responsible for the fall in uterine flow. 3. Despite the uterine ischemia, fetal survival is not affected because fetal temperature falls to the same extent as that of the mother. Hypothermia of the fetus *in utero* probably increases its tolerance to hypoxia.

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1. Westin, B., Sehgal, N., Assali, N. S., *Am. J. Physiol.*, 1961, v201, 485.
2. Allen F. M., *West. J. Surg.*, 1948, v56, 548.
3. Barter, R. H., Albert, S. N., Winshel, A. W., *Am. J. Obst. & Gynec.*, 1958, v76, 1062.

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Release of Norepinephrine from the Heart by Vasoactive Amines. (27244)

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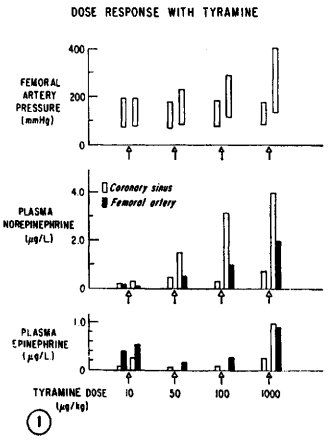
Although it has been suggested that the action of some sympathomimetic amines is mediated by release of endogenous norepinephrine (NE) from adrenergic nerve endings (1,2), little direct evidence supports this view. *In vitro*, tyramine has been shown to increase the rate of release of NE from isolated granules of splenic nerve and adrenal medulla(3,4). Stjarne(5) utilizing a bioassay technic, observed an increase of a pressor substance in the splenic vein effluent following administration of tyramine. On the other hand, no reduction in NE content of guinea pig hearts was observed after prolonged perfusion with tyramine(6) and no increase in NE content of arterial blood of intact animals was detected after injection of tyramine(7, 8). Accordingly, it was suggested that the action of this amine is a direct one and that NE merely plays a facilitating role(6). Therefore, the purpose of the present study was to determine whether, in the intact anesthetized dog, a number of vasoactive amines actually release NE from the heart by direct measurement of catecholamines in coronary sinus blood. The influence of one of these, tyramine, on concentration of NE in cardiac tissue was also determined.

Methods. Fourteen mongrel dogs weighing 18 to 30 kg were lightly anesthetized with morphine (2 mg/kg subcutaneously) and a chloralose-urethane mixture given intravenously. The coronary sinus was catheterized under fluoroscopic control. Blood samples of 30 ml were withdrawn simultaneously from the femoral artery and coronary sinus and were replaced with fresh donor blood which had been mixed with the experimental dog's blood prior to study. In any one experiment one to 4 sympathomimetic amines were administered. After the control samples were obtained, the amine was injected, and at the time of maximal arterial pressure response blood samples were taken from the coronary sinus and femoral artery. Thirty minutes after injection, when arterial pressure had returned to control levels, blood samples were again obtained and another amine injected. In 5 additional open-chest dogs tyramine was infused continuously for one hour at a rate of 200 μg/kg/min., while arterial pressure was recorded continuously. The right atrial appendage was biopsied prior to the infusion and the left atrial appendage at the end of infusion. Control studies were carried out identically in 4 dogs which received saline infusions, and both appendages were biopsied simultaneously in another 5 dogs which had not received vasoactive amines.

Plasma NE was isolated using alumina columns and oxidized to the trihydroxyindole (9,10). Fluorescence measurements were made in an Aminco-Bowman spectrophotofluorimeter, differentiating NE from epinephrine by the spectral characteristics of each (10). NE content of atrial appendage was purified and assayed from a trichloroacetic acid extract(9).



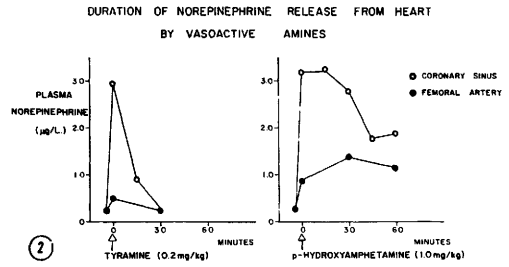


FIG. 1. Effect of varying doses of tyramine on arterial pressure, and plasma catecholamines in one dog.

FIG. 2. Arterial and coronary sinus norepinephrine contents following inj. of 2 vasoactive amines in 2 dogs.

Results. During the control period the coronary veno-arterial (V-A) differences of NE ranged from +0.50 to −0.11 and averaged 0.04 μg/l. The effect of increasing doses of tyramine in one dog is shown in Fig. 1. Concomitantly both concentration of norepinephrine in the coronary sinus blood and the V-A difference became progressively elevated. Gradual increases in arterial concentration of NE occurred as the dose of tyramine was increased. In this dog the concentration of epinephrine in arterial plasma prior to tyramine fell as arterial pressure rose, with injections of 50 and 100 μg/kg tyramine. This was similar to what was seen in experiments in which other vasoactive amines were used. Injection of 1000 μg/kg tyramine, however, resulted in a pronounced increase in epinephrine concentration of both the arterial and coronary sinus samples.

An increase in NE content of coronary sinus blood, associated with a large V-A difference consistently occurred in 3 dogs following injection of tyramine and amphetamine and in 2 dogs each with guanethidine, phenylethylamine, tryptamine, and hydroxyamphetamine, and in one dog each with epedrine, and mephentermine (Table I). On a weight basis tyramine appeared to be the most potent with phenylethylamine, tryptamine, and p-hydroxyamphetamine intermediate and guanethidine, amphetamine, ephedrine, and mephentermine less potent. In 2 dogs each, methoxamine, serotonin, and phenylephrine did not elevate coronary sinus NE content.

In 4 dogs the time course of NE release following a single injection of vasoactive amines was examined. It is shown in Fig. 2 that 30

TABLE I. Influence of Various Vasoactive Amines on Arterial Blood Pressure and Cardiac Release of Norepinephrine.

	Dose (mg/kg)	Increase of arterial systolic pressure above control (%)	Plasma NE (μg/l) CS, B	CS, A	Art., A
Tyramine*	.2	55	.49	2.90	.84
Phenylethylamine†	1.0	56	1.02	3.70	1.42
Tryptamine†	1.0	74	.21	1.63	.56
p-Hydroxyamphetamine†	1.0	116	.40	2.67	.69
Amphetamine*	1.0	38	.56	1.19	1.00
Ephedrine	1.0	32	.61	1.36	.95
Mephentermine	1.0	26	.67	1.15	.57
Guanethidine†	10.0	10	.91	2.65	1.58
Methoxamine†	.5	54	1.19	.72	.36
Phenylephrine†	.2	65	1.19	1.26	—
Serotonin†	.1	30	.43	.54	.41

CS = Coronary sinus Art. = Femoral artery B = Before drug inj. A = After drug inj.
* Values presented are avg of observations on 3 dogs.
† Avg of 2 dogs.

TABLE II. Effect of Tyramine Infusion on NE Content of Atrial Appendage.

Exp.	Condition	Avg NE content of atrial appendage (μg/g)
Tyramine infusion (5 dogs)	Control (R)	2.08
	After (L)	1.17
	Difference (%)	−43 (−37 to −57) %
Saline infusion (4 dogs)	Control (R)	1.99
	After (L)	2.14
	Difference (%)	+8 (+80 to −28) %
Simultaneous biopsy (5 dogs)	Right	2.08
	Left	1.98
	Difference (%)	−5 (−21 to +17) %

Control (R) = Right atrial appendage obtained during control period.
After (L) = Left atrial appendage obtained after tyramine infusion.

minutes following injection of 200 μg/kg tyramine the coronary V-A difference had returned to control levels, but that it was still increased at 60 minutes following injection of 1000 μg/kg p-hydroxyamphetamine. The response to ephedrine was similarly prolonged.

In the 5 dogs which received the infusion of tyramine, NE content of the left atrial appendage obtained at completion of the infusion was consistently lower than that of the right atrial appendage taken prior to the infusion (Table II). The differences averaged 43% whereas in the 9 control dogs no consistent differences between the 2 appendages were observed. It is of interest that in 2 of the dogs no pressor response to a single injection of 1 mg/kg of tyramine occurred when it was given at the end of the tyramine infusion in spite of the presence of significant quantities of residual NE in the heart. In the other 3 dogs the response to tyramine injection was greatly reduced.

Discussion. These studies clearly demonstrate that a number of vasoactive amines release NE from the heart. The duration of this release is short lived with tyramine and more prolonged with p-hydroxyamphetamine and ephedrine. The hypotensive drug Bretylium Tosylate has also been shown to release NE from the heart(11). Serotonin, phenylephrine, and methoxamine produced no clear cut release of NE from the heart. However, in the absence of coronary flow measurements a release of very small quantities cannot be excluded. The secretion of epinephrine from the adrenal gland appeared to be reduced after injection of these vasoactive amines, presumably reflexly due to their pressor effect.

The reduction in NE content of the atrial appendage after tyramine infusion, although not marked, was observed consistently. The observations that the physiologic response to tyramine was abolished or greatly reduced in spite of the presence of residual NE in the heart (57% of control) suggests that the tyramine infusion had depleted a specific tyramine-releasable NE store.

Summary. Intravenous injections of tyramine, phenylethylamine, tryptamine, p-hydroxyamphetamine, amphetamine, ephedrine, mephentermine, and guanethidine were shown to release NE from the heart into the coronary sinus blood of the intact anesthetized dog, while serotonin, methoxamine, and phenylephrine had no such effect. A small but consistent reduction in NE content of atrial appendage was observed following one hour's infusion of tyramine. These observations furnish proof for the concept that release of NE is involved in the pharmacologic action of many of the vasoactive amines.

1. Fleckenstein, A., *Arch. Exp. Path. Pharmak.*, 1953, v218, 117.
2. Burn, J. H., Rand, M. J., *J. Physiol.*, 1958, v144, 314.
3. Euler, U. S. v., Lishajko, F., *Experientia*, 1960, v16, 376.
4. Schuman, H. J., *Arch. Exp. Path. Pharmak.*, 1960, v238, 41.
5. Stjarne, L., *Acta Physiol. Scand.*, 1961, v51, 224.
6. Nasmyth, P. A., *Adrenergic Mechanisms*, Little, Brown & Co., Boston, 1960, 337.
7. Vane, J. R., *ibid.*, 356.
8. Weimer, N., Burack, W. R., Draskoczy, P., *Fed. Proc.*, 1961, v20, 316.
9. Crout J. R., Creveling, C. R., Udenfriend, S., *J. Pharm. & Exp. Ther.*, 1961, v132, 269.
10. Bertler, A., Carlsson, A., Rosengren, E., *Acta. Physiol. Scand.*, 1958, v44, 273.
11. Siegel J., Gilmore, J., *Fed. Proc.*, 1961, v20, 126.

Received November 29, 1961. P.S.E.B.M., 1962, v109.