THE RELEASE OF SYMPATHETIC AMINES BY TYRAMINE FROM THE AORTIC WALLS OF CATS

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Horns of rat uteri in chambers, perfused with the femoral arterial blood of cats, relaxed during pressor responses to intravenous injections of tyramine, but failed to do so when half this weight of tyramine was put directly into the arterial blood bathing the uterine horn. Concentrations of tyramine, evoking only threshold inhibitions of rat uteri, have been shown to release one or more compounds from the isolated perfused aortae of cats. The substances released inhibit the spontaneous and induced contractions of rats' uteri. Finally both adrenaline and noradrenaline have been demonstrated in extracts of cats' aortae.

Chromatographic studies have shown that the concentrations of adrenaline and noradrenaline in the plasma of blood withdrawn from the lower abdominal aortae of cats rise during the pressor response to intravenous injections of tyramine[1]. The first object of this work was the direct biological confirmation of this finding, the second to discover whether these amines were released from the aortic walls.

Experimental

Anaesthesia was induced with ether and maintained by the injection of 7·5 ml. 1·0 per cent chloralose w/v in 0·9 per cent w/v aqueous NaCl per kg. through a right femoral venous cannula. A tracheal cannula was inserted.

The perfusion of a rat uterus from a cat femoral artery. One horn of a rat's dioestrus uterus was anchored in a 5 ml. bath covered with a small rubber dome through which a thread greased with petroleum jelly passed to a frontal writing lever. This horn was continuously perfused with arterial blood which entered the bath from a femoral arterial cannula through 2 inches of rubber tubing; it filled the bath to a constant volume and returned to the cat through a short length of rubber tube and a cannula set in the femoral vein.

The perfusion of the cat's aorta. Positive pressure ventilation was applied to a cat anaesthetised with chloralose, the chest was opened in the midline anteriorly, and the left lung removed after application of hilar ligatures. The thoracic aorta was prepared for perfusion by division of of the 24 intercostal branches between ligatures. The inflow cannula, bearing a side arm, was inserted just distal to the origin of the left subclavian artery after ligation of the arch. The straight outflow cannula was introduced 1 cm. above the diaphragm. The aorta was lifted out after section above the inflow and below the outflow cannulae, and was perfused with Tyrode's fluid, oxygenated and adjusted to pH 6·7, at 37° and a mean pressure of 40 mm. Hg (pulse pressure 80 mm. Hg). In

other experiments the calcium concentration in the Tyrode's fluid was reduced to one-third normal and the temperature to 28°. A diagram of the perfusion circuit, capacity 25–30 ml., is shown in Figure 1. The fluid from reservoir A was driven by a microperfusion pump B (C. F. Palmer Ltd.) through the aorta at a mean pressure registered by manometer M_1, and then traversed a resistance R consisting of thin walled rubber tubing compressed with air from pressure bottle PB at 60–70 mm. Hg pressure as recorded by manometer M_2. From this resistance the aortic effluent was either returned directly to the reservoir A through tube C_1, or was passed via tube C^2 to perfuse a horn of a rat's uterus suspended in a 5 ml. bath (UB) before returning through the overflow to reservoir A. The reservoir A, a moist chamber surrounding the aorta, the uterus bath

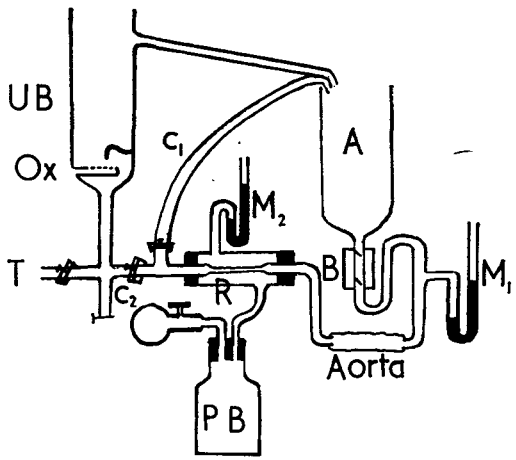

FIG. 1. Circuit diagram for perfusion of the cat aorta *in vitro*. See text for description.

UB, and coils supplying fluid through tube T to this bath whenever it was not in circuit were equipped with warming jackets perfused with water at 37° or at 28°. For the first 15 minutes of each perfusion the Ringer contained 1 mg. harmaline/25 ml.

The preparation of extracts of adrenal glands and aortae. Tissues were stored in the deep freeze for 1–4 hours before mincing with scissors, and grinding with a pinch of silver sand in 2–4 ml. of 0·1N HCl. Extracts and mortar washings were combined, immersed in a boiling water bath for 2 minutes, cooled rapidly and centrifuged. The supernatants were neutralised to litmus as external indicator immediately before bioassay. The method used for the chromatographic separation of amines and their elution has been described[1].

Bioassays. The total quantity of adrenaline plus noradrenaline in each extract was assayed as adrenaline on the rat colon. The adrenaline was assayed on the rat uterus. The relative potency of adrenaline and noradrenaline was determined for each preparation, and the absolute

quantities of the two amines in the extract were calculated by the use of simultaneous equations. Both the colon and the uterus were suspended in aerated de Jalon's Ringer and the assays were made as described by Gaddum, Peart and Vogt[2].

Drugs. Tyramine hydrochloride and harmaline (L. Light and Co. Ltd.), (−)adrenaline and (−)noradrenaline bitartrates (Burroughs Wellcome Ltd.), hexamethonium bromide (May and Baker Ltd.) and heparin (Liquemin, Roche Products Ltd.) were obtained commercially.

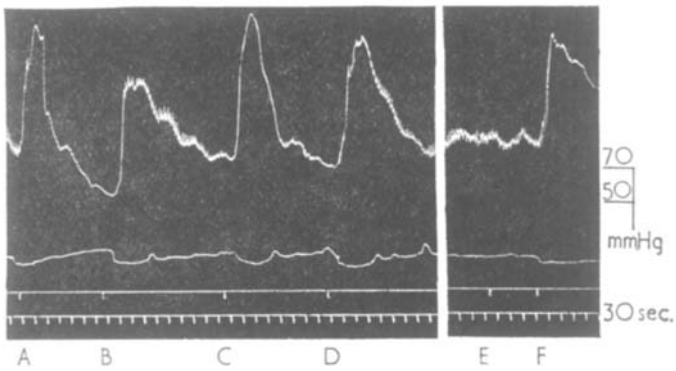

FIG. 2. The upper trace is of the mean arterial pressure of a cat under chloralose anaesthesia, in which the adrenals had been excluded from the circulation and lasting block of autonomic ganglia induced with hexamethonium; the lower traces show changes in the tone of a rat uterus perfused with the cat's femoral arterial blood. A to D intravenous injection to cat. E and F close arterial injections to uterus. A = 5 μg. (−)-adrenaline, B and F = 200 μg., E = 100 μg., and D = 300 μg. tyramine hydrochloride, C = 3 μg. (−)-noradrenaline.

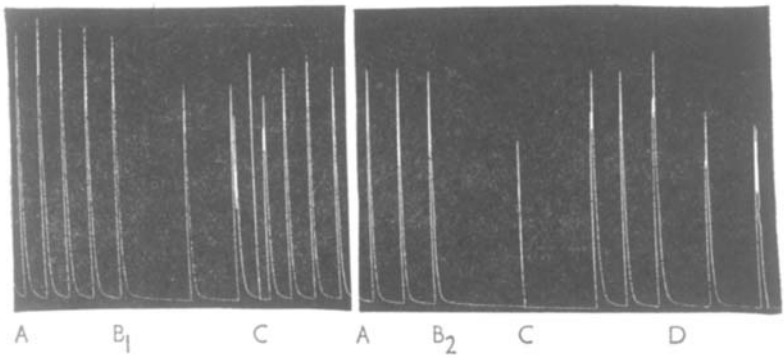

FIG. 3. The tracings show the spontaneous contractions of a rat's uterine horn suspended in oxygenated Tyrode's fluid at 37°. A to B_1 and B_2, the Tyrode's fluid had perfused a cat thoracic aorta for 15 minutes. B_1 and B_2 to C, the fluid contained 1·0 and 1·5 μg. respectively of tyramine-HCl/30 ml. which had perfused the aorta for 10 minutes. At C, the bath was emptied and filled with fresh Tyrode's fluid. At D, 0·5 μg. tyramine HCl was added to 5·4 ml. normal Tyrode's fluid in the uterus bath.

RESULTS

Horns of rats' dioestrus uteri bathed in the femoral arterial blood of chloralosed, acutely adrenalectomised, hexamethonium treated cats became quiescent after some minutes of perfusion but developed tone. They relaxed similarly whether adrenaline or noradrenaline was injected intravenously (Fig. 2) or directly into the tube leading to the chamber containing the uterus. Whereas the intravenous injection of 200 μg. tyramine hydrochloride caused relaxation of the uterus, the close arterial injection of 100 μg. was without effect (Fig. 2).

The horn of a rat's dioestrus uterus perfused with oxygenated Tyrode's fluid at 37° in series with the thoracic aorta of a cat contracted rythmically (Fig. 3) but was inhibited when 1·5 μg. tyramine hydrochloride was

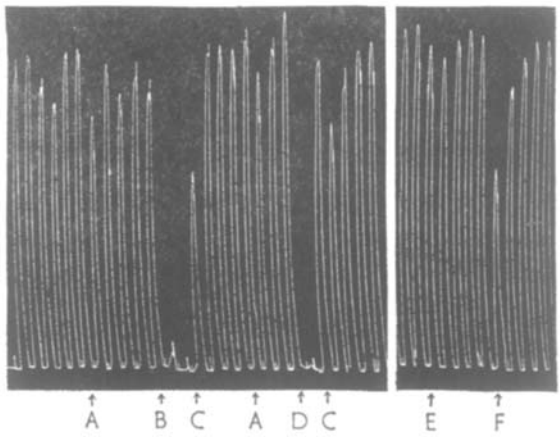

FIG. 4. The tracings show contractions of a horn of rat uterus in deJalon's fluid at 28° in response to 1 µg. acetylcholine added to the bath (5 ml.) at 3-minute intervals, then washed out. At A, the uterus is put in circuit with a perfused cat aorta. At B, 5 µg. and at D, 2·5 µg. tyramine HCl were added to the perfusion reservoir (circuit vol. 30 ml.). At C the uterus was washed and isolated from the perfusion circuit. At E 1 µg. tyramine HCl and at F 0·04 ng. adrenaline were added to the 5 ml. uterine bath (in isolation from the circuit) 1 minute before the next addition of acetylcholine.

added to the reservoir to yield a final concentration of 0·05 μg./ml. When the horn was isolated from the perfusion circuit the addition of 0·5 μg. tyramine hydrochloride to 5 ml. of bath fluid caused only slowing of spontaneous activity and some lessening of the tension developed during contractions. Similarly, when the temperature and the concentration of calcium and glucose were reduced in the perfusion fluid so that a uterus became quiescent, but could be stimulated to contract at intervals of 3 minutes by the addition of 1 μg. acetylcholine to the bath, no inhibition of these contractions resulted from the filling of the bath by fluid which had circulated through the aorta until tyramine hydrochloride was added to the circuit reservoir. Then, 2·5 μg. tyramine hydrochloride in 30 ml. produced complete inhibition of the response to acetylchloine. But, when the uterus was excluded from the circuit containing the aorta,

1 μg. tyramine hydrochloride in 5 ml. bath fluid caused only threshold reduction in the response to the fixed dose of acetylcholine (Fig. 4).

Extracts of aortae taken from ten anaesthetised exsanguinated cats and assayed biologically without prior chromatographic separation of the amines were found to contain both adrenaline and noradrenaline (Table I). This was twice confirmed by chromatographic separation of these compounds. The total quantities of these amines per g. tissue and the relative proportions in which they were present varied greatly and were not affected by the induction of lasting ganglion block with hexamethomium

TABLE I

AMINES OF THE SYMPATHETIC NERVOUS SYSTEM EXTRACTED FROM THE AORTAE OF CATS

Weight of cat (kg.)	Procedure before removal of the aorta	μg./g. tissue	
		(−)Noradrenaline	(−)Adrenaline
3·8	Anaesthesia induced with ether and maintained with chloralose. Exsanguinated	0·018	0·265
3·4		0·023	0·500
4·2		0·396	0·042
3·7		0·690	0·061
2·1		0·036	2·750
1·3		0·630	2·500
3·6	As above thoracic aorta*	0·270	2·560
	abdominal aorta*	0·108	2·104
3·1	Anaesthesia induced with ether and maintained with chloralose. Adrenals excluded from the circulation. Lasting ganglion block induced with hexamethonium 30 to 40 minutes before bleeding	0·167	2·430
2·2		1·010	0·255
2·3		0·610	0·464
2·6		0·995	0·600
3·7		0·542	0·208
2·4		1·013	2·872

* Chromatographic separation of amines and elution proceded bioassay.

TABLE II

COMPARISON OF THE SYMPATHETIC AMINES PRESENT IN THE AORTIC WALLS AND THE ADRENAL GLANDS OF CATS

Weight of cat (kg.)	μg. Amine/g. tissue				Ratio, adrenaline to noradrenaline	
	Aorta		Adrenal gland			
	(−)Noradren.	(−)Adren.	(−)Noradren.	(−)Adren.	Aorta	Adrenal gland
2·1	1·57	1·62	20·2	224·0	1·03	11·2
4·3	0·96	0·50	98·0	998·0	0·54	10·2
1·1	6·15	2·26	215·0	500·0	0·36	2·3
0·9	2·55	2·91	83·3	517·0	1·14	6·3
2·1	0·43	0·85	225·0	860·0	1·98	3·8

(3 mg./kg. i.v. and 6 mg./kg. subcutaneously) and exclusion of the adrenals from the circulation 30–40 minutes before bleeding (Table I). No relation was found either between the total weight of the stores of adrenaline and noradrenaline per g. tissue or between the ratios of adrenaline to noradrenaline present in the adrenal glands on the one hand and in the aortic walls on the other (Table II).

DISCUSSION

Intravenous injections of tyramine increased the concentrations of adrenaline and noradrenaline of heparinised blood withdrawn from the

lower abdominal aortae of cats anaesthetised with chloralose which had been rested for 30–40 minutes after induction of ganglion block with hexamethomium and exclusion of the adrenals from the circulation. The identity of the adrenaline fraction, separated chromatographically has since been proved by parallel assay on rat colon, rat uterus, and the blood pressure of the guinea pig and the rat[1]. Confirmation of this observation has been obtained since horns of dioestrus uteri of rats perfused with the femoral arterial blood of cats, similarly adrenalectomised and treated with hexamethonium, relaxed during pressor responses to intravenous injections of tyramine but were unaffected when half of this intravenous dose was injected into the arterial blood approaching the uteri (Fig. 2).

It has been shown that tyramine liberates a substance from the walls of the cat aorta which inhibits both the spontaneous contractions of the rats uterus in fluid at 37° (Fig. 3), and the responses of the quiescent rat uterus in de Jalon's fluid at 28° to acetylcholine (Fig. 4) as does adrenaline[2]. Since the walls of the aorta contain adrenaline (Tables I and II) it is probable that the uterine inhibitor liberated from the aorta by tyramine was adrenaline. In the two experiments in which it was measured the final content of adrenaline per g. tissue has been lower in the tyramine treated perfused thoracic aorta than in the unperfused untreated abdominal aorta.

Intravenous tyramine was not found to cause increase in the adrenaline and noradrenaline of plasma from carotid blood[1], and did not cause relaxation of the rats uterus perfused with carotid blood[3]. Since adrenaline and noradrenaline are found in the walls both of the thoracic and the abdominal aorta (Table I) it seems probable that the high concentrations of these amines found previously in the plasma of lower aortic blood during pressor responses to tyramine are caused, at least in part, by the steady addition of these amines to the blood as it traverses the length of the aorta.

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