left atria or the ventricles in the dog heart. Other studies have reported on epinephrine and dopamine[6,7].

In the last few years, analytical methods for catecholamine measurements have impro-

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ved in both sensitivity and selectivity[8-14]. In the study here, on the regional distribution of catecholamines in the heart, I measured catecholamines by high-performance liquid chromatography (HPLC) with an electrochemical detection (ED).

Materials and Methods

Chemicals

Epinephrine bitartrate and 3, 4-dihydroxybenzylamine (DHBA) hydrobromide were purchased from Sigma (St. Louis, MO, U.S.A.). Norepinephrine hydrochloride and dopamine hydrochloride were purchased from Katayama Chemicals (Osaka, Japan). Alumina (Woelm neutral grade I) was activated as described by Anton and Sayre[15]. Other chemicals were all of reagent grade.

Reversed-Phase High-Performance Liquid Chromatography

The LC-304 liquid chromatograph [Bioanalytical System (BAS), West Lafayette, IN, U.S.A.] was composed of a PM-29 dual piston pump, an injection valve Rheodyne 7125 (Berkeley, CA, U.S.A.) with a 200 μl loop, a Biophase ODS column (250 mm×4 mm I.D., 5 μm average particle size) and an electrochemical detection LC-4 (BAS). The detector was operated at+0.65 V with an Ag/AgCl reference electrode (Model BAS TL 5) consisting of a paraffin-oil-based carbon paste (CPO) working electrode. Analysis was performed with 0.1 M sodium pentansulfonate and 0.1 mM Na_2-EDTA at a constant flow-rate of 1.0 ml/min and a column temperature of 20°C. The mobile phase was degassed by vacuum.

Sample Preparation

Wister rats (130-250g), guinea pigs (350-400g), rabbits (3.0-3.5Kg) and mice (15-20g) of either sex were used. The guinea pigs and rabbits were killed under pentobarbital anesthesia, and the other animals were killed by decapitation. The hearts were removed immediately, and the heart regions were dissected out on a glass plate placed over ice. The right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), and interventricular septum (IVS) were separated. The preparations were quickly frozen on dry ice and stored at −80°C until assay.

Analytical Procedure

The frozen tissues were cut into small pieces and homogenized in 350 μl of 0.2 M perchloric acid containing 0.1% Na_2EDTA and 0.1% $Na_2S_2O_5$, and 50 μl of DHBA (1 pmol/10 μl) added as internal standard. The mixture was homogenized in an ice-bath for 10 sec with an ultrasonic probe homogenizer (Model UR-200P, Tomy Seiko Co., LTD., Tokyo, Japan) at a setting of 5. The homogenates were placed in a 1.5 ml conical centrifuge tube with 5-10 mg activated alumina and adjusted to pH 8.5 by addition of 80 μl 2 M Tris. The catecholamines were adsorbed on the alumina by shaking at room temperature for 10 min; the alumina was washed three times for 30 sec with 500 μl distilled water. After the final washing, the tube was centrifuged (3,000 r.p.m., 1 min) and any excess liquid was discarded. The amines were eluted from the alumina by voltexing for 10 min with 50 μl of 0.5 M hydrochloric acid. After centrifugation, a 10 μl aliquot of the supernatant was injected into HPLC with a Hamilton microsyringe. The system allowed injecting up to 30 μl.

Protein concentration was measured according to the method of Hartree[16], using bovine serum albumin as the standard. Statistical analyses were performed by the Student's t-test.

Results

Fig. 1A shows the chromatogram of the standard mixture containing 1 pmol each of NE, E, DA and DHBA. NE, E, DHBA and DA were eluted at 3.6, 5.2, 6.1 and 9.6 min, respectively. Fig. 1B is a chromatogram of catecholamines assayed from the rat right atrium. Both NE and DA were detected in all examined heart regions. E was not always detectable but trace amounts were occasionally present. E could not be often detected in the nonspecific peaks (NS) derived from heart tissues.

The coefficients of variation of NE, E and DA were 2.1, 3.6 and 4.0%, respectively (n=10). NE, E and DA were recovered at 76.7, 73.3 and 68.5%, respectively (n=10).

As shown in Fig. 2, all substances analyzed had linear curves of a peak height proportional to the concentration added (bet-

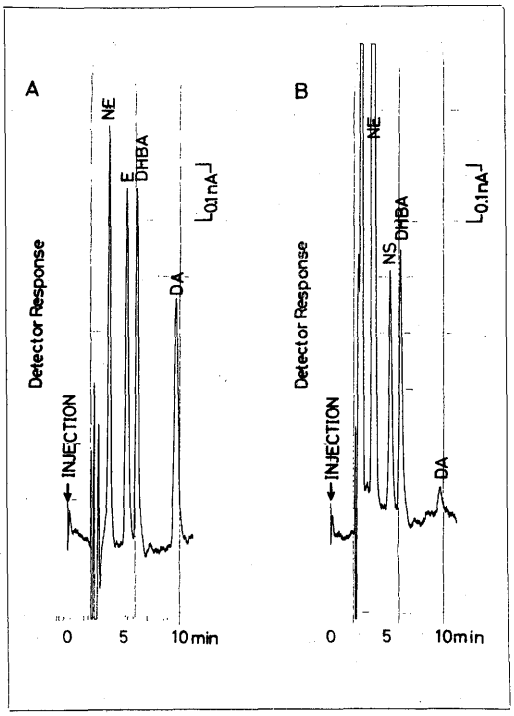

Fig. 1 Chromatograms of HPLC assays of tissue catecholamines.
(A) Standard mixture containing 1 pmol each of injected norepinephrine (NE), epinephrine (E), 3, 4-dihydroxybenzylamine (DHBA) and dopamine (DA).
(B) Tissue extract from the rat right atrium. E was not detectable at the nonspecific peak (NS). Tissue=0. 38 mg protein; NE=40. 8 pmol/mg protein; DA=1. 80 pmol/mg protein.

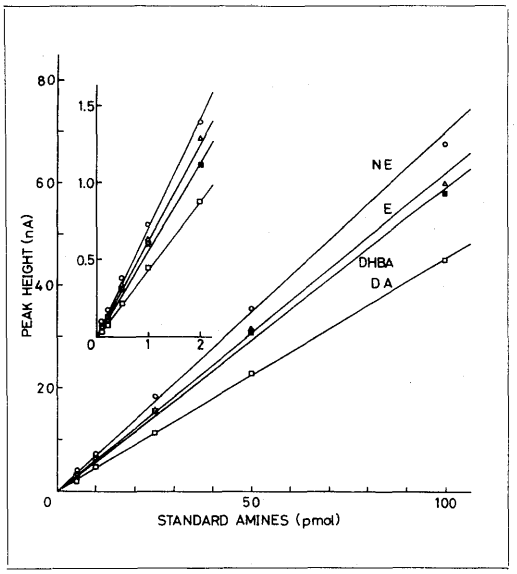

Fig. 2 Standard curves of norepinephrine (NE), epinephrine (E), 3, 4-dihydroxybenzylamine (DHBA) and dopamine (DA) in HPLC with electrochemical detection (ED) for peak height. Ten microliters of a sample containing various amounts (0. 01 pmol to 100 pmol) of NE (○), E (△), DHBA (■) and DA (□) were injected into the column, and each was detected by HPLC-ED. The HPLC conditions are described in the Methods.

ween 0 and 100 pmol). The minimum assay range for NE, E and DA was about 0. 03, 0. 04 and 0. 05 pmol, respectively.

The time course of catecholamine degradation was also studied (Fig. 3). Catecholamines in acidic extract were stable in ice-cold temperature for 8 hours, and the assay procedure was completed within this time. Catecholamines dissolved when left at room temperature, and about 60% of DA dissolved in 8 hours at room temperature.

Table 1 and Fig. 4 show the contents of NE and DA by heart regions in the examined species. In all species the NE concentration was higher in the atria than in the ventricles. DA had almost the same distribution as NE. In the guinea pig atria, NE concentration was markedly higher in RA than in LA ($p<0.02$). In the mouse the average NE concentration was higher in LA than in RA but not significantly. There was no difference of NE content between RA and LA in the rat and rabbit. The average DA concentration was higher in LA than in RA in only the mouse. Furthermore in this species, the DA concentration was much higher in LA than in RV. In the guinea pig the ventricle NE concentration was significantly higher in RV than LV and in IVS than LV ($p<0.01$, RV vs LV; $p<0.02$, IVS vs LV). In the rat and rabbit the

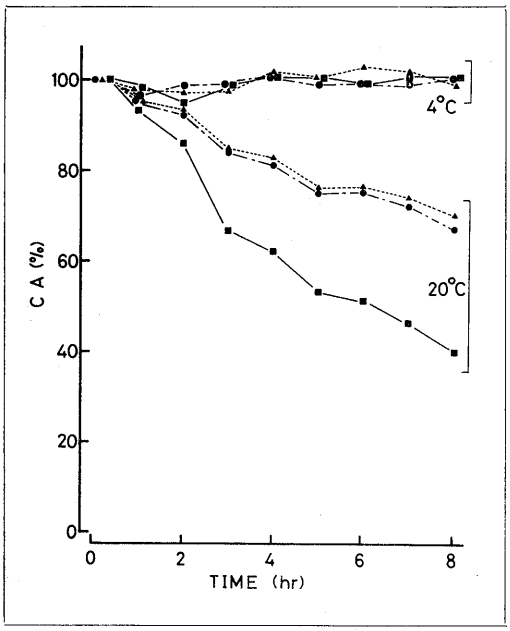

Fig. 3 Time course of degradating catecholamines.
NE=norepinephrine (●); E=epinephrine (▲); DA=dopamine (■); CA=catecholamine.

average NE concentration was higher in RV than LV but not significantly. In the guinea pig DA concentration was significantly higher in IVS than in LV ($p<0.01$), but the average DA concentration was higher in RV than LV in the guinea pig, rabbit and mouse but was similar in all three regions of the rat ventricle.

Discussion

The trihydroxyindole fluorometric and radioenzymatic assay procedures have been widely used to measure small amounts of catecholamines[8,9,14]. An improved fluorometric assay has been used for biopsy studies in diagnostic cardiac catheterization[2]. In 1975 Kissinger et al[17]. reported on a new method of catecholamine analysis which combined HPLC and ED, and this method has been used for the catecholamine determinations in brain tissues[10,11] and for plasma samples[12,13]. The quantitative method of HPLC-ED described here enabled measurements of picomoles of catecholamines with

Table 1 Heart levels of NE and DA in four mammalian species

Species	Amine	Amine concentration (pmol/mg protein)				
		RA	LA	RV	LV	IVS
Rat	NE	87.8±7.24 (14)	82.2±3.86 (14)	44.8±4.53 (15)	36.2±3.50 (13)	26.9±3.95 (14)
	DA	1.71±0.27 (13)	1.76±0.38 (14)	0.82±0.19 (15)	0.93±0.29 (13)	0.97±0.18 (14)
Guinea pig	NE	324±63.2	150±13.9	158±35.2	71.8±5.98	110±11.4
	DA	2.00±0.30 (6)	1.91±0.43 (6)	1.44±0.60 (6)	0.33±0.20 (6)	0.18±0.09 (6)
Rabbit	NE	114±27.0	93.1±17.9	88.3±18.8	50.6±9.28	48.6±8.86
	DA	4.50±0.80 (8)	3.07±0.89 (8)	3.90±1.06 (8)	2.41±0.67 (8)	1.92±0.35 (8)
Mouse	NE	117±35.8	153±57.3	94.2±26.1	109±43.8	47.7±10.2
	DA	3.75±1.90 (9)	5.72±3.90 (8)	3.57±1.41 (9)	2.57±1.22 (9)	1.21±0.49 (9)

Values represent means±S.E.M. Numbers in parentheses refer to the number of animals used. NE=norepinephrine; DA=dopamine, RA=right atrium; LA=left atrium; RV=right ventricle; LV=left ventricle; IVS=interventricular septum.

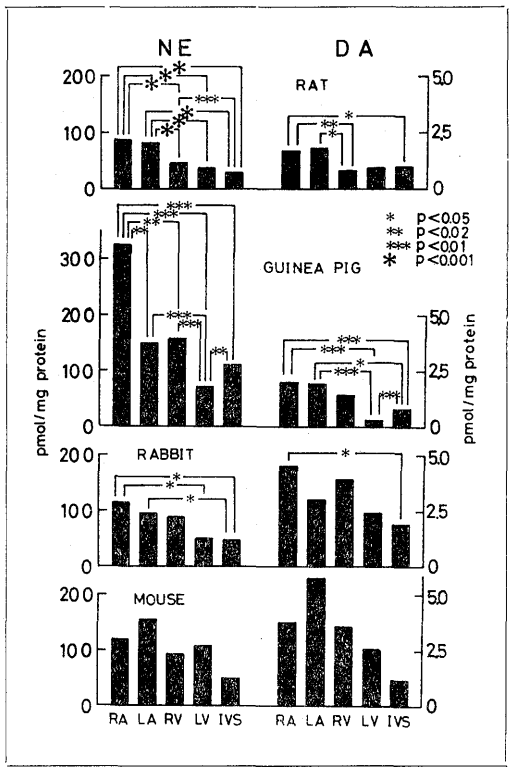

Fig. 4 NE and DA concentrations in various heart regions in different mammalian species. Values are means from Table 1. NE=norepinephrine; DA=dopamine; RA=right atrium; LA=left atrium; RV=right ventricle; LV=left ventricle; IVS=interventricular septum.

small amounts of heart tissues.

The present study has shown that the predominant catecholamine in the heart was NE. NE and DA were present in all samples but E was not always detected. Moreover, the NE concentration was higher in the atrium than in the ventricle and was markedly higher in RA than in LA in the guinea pig. In the rat, rabbit and mouse a statistically significant difference was not found between RA and LA. In the ventricles of the rat, guinea pig and rabbit the average NE concentration was higher in RV than in LV.

Shore et al[5]. who used the spectrofluorometric method on dogs found no differences in the NE content between RA and LA. In contrast Angelakos et al[3]. used the trihydroxyindole fluorometric method and reported that there was a small but significant difference between NE concentration in RA and LA in rabbits and guinea pigs. Others have reported on differences in NE content between RA and LA and between the atria and ventricles in rats, rabbits, guinea pigs and cats[3,6].

A higher NE concentration in RV was found in the rat, guinea pig, rabbit and cat[3,6]. Our results on the rat and rabbit have demonstrated that the average NE concentration was higher in RV than LV but not significantly. Angelakos[4] and Shore et al[5]. reported that the difference in NE concentration between RV and LV was small and not statistically significant in the dog. However, Angelakos et al[18]. reported that the NE concentration was highest in the rabbit RV. In the contrast, in the mouse, NE was found in larger amounts in LA and LV than in RA and RV, although the contrasting differences were not statistically significant. Therefore, some differences seem to exist in NE heart concentrations by animals, but the analytical methodology used probably account for some of the reported discrepancies.

The DA levels in the present study were higher in the atrium than in the ventricle and were higher in the right heart regions than in the left heart regions in rats, guinea pigs and rabbits. In contrast, in the mouse, the DA level was highest in LA. These results agree with other investigators[3,4,18].

In this study E was not always detectable, and even when found, it was at trace levels. Angelakos et al[3]. reported that E was absent or very low in rabbit atria but present at small but definite concentrations in ventricles, with RV containing more than LV. On the other hand, Serrano et al[5]. reported that the concentration of E was higher in

the atria than in the ventricles. The heart has measurable but trace amounts of enzyme phenylethanolamine-N-methyltransferase (PN -MT)[19]. But de novo synthesis of heart E seems to be at trace levels. E in the heart reflects blood-borne amine and in our chromatographic data (Fig. 2) it was not always detected in the nonspecific peak.

Histochemical studies[3,18] have indicated that in most areas of the heart the density of the catecholamine-containing fibers, which are postganglionic sympathetic fibers roughly equalled the chemically determined catecholamine concentration. So, differences in NE and DA concentrations in various heart regions of the same species may reflect distributions of adrenergic fibers and/or the turnover rate of catecholamines of those regions.

Thus, these results suggest that it is necessary to consider differences in catecholamine concentrations by species and regions, in addition to catecholamine metabolism when examining myocardial catecholamines.

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