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THE NATURE OF CARDIAC SYMPATHIN IN THE DOG

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Since the demonstration by Peart (1949) that stimulation of the splenic nerves of the cat releases a " sympathin " consisting predominantly of noradrenaline with a small admixture of adrenaline, similar results have been obtained for liver sympathin by Mann and West (1950) and for the substance released by stimulation of the hypogastric nerves by the same authors (1951).

Evidence on the nature of cardiac sympathin is available only for the frog's heart. Loewi (1936) demonstrated the actual release, on nervous stimulation, of a substance having the properties of adrenaline, and supporting evidence for its identity with adrenaline was obtained on extracts of heart muscle by Shaw (1938) and v. Euler (1946a). Cannon and Rosenblueth (1933) showed in the cat that stimulation of the cardio-accelerator strands from the right stellate ganglion caused the liberation into the blood mainly of a sympathin with excitor properties (sympathin E). Since that work, the identification of the sympathin released on stimulation of the nervi accelerantes has, as far as we know, not been attempted in the mammal. Mammalian heart extracts examined by v. Euler (1946b) were shown to contain a sympathin differing in its properties from pure adrenaline ; this was later shown in cattle to consist of a mixture of noradrenaline with from 8 to 20 per cent adrenaline (Goodall, 1950).

The present paper is concerned with the nature of the sympathin released into the coronary blood of dogs on stimulation of the cardiac sympathetic nerves.

METHODS

Dogs were anaesthetized with ether followed by chloralose. Except for the first experiments, the splanchnic nerves were cut bilaterally below the diaphragm, the abdominal wound was closed, artificial respiration started, and the chest opened. The branches of the sympathetic leaving the stellate ganglia for the heart were traced on both sides and a ligature placed around the nerves. The pericardium was opened, both vagi cut, heparin (600–650 u./kg.) injected intravenously, and a Morawitz cannula introduced into the coronary sinus. The cannula was coated with a thin layer of hard paraffin.

Except for the periods when blood samples were being collected, the coronary blood was returned through a by-pass into a jugular vein. The sympathetic nerves were stimulated by alternating current from a Ritchie–Sneath stimulator (Ritchie, 1944), 7.5–10 V. and shielded platinum electrodes being used. Stimulation was started 5 sec. before the

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collection of a "stimulation sample" of blood and continued during the whole period of collection. Samples of pulmonary venous blood were taken from the right atrium and of arterial blood from the carotid or femoral artery, and collected sometimes during the stimulation and sometimes during the control periods. The blood was drained into ice-cooled centrifuge tubes and the plasma extracted. The extracts were chromatographed on paper, either by the procedure of Crawford and Outschoorn (1950) or by its modification which employs HCl-phenol instead of SO_2-phenol (see Vogt, 1952, for details concerning the preparation of the extracts and the chromatographic procedure). The eluates were tested for noradrenaline on the blood pressure of the atropinized hexamethonium-treated rat (Crawford and Outschoorn, 1950; Outschoorn, 1952) and for adrenaline occasionally on the rat's blood pressure but usually on the rat's uterus (Gaddum, Peart, and Vogt, 1949). Assays of native plasma on the rat's uterus proved impracticable owing to a high concentration of interfering substances in the coronary blood. The final eluates were concentrated so as to contain in 0.5 ml. the amines from 5 ml. plasma. The yield of recovery experiments and the precautions to be taken to check for substances in the eluates which may interfere with the assays are described in the papers to which reference has been made.

RESULTS

During stimulation of the two nervi accelerantes, the heart rate became faster and there was an increase in the coronary flow and in the force of the beat. The acceleration was more obvious on stimulating the right nerve. It did not reach its maximum before a latent period of some seconds. On stimulating the left nerve acceleration was often absent, but an inotropic effect could always be seen.

In three preliminary experiments, in which the splanchnic nerves were left intact, all samples of coronary plasma contained fairly high concentrations of adrenaline (up to 22 mμg./ml.), and on stimulation of the accelerator nerves these concentrations sometimes increased even further. Since at least part of this adrenaline seemed to be of medullary origin, the splanchnic nerves were cut in the remaining experiments, whereafter the highest concentration of adrenaline which could ever be ascertained in any sample was 1.5 mμg./ml.

Noradrenaline.—In all experiments, in which splanchnotomy had been performed, it was possible to demonstrate the release of noradrenaline on stimulation of the nervi accelerantes (see Tables I and II). The amounts ranged from 50 to over 700 mμg./min. The output per min. was approximately doubled by stimulating the nerves on both sides instead of on the right side only (Exp. 10).

Adrenaline.—In Exps. 4–6 the assay of adrenaline was attempted on the native plasma (using the rat's uterus) and on the concentrated eluates (using the rat's blood pressure). Neither of these tests proved sensitive enough to detect any adrenaline. By determining the threshold of the assay preparation, it was possible to establish that no more than one-third of the amines released might be adrenaline (compare the released noradrenaline recorded in the last column of Table I with the threshold for adrenaline listed in columns 5 and 6 and representing the maximum amount of adrenaline which might have been released on stimulation).

More information was obtained in Exps. 7–10, in which, after chromatography, the concentrated eluates were tested on the rat's uterus. The results are shown in Table II. Only in Exps. 9 and 10 were there traces of adrenaline (1–1.5 mμg./ml.) found in the coronary blood, but their occurrence was not cor-

TABLE I

ESTIMATION OF ADRENALINE AND NORADRENALINE IN PLASMA OBTAINED FROM THE CORONARY SINUS

Exp. No.	Nature of sample	Duration of collection (sec.)	Volume of plasma (ml.)	Output per min. (in mμg.)			
				Adrenaline*		Noradrenaline	
				Native plasma, rat's uterus	Eluates, rat's B.P.	Eluates, rat's B.P.	Increase during stimulation
4	Control	25	12.5	<300	<375	<186	
	Stimulation	28	21.0	<450	<562	279	>93
	Stimulation	25	16.0	<384	<480	960	>719
	Control	25	16.2	<389	<486	<241	
5	Control	30	7.0	<70	<175	—	
	Stimulation	30	6.0	<60	<150	—	
	Stimulation	30	7.0	<70	<175	175	>88
	Control	30	7.0	<70	<175	<87	
	Stimulation	30	4.0	<40	<144	144	>80
	Control	36	6.2	<52	<129	<64	
6	Control	30	8.0	S	<200	80	
	Stimulation	30	11.0	S	<275	136	56
	Stimulation	30	11.0	S	<275	550	>520
	Control	30	6.0	S	<150	<30	

S = stimulating effect on the rat's uterus by interfering substances.

* The figures in the columns headed "Adrenaline" represent the threshold amounts detectable in each instance by the particular preparation and thus the maximum amount of that substance which might have been released into the coronary blood.

TABLE II

ADRENALINE AND NORADRENALINE IN PLASMA OBTAINED FROM THE CORONARY SINUS

(Estimation after chromatographic separation)

Exp. No.	Nature of sample	Duration of collection (sec.)	Volume of plasma (ml.)	Output per min. (in mμg.)				Percentage methylation
				Total		Change during stimulation		
				Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	
7	Control	35	13.3	<22.6	<141			
	Stimulation	60	10.2	<10.2	63	<10.2		
	Stimulation	60	8.0	—	100	—		
8	Control	60	12.0	<24	30			
	Stimulation	30	10.0	<40	266	<40	236	<15.6
	Stimulation	30	5.0	<20	500	<20	470	<4.2
	Stimulation	60	9.2	—	(560†)	—	—	
9	Control	45	9.0	12–18	30			
	Stimulation	45	8.0	<5.3	80	−10	50	(0)
	Stimulation	45	10.0	<26.7	133	<11.7	103	<10.2
10	Control	40	9.0	16.9	<8			
	Stimulation (R. nerve)	25	10.0	36.0	151	19.1	>143	<11.8
	Stimulation (Both nerves)	25	10.0	24.0	240	7.1	>232	<3.0
	Control	40	10.0	15.0	<38			
	Stimulation (Both nerves)	25	7.0	16.8	336	1.8	>298	<0.6

† Estimation in native plasma.

related with the activity of the nervi accelerantes, since they were found both before and during stimulation of the nerves and were also present in the arterial blood. Once, in fact, the quantity of adrenaline in the coronary blood became less during stimulation. The origin of these small amounts was obviously extracardiac and probably the result of direct stimulation of the adrenal medulla by anoxia due to low blood pressure. One possible exception was the finding of a release of 19 mμg./min. on stimulating the right accelerator nerve in Exp. 10. Even this figure, however, is based on a difference in adrenaline concentrations between the sample (1.5 mμg./ml.) and its control (1.25 mμg./ml.) which cannot be considered significant.

In the other experiments, no adrenaline was found, and the Table gives the upper limits for a possible output of this substance. From these figures the maximum possible percentage methylation of the released sympathin was calculated. The figures necessarily vary with the sensitivity of the assaying organ and the amount of noradrenaline produced, and range from 0–16 per cent. It is evident that, if any adrenaline is released on stimulation of the accelerator nerves, its quantity does not exceed a few per cent of the total amines.

When samples of pulmonary venous blood or of arterial blood were tested, the pulmonary venous plasma (collected during control periods) had no detectable adrenaline (<2 mμg./ml.), but, in Exps. 9 and 10, the arterial plasma contained a small quantity of adrenaline which was the same whether the plasma was collected during control periods or during nerve stimulation. Whenever the quantity of noradrenaline released into the coronary blood was high, there was, however, some noradrenaline to be found in those samples of arterial blood which were collected during stimulation of the cardiac nerves. Table III shows this phenomenon in Exp. 9.

TABLE III

Comparison of amine concentrations in plasma obtained simultaneously from the femoral artery and from the coronary sinus (Exp. 9). Estimation after chromatographic separation

Nature of sample	Concentration of amines (mμg./ml.)			
	Coronary plasma		Arterial plasma	
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Control	1.0–1.5	2.5	1.0–1.5	<2.5
Stimulation	<0.5	7.5	1.0–1.5	<2.5
Stimulation	<2.0	10.0	<2.0	2.5

Noradrenaline became detectable in the third sample of arterial plasma, when the concentration in the coronary plasma had reached 10 mμg./ml. (The samples of coronary blood were taken over a period of 45 seconds and an equal volume of arterial blood was collected in 10–15 seconds as soon as the accelerator effect was fully developed.

Discussion

The experiments show that stimulation of the nervi accelerantes in the dog causes the liberation of noradrenaline into the coronary blood. In no instance was there

evidence for the release of adrenaline on nerve stimulation, so that the methylated product is either not released at all or produced in amounts lying below the thresholds of the assays. In favourable experiments, this threshold was sufficiently low to exclude a percentage methylation above 3 per cent of the noradrenaline released. No attempt was made at identifying any other compounds which might be released. It is of interest that, of the two amines, *l*-noradrenaline is the more potent dilator of the coronaries (Smith and Coxe, 1950), that it has a greater inotropic action (Garb, 1950), and that, in the " fully " atropinized dog (0.4–0.6 mg./kg. of atropine sulphate), it is also the more potent accelerator of the heart (Lockett, 1950). Statements to the contrary regarding the effect on the heart rate are only in apparent contradiction to this, as the dose of atropine required to abolish the reflex slowing of the heart after a dose of noradrenaline is greater than that required to abolish the similar slowing produced by adrenaline. Thus the experiment reported by Pickford and Watt (1951), in which 0.13 mg. atropine sulphate per kg. did not prevent a slowing of the heart rate in a dog, is in good agreement with Lockett's observation that, in one animal, noradrenaline slowed the heart after 0.2 mg. atropine sulphate per kg., but accelerated it after 0.4 mg. per kg. The frog's heart, on the other hand, not only appears to use adrenaline as the sympathetic transmitter, but also to be more sensitive to the methylated product (West, 1947 ; Gaddum *et al.*, 1949).

The amounts of noradrenaline released on electrical stimulation of the cardiac nerves were occasionally sufficient to pass from the coronary sinus through the lungs and produce in the peripheral blood a detectable rise in circulating " sympathin."

The percentage methylation of extracts of cattle heart was reported by Goodall (1950) to amount to 8–20 per cent. Similar figures have been reported (Euler, 1951) for the sheep, and probably will also hold for the dog. Since no evidence for the release of adrenaline was obtained on stimulation of the nervi accelerantes, other sources of this amine may have to be considered, for instance, chromaffine tissue in the heart. The existence of such tissue was suggested by Hoffman, Hoffman, Middleton, and Talesnik (1945), who reported the release of an adrenaline-like substance when they injected acetylcholine or nicotine into atropinized perfused mammalian hearts. More evidence is, however, required to confirm that the substance released in this way does consist mainly of adrenaline.

Summary

Stimulation of the nervi accelerantes in the dog causes the release into the coronary blood of noradrenaline. Adrenaline is not liberated in detectable amounts by stimulation of the heart nerves. If any of the cardiac sympathin is adrenaline, its amount does not exceed 3 per cent of the noradrenaline released.

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