DETERMINATION OF MONOAMINES AND TEN OF THEIR METABOLITES IN THE RAT BRAIN AND HEART BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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The method of high performance liquid chromatography with electrochemical detection (HPLC-ED) is spreading ever more widely as a means of determining the content of monoamines and their metabolites in biological objects [5-7]. However, despite the evident advantages of this approach (high sensitivity, selectivity, comparative ease of reproduction), it has not yet been finally researched. For instance, the total number of components which can be determined simultaneously does not exceed 6-8, due to the difficulty of selecting adequate conditions for chromatographic separation: parameters of the column, properties of the mobile phase (composition, pH, ionic strength, character of the ion-pair reagent).

In the present investigation the following substances were determined by HPLC-ED in the rat brain and heart: noradrenalin (NA) and its metabolites — normetanephrine (met-NA), 3-methoxy-4-hydroxyphenylglycol (MOPEG), 3,4-dihydroxyphenylglycol (DOPEG), vanilylmandelic acid (VMA); adrenalin and its metabolite metanephrine (met-A); dopamine (DA) and its metabolites 3-methoxytyramine (met-DA) and 3,4-dihydroxyphenylacetic acid (DOPAA), the DA precursor 3,4-dihydroxyphenylalanine (DOPA); the serotonin precursor 5-hydroxytryptophan (OTP), and its metabolite 5-hydroxyindoleacetic acid (OIAA). Changes in the balance of monoamines and their metabolites in the heart and brain of rats were caused by injections of the following preparations: the monoamine oxidase (MAO) inhibitor iproniazid [2]; reserpine, which disturbs intraneuronal storage of monoamines [3], and imipramine, an inhibitor of monoamine reuptake into nerve endings [1].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 180-200 g. The preparations were injected intraperitoneally in the following doses: reserpine 5 mg/kg, iproniazid 100 mg/kg, and imipramine 10 mg/kg. The rats were decapitated 24, 4, and 2 h after injection of reserpine, iproniazid, and imipramine respectively. The animals' brain and heart were removed in the cold and homogenized in cold water (0-4°C) in the ratio of 1:4 (w/v) by means of an "Ultra Turrax" homogenizer for 2 min at 20,000 rpm. The tissue homogenate was diluted twice with 0.2 N perchloric acid and centrifuged at 15,000 g for 30 min on the K-24 centrifuge at 0-4°C. The supernatant was filtered through Millipore filters (type GS, pore diameter 0.22 μ). A sample of 50 μl was taken from the resulting filtrate and introduced into the chromatographic system. The system for HPLC consisted of the following components: A type SP 8700 high-performance liquid chromatograph from Spectra Physica, USA, an electrochemical detector from Metrohm, Switzerland, an "Ultrasphere ODS C_{18}" column (250 × 4.6 mm, particle diameter 5 μ) from Beckman, USA. The composition of the mobile phase was as follows (in mM): Na-phosphate buffer (pH 2.7) 100, the disodium salt of EDTA 0.1, triethylamine 0.5, the sodium salt of heptane-sulfonic acid 1. The concentration gradient of methanol in this mobile phase was 0.2-20% from the 3rd until the 35th minute, from the 35th to the 45th minute the mobile

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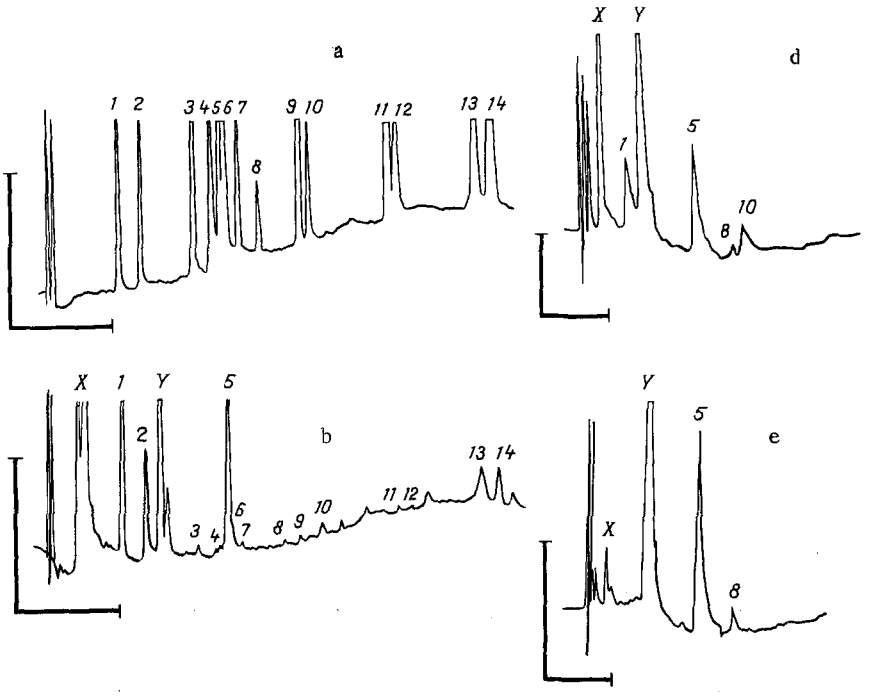

Fig. 1. Chromatograms of gradient separation of a standard mixture of monoamines and some of their metabolites. *a*) Standard solution of monoamines, b) control (rat heart), d) rat brain (4 h after intraperitoneal injection of 10 mg/kg of imipramine), e) rat heart (24 h after intraperitoneal injection of 10 mg/kg reserpine). 1) Na, 2) DOPEG, 3) adrenalin, 4) VMA, 5) DBA, 6) DOPA, 7) met-NA, 8) MOPEG, 9) DA, 10) met A, 11) OTP, 12) DOPAA, 13) Met-DA, 14) OIAA. Calibration: 0.01 and 10 min.

TABLE 1. Effect of Reserpine, Iproniazid, and Imipramine on Concentrations of Monoamines and Their Metabolites (in μg/g tissue) in Rat Heart and Brain (M ± m)

Substance	Heart				Brain			
	control	reserpine	iproniazid	imipramine	control	reserpine	iproniazid	imipramine
DA	40±6	0	0	15±3	48±8	0	0	0
met-DA	200±23	0	300±40	240±34	300±50	0	1050±150	290±36
DOPAA	50±20	0	0	25±10	90±25	0	60±15	130±30
NA	730±90	20±6	190±20	660±80	480±60	100±20	300±45	520±70
Met-NA	40±8	0	0	40±9	10±3	0	70±14	1±0,4
MOPEG	75±10	10±3	410±47	320±28	80±10	20±5	200±23	110±15
Adrenalin	15±5	0	5±3	0	1±0,3	0	1±0,5	0
Met-A	200±21	0	0	100±13	400±43	0	800±87	400±45
OTP	50±8	0	0	60±7	150±20	0	0	80±12
OIAA	490±56	0	0	400±48	600±67	0	0	430±50

Legend. 0) Under 1 ng/g tissue, after intraperitoneal injection; iproniazid 100 mg/kg, imipramine 10 mg/kg, both 4 h after intraperitoneal injection.

phase contained 20% of methanol, and from the 45th to the 65th minute its concentration fell to 0.2%. The rate of flow was 0.7 ml/min. The data were recorded in an electrochemical cell by means of working and additional electrodes, made from carbon glass, at a potential of 0.8 V relative to the Ag-AgCl electrode. The data were processed by SP 4200 integrator (Spectra Physica). The experimental results were subjected to statistical analysis with calculation of mean values and their confidence intervals at the P = 0.05 level. The reagents used were from Sigma (USA).

EXPERIMENTAL RESULTS

A chromatogram of gradient separation of a standard mixture of monoamines and some of their metabolites, when the concentration of each component of the mixture was 0.1 mg/ml, is shown in Fig. 1. Dihydroxybenzylamine (DBA, 0.1 mg/kg) was used as the internal standard, because of the similarity of its structure to that of natural catecholamines. The area of the

TABLE 2. Postmortem Changes in Concentrations of Monoamines and Their Metabolites (in ng/g tissue) in Rat Heart (M ± m)

Time, h	DA	DOPAA	NA	Met-NA	MOPEG	DOPEG	Adrenalin	Met-A	DOPA	OTP	OIAA
0	30±6	60±9	660±90	30±9	80±10	610±80	10±3	220±23	10±3	50±7	450±60
3	40±7	60±8	340±70	60±20	70±9	510±70	30±9	600±80	20±7	60±8	600±80
6	10±3	20±7	410±75	70±23	80±10	510±70	40±11	800±100	10±3	20±6	490±70

DBA peak in each chromatogram was taken as 1, and the areas of the peaks for other substances, proportional to their concentrations, were calculated relative to it. All the test monoamines and their metabolites were determined in the rat heart (Fig. 1a, Table 1) and brain (Table 1). In addition, as Fig. 1b shows, unidentified peaks also were discovered in the homogenate of rat heart by the HPLC-ED method; the most important of them from the point of view of area were designated X and Y. These peaks are probably different in nature. For instance, the area of peak X decreased in response to injection of reserpine into the rats (Fig. 1e), and reserpine is known to deplete the intracellular monoamine depots [3, 4], and the peak itself assumed a rather different outline under the influence of imipramine (Fig. 1d). The character of the Y peak was unchanged in the rat heart (Table 1) under the influence of reserpine (Fig. 1e) and imipramine (Fig. 1d), and this makes it unlikely that the substance represented by the Y peak is a compound of monoamine nature.

As Table 1 shows, reserpine (10 mg/kg) considerably reduced the concentration of catecholamines and their metabolites in the brain and heart 24 h after its injection into rats. This is in agreement with results obtained by other workers [3, 4] who found that reserpine depletes monoamine reserves. Levels of adrenalin, NA, DA, DOPAA, OTP, and OIAA in the heart and brain also fell 4 h after injection of the MAO inhibitor iproniazid (100 mg/kg) into rats, more especially in the heart (Table 1). Under the influence of iproniazid the concentrations of methylated derivatives of catecholamines (met-A, met-NA, met-DA) in the brain increased, whereas in the heart the met-A and met-NA levels fell.

Imipramine (10 mg/kg) caused the concentrations of DA, met-NA, and OTP in the brain and also the levels of DA, DOPAA, adrenalin, and met-A in the heart to fall 4 h after injection into rats (Table 1). Under the influence of imipramine the MOPEG concentration rose in both organs, by a greater degree in the heart than in the brain.

Table 2 gives data on postmortem changes in concentrations of biogenic amines and their metabolites in the rat heart. As Table 2 shows, during the 3 h which elapsed after decapitation, the NA concentration in the heart fell by 30-40%, the DOPEG level fell by 10%, whereas the concentrations of adrenalin, met-NA, and met-A rose. During the next 3 h the concentrations of DA, DOPAA, and OTP in the rats' heart fell. Thus the HPLC-ED method used in this investigation can ensure reliable separation and identification of more than 10 monoamines and their metabolic products in the tissues of animal organs simultaneously. The broad spectrum of substances detected can give a deeper insight into the biochemical basis of the normal state and pathological states of different genesis, and can reveal changes in the balance of monoamines and their metabolites during administration of drugs, and on that basis, conclusions regarding the direction of their action *in vivo* can be drawn.

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