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NORADRENALINE CONTENT IN THE HEART AND SPLEEN OF THE MOUSE UNDER NORMAL CONDITIONS AND AFTER ADMINISTRATION OF SOME DRUGS

BY

D. F. SHARMAN, S. VANOV AND MARTHE VOGT

From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge

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The noradrenaline content of the heart and spleen was investigated in normal mice and in mice treated with drugs. A modification of the methods of Bertler, Carlsson & Rosengren (1958) was used for extraction, and of v. Euler & Floding (1955) for fluorimetric estimation of the amine. In normal mice the mean noradrenaline content of the heart was 0.55 μg/g and that of the spleen 0.26 μg/g fresh tissue. Iproniazid (100 mg/kg), nicotine (0.1 mg/kg) and histamine (0.5 mg/kg), given 1 and 3 hr before killing the mice, did not significantly change the concentration of noradrenaline in the heart. Neither did nicotine and histamine, administered 1 hr before death, significantly alter the noradrenaline content of the spleen. The rapid changes in the catechol amine content of mouse tissues reported with these drugs by De Schaepdryver & Preziosi (1959) were not observed. In contrast, reserpine (2.5 mg/kg), methyl reserpate methyl ether (1 mg/kg), and methyl 18-epireserpate methyl ether (2 mg/kg) caused severe depletion of noradrenaline from the heart and spleen of the mice.

Of the numerous reports on the normal catechol amine content of mammalian tissues few concern mice. The effect of drugs on the amine stores in mice has also been studied very little. In 1959 De Schaepdryver & Preziosi, using adsorption of adrenaline and noradrenaline on aluminium oxide, elution by sulphuric acid and fluorimetric estimation, analysed the catechol amine concentrations of the suprarenal, heart, liver and spleen in normal mice. They found that a number of drugs rapidly deplete the amines from these tissues. Thus, 1 hr after the intraperitoneal injection of nicotine (0.1 mg/kg) or iproniazid (100 mg/kg) complete loss of both amines from the heart was observed. Similarly, 1 hr after administration of nicotine or histamine (0.5 mg/kg), no noradrenaline was detected in the spleen. Very rapid recovery of both cardiac and splenic noradrenaline to normal and to levels above normal was observed within a few hours. Even after reserpine, recovery of the cardiac noradrenaline was nearly complete in 48 hr. However, experiments of several authors (Carlsson, Rosengren, Bertler & Nilsson, 1957; Muscholl & Vogt, 1958; Paasonen & Krayer, 1958) have shown in other species that, after depletion by reserpine, it took many days for the concentration of tissue noradrenaline to recover. These results suggested that, in contrast to other species, the stores of noradrenaline in mouse tissues are labile and easily affected by drugs. Other species differences in the metabolism and storage of catechol amines have been recently demonstrated by Sanan & Vogt (1962).

The present study was made in order to investigate further what appeared to be unusual behaviour of the tissue catechol amines in the mouse and to repeat the experiments of De Schaepdryver & Preziosi (1958) by using another method of estimation. The noradrenaline content of the heart and spleen was estimated in normal mice and in mice treated with iproniazid, nicotine, histamine, reserpine and two of its analogues.

METHODS

Animals and drugs. Albino mice of both sexes (predominantly male) were used. The body weight of the animals ranged from 20 to 40 g. The animals were kept in groups of 10 or 20 at room temperature. Food in pellets and tap-water were allowed *ad libitum.*

Special precautions were taken to minimize the biological variations related to the origin of the animals. The majority of the experiments were carried out on one breed of mice reared in the laboratories; for the remaining experiments animals from a single strain were obtained from a dealer. The organs of a group of five or six mice were pooled, since this number provided sufficient tissue for an accurate estimation of noradrenaline. The groups were made as uniform as possible in respect of body weight and sex.

The following drugs were used: iproniazid phosphate (Marsilid, Roche Products), nicotine hydrogen tartrate (British Drug Houses), histamine acid phosphate (British Drug Houses), reserpine (Serpasil, Ciba), methyl reserpate methyl ether hydrochloride and its 18-epi isomeride (Su-8842 and Su-9064, Ciba). The doses of nicotine and histamine are expressed in terms of the base and those of all other drugs as weight of the salts.

All drugs were dissolved in an isotonic solution of sodium chloride and injected intraperitoneally in a volume of 0.5 ml./20 g body weight. Controls were injected with the same volume of sodium chloride solution.

The animals were killed by decapitation and exsanguinated. The hearts were dissected as rapidly as possible. The blood and the blood clots were removed by blotting on filter paper. Before excising the spleens, electrical stimulation of the splenic nerves (square waves of 100 msec duration, 12/sec, 6 V) was carried out for a few sec in an attempt to induce contraction of the organ.

General procedure. Immediately after their removal the tissues were weighed and homogenized in 0.1 N hydrochloric acid (2 ml./g tissue) in the presence of a few mg ascorbic acid. The homogenate was diluted with about twice its volume of water. The proteins were precipitated by adding 1 vol. 0.8 N perchloric acid. The further treatment of the extract was essentially the same as described by Bertler *et al.* (1958). The main characteristic of this method is the absorption of the catechol amines on the cation exchange resin Dowex-50×8 in a column. The elution was done by gravity, and 10 ml. of 1.2% hydrochloric acid was used. The eluates were analysed fluorimetrically for noradrenaline with an Aminco-Bowman spectrophotofluorimeter. The trihydroxyindole method of von Euler & Floding (1955) was used in a somewhat modified way: the reaction (oxidation with potassium ferricyanide and rearrangement with alkali) was performed in the dark and the *p*H of the M acetate buffer was 6.5 (Sharman, 1960). Von Euler & Lishajko (1961) improved the original method by addition of ethylene diamine to the mixture of sodium hydroxide and ascorbic acid. In the majority of the estimations, ethylene diamine was used but was incorporated in the sodium hydroxide solution, since this was added separately from ascorbic acid, as suggested by Crawford & Law (1958).

The samples were activated at 400 mμ, and the resulting fluorescence was read at 500 mμ. The catechol amines in the extracts were read against a standard noradrenaline solution treated in the same way as the sample. The results were expressed as μg of noradrenaline base. Extract blanks were obtained after allowing a portion of the eluates to fade for 7 min in the presence of potassium ferricyanide and sodium hydroxide and adding ascorbic acid afterwards.

A polished Araldite rod was used as a standard for the calibration of the spectrophotofluorimeter. The sensitivity was checked and corrected if necessary before each reading.

All solutions for the fluorimetric analysis were made with deionized water. Also, all glassware was rinsed with deionized water in order to prevent possible interference by some fluorescent material contaminating the ordinary distilled water.

Test for sensitization or masking. To 1 ml. of each eluate a known amount of noradrenaline was added. The fluorescence was developed in the usual way, and the increase in fluorescence above that obtained with 1 ml. of eluate only was measured. This increase should theoretically be identical with the fluorescence produced by the added amount of noradrenaline in pure solution. Any deviation from the expected reading would disclose either masking or intensification of the reaction by some material in the tissue. Masking was hardly ever found, but the fluorescence produced in the extracts was sometimes slightly higher than in pure solution; the difference never exceeded the equivalent of 2.0 ng noradrenaline.

Recovery. The recovery was checked in every single experiment. For this purpose two portions of 3 ml. each were pipetted off from the homogenates which had been diluted with water to either 6.5 or 7 ml. To one of these portions 0.1 μg of noradrenaline was added. The recovery was determined by comparing the fluorescence of the two portions. The mean recoveries were of the order of 80%.

Sensitivity. Because of these various controls, the fluorescence was measured on about 3/70th of the original homogenate, as seen from the following plan:

6 hearts (spleens) homogenized with twice their weight of 0.1 N HCl → Dilution to 6.5 or 7 ml. → 3 ml. = A (test); 3 ml. + 0.1 μg noradrenaline = B (recovery)

A and B each yielded 10 ml. eluates, which were used as follows:

2 ml. for duplicate estimation on 1 ml. each;

2 ml. for duplicate check on masking or sensitization (1 ml. eluate + known amount of noradrenaline);

1 ml. for faded (extract) blank.

If 1 g of tissue is worked up, reliable readings can be obtained by this procedure down to a tissue concentration of noradrenaline of 0.1 μg/g. Estimations of lower concentrations are only approximate.

RESULTS

The noradrenaline content of the heart and spleen in normal mice. The animals referred to here as "normal" are either untreated mice or controls injected with 0.9% sodium chloride solution. The noradrenaline content of the heart and spleen for these is summarized in Table 1. The penultimate column shows the individual concentrations, means, standard deviations and standard errors of the mean, corrected for recovery. The percentage recovery will be found in the preceding column.

The coefficient of variation was not significantly changed when the concentrations were expressed as μg noradrenaline/100 g body weight instead of μg/g fresh tissue. No consistent relationship was found between body weight and noradrenaline concentration. Since estimations of noradrenaline concentrations below 0.1 μg/g tissue have a low degree of accuracy, the second decimal in the table is only an approximation.

TABLE 1

NORADRENALINE CONTENT OF THE HEART AND SPLEEN IN NORMAL MICE

s.d.=standard deviation. s.e.=standard error of the mean

Group	No. of animals and sex	Total body wt. in g	Tissue	Total wt. of tissue in mg	Noradrenaline µg/g tiss.	Noradrenaline µg/100 g body wt.	Recovery %	Noradrenaline, µg/g tissue, corrected for recovery	Remarks
1	6 ♂	183	Heart	860	0·45	0·21	63	0·71	
2	6 ♂	166	Heart	850	0·37	0·18	65	0·57	No
3	6 ♂	231	Heart	1046	0·52	0·23	92	0·56	treatment
4	6 ♂	237	Heart	1072	0·53	0·24	87	0·61	
	Mean	204		957	0·47	0·21	76·7	0·61	
	s.d.				0·07	0·026		0·07	
	s.e.				0·035	0·013		0·03	
5	6 ♂	229	Heart	1006	0·56	0·24	96	0·58	
6	5 ♂	223	Heart	936	0·59	0·25	71	0·83	
7	6 ♂	214	Heart	904	0·39	0·16	60	0·65	
8	6 ♂	233	Heart	1163	0·59	0·29	70	0·84	Saline-
9	6 ♀	194	Heart	914	0·59	0·27	108	0·55	treated
10	6 ♀	185	Heart	845	0·84	0·37	95	0·88	controls
11	6 ♀	186	Heart	890	0·72	0·34	88	0·82	
12	6 ♂	176	Heart	895	0·43	0·21	69	0·61	
	Mean	205		944	0·59	0·28	82·1	0·72	
	s.d.				0·14	0·07		0·13	
	s.e.				0·05	0·03		0·05	
				Overall mean	0·55	0·26	80·3	0·68	
				s.d.	0·14	0·06		0·13	
				s.e.	0·04	0·02		0·04	
1	6 ♀	194	Spleen	943	0·29	0·13	100	0·29	
2	6 ♀	186	Spleen	1263	0·21	0·13	82	0·25	Saline-
3	6 ♀	188	Spleen	860	0·33	0·15	103	0·32	treated
4	5 ♀	147	Spleen	690	0·38	0·17	69	0·55	controls
5	6 ♂	195	Spleen	979	0·26	0·12	65	0·40	
6	6 ♂	171	Spleen	1006	0·17	0·09	75	0·22	
7	6 ♂	147	Spleen	922	0·16	0·09	53	0·30	
	Mean	176		952	0·26	0·12	78·1	0·33	
	s.d.				0·08	0·03		0·11	
	s.e.				0·03	0·01		0·04	

The mean for the noradrenaline content of the *heart* of the untreated mice was 0.47 µg/g tissue with a standard deviation of ±0.07 (Table 1). The corresponding figures for the saline-treated controls were 0.59±0.14 µg/g, and the mean of both groups 0.55±0.14 µg/g. The difference between the two groups becomes smaller (and non-significant) when correction is made for recovery (Table 1, penultimate column).

The *spleen* was only analysed in saline-treated mice. The mean noradrenaline concentration in seven groups was 0.26±0.08 µg/g tissue. This figure is lower than that obtained by De Schaepdryver & Preziosi (0.686 µg/g tissue). In order to obtain the highest concentration possible, the splenic nerves were stimulated for a few sec so as to reduce the spleen volume ; not much contraction was, however, observed, probably because the spleen was already contracted as a result of the exsanguination.

The results both for the heart and spleen show considerable variations which are reduced by correcting for recovery. Consistent differences between the two sexes were not found, but an occasional group of female mice had a slightly higher noradrenaline content of their spleens.

The noradrenaline content of the heart and spleen of the mouse after administration of drugs. The effects of the drugs studied are presented in Table 2. These drugs might be conveniently divided into two groups. Firstly, iproniazid, nicotine and histamine, and, secondly, reserpine and two of its analogues—methyl reserpate methyl ether and methyl 18-epireserpate methyl ether. The effect of reserpine served to confirm that the method would demonstrate a depletion of catechol amines if this were to occur.

TABLE 2

NORADRENALINE CONTENT OF THE HEART AND SPLEEN OF THE MOUSE AFTER TREATMENT WITH DRUGS

Group	No. of animals and sex	Total body wt. in g	Tissue	Total wt. of tissue in mg	Dose of drug (mg/kg)	Duration of exp. (hr)	Noradrenaline µg/g tiss.	Noradrenaline µg/100 g body wt.	Recovery %
1	6 ♂	214	Heart	1002	Iproniazid, 100	1	0·54	0·25	87
2	6 ♂	252	Heart	1002		1	0·64	0·25	80
3	6 ♂	217	Heart	894		1	0·31	0·12	100
4	6 ♂	227	Heart	981		3	0·60	0·26	85
5	6 ♀	190	Heart	863	Nicotine, 0·1	1	0·74	0·33	134
6	6 ♀	185	Heart	891		1	0·66	0·31	77
7	5 ♀	141	Heart	659		1	0·76	0·35	92
8	6 ♂	180	Heart	832		3	0·50	0·22	70
9	6 ♂	165	Heart	978	Histamine, 0·5	1	0·35	0·20	62
10	6 ♂	154	Heart	765		1	0·48	0·23	78
11	6 ♂	239	Heart	917	Reserpine, 2·5	18	0·013	0·005	58
12	6 ♂	235	Heart	1058	Su-8842, 1	2	0·03	0·01	93
13	6 ♂	194	Heart	1052	Su-8842, 1	2	0·03	0·01	80
14	6 ♂	242	Heart	1264	Su-9064, 2	2	0·03	0·01	136
1	5 ♀	145	Spleen	708	Nicotine, 0·1	1	0·34	0·16	105
2	6 ♂	180	Spleen	797	Nicotine, 0·1	3	0·30	0·12	68
3	6 ♂	165	Spleen	1160	Histamine, 0·5	1	0·15	0·10	63
4	6 ♂	194	Spleen	960	Su-8842, 1	2	Less than 0·01		61

Table 2 shows that iproniazid, nicotine and histamine in the doses used did not produce significant changes in the noradrenaline content of the heart and spleen in the course of 1 or 3 hr.

In contrast with this, a single injection of reserpine provoked in 18 hr a profound fall in the noradrenaline content of the heart. Similarly, the two reserpine analogues, methyl reserpate methyl ether and its 18-epi isomeride, caused severe loss of noradrenaline from heart and spleen. In confirmation of Robison, Lucas, MacPhillamy, Barrett & Plummer (1961), the action of these compounds was found to be very fast, negligible amounts of the amine being left in the tissues after as little as 2 hr. The behavioural and autonomic effects of the doses used were less marked than those of reserpine, 2.5 mg/kg. There was obvious sedation and reduced reactivity to external stimuli, closure of the eyelids and tremor.

DISCUSSION

As Udenfriend (1959) stated, specificity and sensitivity are the most important criteria in the choice of a chemical method for the estimation of catechol amines. The specificity of the present method is based on the combination of selective adsorption and elution with a characteristic fluorescence. Of the catechol amines which might be mistaken for noradrenaline, isoprenaline has never been found in

the tissues examined, while adrenaline is present in such small amounts that it cannot seriously affect the results. The fluorescence derived from dopamine with this method is less than 1% of that derived from noradrenaline (Crawford & Law, 1958).

The sensitivity of the method is sufficient to measure accurately 0.1 μg noradrenaline in 1 g of fresh tissue, and is, therefore, adequate for the estimation of noradrenaline in 1 g of normal heart which is about 5 times that figure. Since the concentration of adrenaline in all mammalian species examined, including the rat, is only a few per cent. of that of noradrenaline, it was obvious that reliable figures for the adrenaline content of the organs of mice could not be expected with this method. In addition, it is known that the concentration of adrenaline in tissues supplied with sympathetic nerves varies much more than that of noradrenaline. No attempt was therefore made to estimate any adrenaline in the tissues analysed.

As a precaution, recoveries were carried out in each estimation to check on accidental losses. Though, on the average, the recovery was of the order of 80%, the variation of the losses suggests that the precision is greatly increased by estimating the recovery from each homogenate.

The noradrenaline content of the heart of normal mice found in the present work (overall mean 0.55 μg/g) is in good agreement with the figures reported by De Schaepdryver & Preziosi (1959) and by Porter *et al.* (1961).

The concentration in the spleen of normal mice, however (0.26 μg/g), was significantly lower than that found by De Schaepdryver & Preziosi (0.69 μg/g). In order to stabilize the weight of the spleen by expelling as much blood as possible, electrical stimulus was applied for a few sec to the splenic pedicle in the present experiments. It is most unlikely that this should have reduced the noradrenaline content of the splenic tissue, since Vogt (1954) has shown that stimulation of the superior cervical sympathetic nerve for 2 hr does not change the noradrenaline content of the ganglion. The reason for the lower figure may lie in a strain difference.

After reserpine and two of its analogues, the noradrenaline content of the heart and spleen fell to values so low that they were not significantly different from zero. In this the present results agree with those of De Schaepdryver & Preziosi with reserpine. When, however, iproniazid, nicotine and histamine were injected, and the tissues examined 1 and 3 hr later, the results obtained were at variance with those of De Schaepdryver & Preziosi (1959). These authors found no noradrenaline in the heart after iproniazid and nicotine, and none in the spleen after nicotine and histamine, whereas the foregoing experiments gave normal concentrations for the noradrenaline in all these instances. It is very difficult to explain these discrepancies. Our conclusion is that the noradrenaline content of the mouse organs is not changed more readily than that of other species and that the stores are just as stable in the mouse as in the rabbit or cat.

Nicotine and histamine are drugs which deplete catechol amines from the adrenal gland by a direct action on the medullary tissue. They are also known to cause stimulation of sympathetic ganglia. The present experiments with these drugs show that such stimulation is not accompanied by a depletion of the amines from their

peripheral storage sites. This is in agreement with the findings of Muscholl (1961) on the effect of nicotine and dimethylphenylpiperazinium iodide on cat atria, and of Sanan & Vogt (1962) with the latter drug on sympathetic ganglia.

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