RESEARCH PAPERS

CHROMATOGRAPHIC STUDIES OF THE EFFECT OF INTRAVENOUS INJECTIONS OF TYRAMINE ON THE CONCENTRATIONS OF ADRENALINE AND NORADRENALINE IN PLASMA

BY MARY F. LOCKETT AND K. E. EAKINS

From the Department of Physiology and Pharmacology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3
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Intravenous injections of tyramine, increased the concentrations of adrenaline and noradrenaline in the plasma of heparinised blood withdrawn from the lower aortae of chloralosed cats, which had been rested for 30–40 minutes after induction of lasting ganglion block with hexamethonium and exclusion of the adrenals from the circulation. Both the pressor response to tyramine and this increase in catechol amine was abolished either by pretreatment of the animal with reserpine or by the intramuscular injection of cocaine. Only one tenth of the increase in adrenaline and noradrenaline in the plasma of the lower aorta reached the inferior vena cava, and there was only a very small accompanying rise in the catechol amine content of plasma from blood taken from the base of the carotid arteries when the tyramine injected did not exceed 120 μg./kg.

IT is now well known that postganglionic sympathetic denervation of a tissue greatly reduces or abolishes its responsiveness to tyramine. For example, tyramine fails to dilate the denervated pupil[1] and no longer causes vasoconstriction in the denervated forelimb[2] or contraction of the denervated nictitating membrane[3] in cats. Reserpine also reduces or abolishes the pressor action of tyramine[4] and depletes[5] the chromaffin tissue in the walls of large blood vessels[6] of their stores of noradrenaline-like material. The pressor action of tyramine is re-established after treatment with reserpine by prolonged infusion of noradrenaline[7]. It was predominantly these facts which led Burn and Rand to advance the hypothesis that at least part of the pressor activity of tyramine might be attributed to a release of noradrenaline-like material from the walls of the blood vessels. The object of the present work has been to make direct test of this hypothesis, since it rested on indirect evidence alone, and to measure the changes in the plasma concentrations of catechol amines which result from the intravenous injection of tyramine.

EXPERIMENTAL

Methods

Male, female or neuter cats were used. Anaesthesia, induced with ether, was maintained by the intravenous injection of 7 ml./kg. of 1·0 per cent chloralose in 0·9 per cent aqueous sodium chloride. The trachea was cannulated, the adrenals were excluded from the circulation and the mean arterial pressure was recorded in every experiment. Heparin, 500 units/kg. was used throughout as anticoagulant. In five experiments

of series 1 and in all other experiments records were made of the contractions of the right nictitating membrane in response to rectangular stimuli of 0·5 milliseconds duration delivered to the right cervical sympathetic chain at rates up to 30/second. Hexamethonium bromide 10 mg./kg. intravenously, supported by 10 mg./kg. given subcutaneously, blocked transmission in the superior cervical ganglion for the duration of these experiments. The supporting dose of hexamethonium was doubled in those three experiments of group 1 in which block of the superior cervical ganglion was not demonstrated.

Series 1. *Eight experiments.* Intravenous injections were made through a glass cannula tied into the right femoral vein. Samples of arterial blood were withdrawn through a polythene cannula inserted into the aorta through the right femoral artery so that the tip lay 0·5 to 2·0 cm. above the bifurcation. Mean arterial pressure was recorded from a carotid artery.

Series 2. *Four experiments.* Injection and aortic cannulae as in series 1. An additional cannula for arterial sampling was introduced into the left carotid artery, the tip reaching a point within 1 cm. of its origin. Mean arterial pressure was recorded from the left femoral artery.

Series 3. *Three experiments.* The cannula for intravenous injections was inserted into the right external jugular vein. Mean arterial pressure was recorded from the left carotid artery. Polythene cannulae for the collection of blood samples were introduced into the lower aorta (as in series 1) and through the right femoral vein to extend for 1·0 to 2·5 cm. above the junction of the common iliac veins.

Series 4. *Two experiments.* As for series 1 except that each animal received reserpine 1 mg./kg. by intramuscular injection 36 hours before the experiment. Experiments began with the withdrawl of control blood samples 30–40 minutes after ganglion block had been established (see above). Further blood samples were withdrawn ten minutes later during a pressor response to an intravenous injection of tyramine. The whole process was repeated 20 minutes after the pressor effect of the first dose of tyramine had disappeared. Plasma from blood collected from each site before and between responses to tyramine was separately pooled. That from blood collected during tyramine effects was similarly but separately treated. Blood samples varied from 4 to 8 ml. and were taken into cooled heparinised tubes. The plasma was removed without delay. Adrenaline and noradrenaline were separated by ascending paper chromatography, using phenol-hydrochloric acid as solvent in an atmosphere of carbon dioxide, from protein free extracts of plasma, and eluates were prepared for bioassay as previously described[8].

Noradrenaline was assayed on the blood pressure of rats anaesthetised with 1·5 ml. 15 per cent urethane per 100 g. intraperitoneally, and treated with 0·5 mg. hexamethonium intravenously and 1·0 mg. subcutaneously shortly before the assay began.

Adrenaline was always assayed by inhibition of the responses of the rat's quiescent uterus to a fixed dose of acetylcholine[10] and was also assayed on the mean arterial pressure of the rat in many experiments of group 1.

Drugs

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

RESULTS

The plasma of lower aortic blood contained only low concentrations of adrenaline and noradrenaline when taken from acutely adrenalectomised chloralose cats which had been rested for 30–40 minutes under the full and lasting ganglion blocking action of hexamethonium bromide. These concentrations were very markedly increased during pressor responses to intravenous injections of tyramine hydrochloride (Table I). Both the pressor effect of tyramine and the raised concentrations of adrenaline and noradrenaline which it caused in lower aortic blood were abolished or greatly reduced either by the intramuscular injection of 10 mg./kg. cocaine hydrochloride or pretreatment of the cats with reserpine (Table I).

TABLE I

COMPARISON OF THE EFFECT OF TYRAMINE ON THE MEAN ARTERIAL PRESSURE AND THE CONCENTRATIONS OF (−)-ADRENALINE AND (−)-NORADRENALINE IN PLASMA SEPARATED FROM BLOOD WITHDRAWN FROM THE LOWER ABDOMINAL AORTAE OF CATS UNDER CHLORALOSE ANAESTHESIA AND TREATED WITH HEXAMETHONIUM

Experiment		Tyramine HCl μg./kg. i.v.	Resting		μg./100 ml. plasma after tyramine		Rise in blood pressure after tyramine mm. Hg
Series	No.		Adren.	Noradren.	Adren.	Noradren.	
I	1	100	0·03	0·09	6·0	5·5	71
	2	100	<0·10	<0·80	5·0	6·0	76
	3	100	0·07	0·41	7·2	3·0	68
			0·06	<0·03	0·05	<0·3	2 after cocaine
	4	100	<0·06	<0·75	6·5	2·4	82
			0·32	<0·20	0·9	0·4	4 after cocaine
	5	100	0·80	1·20	10·5	6·5	108
			1·10	0·84	2·0	1·3	28 after cocaine
	6	120	0·04	0·93	8·6	2·1	46
			2·68	1·00	1·6	0·9	10
	7	140	0·40	0·27	8·9	1·1	98
			0·92	<0·20	0·8	<0·2	12 after cocaine
Cats pretreated with reserpine							
VI	1	100	0·43	1·57	0·4	1·6	4
	2	100	0·32	0·98	0·4	1·2	6

Whereas intravenous injections of tyramine caused a large increase in the concentrations of adrenaline and noradrenaline in plasma from lower aortic blood they evoked little change in that drawn from a carotid artery close to its origin (Table II). Only a fraction of that adrenaline and noradrenaline liberated by tyramine into the lower aortic blood returned from the leg and tail to be found in blood drawn from the inferior vena cava a little above its origin (Table II).

DISCUSSION

The trace amounts of catechol amine found in the blood of chloralosed cats, which had been rested for 30–40 minutes after exclusion of the

adrenal glands from the circulation and induction of a lasting block to transmission in autonomic ganglia, did not differ significantly from the normal concentrations of these amines in blood withdrawn from man by venipuncture[11–14]. Neither did the site of withdrawal of these resting samples much affect the concentrations of amines they contained (Tables I and II). These, and the blood samples taken from man, contained much more noradrenaline than adrenaline. It is therefore probable that a lasting block of transmission in sympathetic ganglia does not prevent a slow leak of catechol amine from the region of the terminations of adrenergic fibres. This last statement does however rest on the assumption that the low concentrations of adrenaline and noradrenaline found yield adequate substrate concentrations for the *o*-methylating enzyme system[15].

TABLE II

COMPARISON OF THE EFFECTS OF TYRAMINE ON THE MEAN ARTERIAL PRESSURE AND ON THE CONCENTRATIONS OF (−)-ADRENALINE AND (−)-NORADRENALINE IN PLASMA SEPARATED FROM BLOOD WITHDRAWN FROM THE LOWER AORTA, NEAR THE ORIGIN OF THE CAROTID ARTERY, AND FROM THE LOWER PART OF THE INFERIOR VENA CAVA

Experiment		Tyramine HCl mg./kg.	µg./100 ml. plasma								Rise in blood pressure after tyramine mm./Hg
			Lower aorta				Carotid artery				
			Resting		After tyramine		Resting		After tyramine		
Series	No.		Adren.	Noradren.	Adren.	Noradren.	Adren.	Noradren.	Adren.	Noradren.	
II	1	188	0·65	<0·9	9·9	—	0·02	<0·8	0·35	<0·9	42
	2	100	0·34	1·4	4·2	2·6	0·06	1·8	0·45	1·3	46
	3	115	0·84	<0·3	9·5	4·0	0·11	<0·9	1·03	<0·4	88
	4	146	1·48	<0·9	11·8	5·7	0·21	<0·5	1·19	<0·9	51
			Lower aorta				Inferior vena cava				
III	1	165	0·57	<0·4	16·7	8·4	1·26	<0·3	7·3	<0·4	48
	2	130	0·07	<0·04	6·1	4·3	0·10	<0·1	0·7	<0·1	43
	3	100	0·34	1·4	4·2	2·6	0·21	1·5	2·1	1·3	44

Intravenous injections of tyramine caused very considerable increase in the concentrations of adrenaline and noradrenaline in the plasma of blood withdrawn from the lower aorta but evoked only small change in the levels of these amines in plasma taken from the base of a carotid artery (Table II). Blood plasma therefore gained in catechol amine whilst in transit from the arch to the bifurcation of the aorta. The gain in adrenaline exceeded that in noradrenaline. This fact may indicate a different origin for the tyramine-liberated amine and that found normally in plasma. There are two possible sites of origin of the tyramine liberated base which require exclusion before a third is sought. First, the stores of a material described as noradrenaline-like by Schmiterlow[6] and accepted as noradrenaline by Burn and Rand[7,8]. The fact that reserpine depletes these arterial stores of noradrenaline[7,8] and abolishes the pressor effect of tyramine is not evidence that the amines liberated by tyramine emerge from them, because reserpine depletes other stores of their catechol amine[7,8] also. If the material in the arterial stores is solely noradrenaline it could not explain the appearance of adrenaline in aortic plasma. The

second site from which adrenaline and noradrenaline may have been liberated by tyramine is from the blood cells themselves, for Weil-Malherbe and Bone[12] have shown that the red cells, for instance, contain more adrenaline, but less noradrenaline, than plasma. Whatever the source of the amines which appear in the aortic blood during a pressor response to tyramine, the concentrations resulting in the arterial blood are certainly sufficient to have peripheral constrictor effect (Table I) and only a small fraction of the catechol amine present in the arterial blood returns to the inferior vena cava from the legs and tail.

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