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TYRAMINE INDUCED LIPOLYSIS FOLLOWING PRETREATMENT OF GUINEA PIGS AND RATS WITH METARAMINOL

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Abstract—α-Methyl-norepinephrine, norepinephrine and epinephrine stimulate the mobilization of fatty acids from epididymal fat of guinea pigs, when incubated *in vitro*, to the same order of magnitude. Metaraminol, however, is at least a thousand times less potent.

The *in vivo* lipolytic potency of tyramine was investigated in guinea pigs and rats treated with metaraminol. In rats tyramine had no effect on the mobilization of fatty acids, whereas guinea pigs responded to tyramine with an additional increase of plasma fatty acids. α-Methyl-norepinephrine was found in heart tissue of guinea pigs but not in that of rats. The conversion of metaraminol into α-methyl-norepinephrine is postulated and made responsible for the 'tyramine effect' in the guinea pig.

A HOST of natural and chemical compounds as well as nutritional and environmental circumstances elevate the albumin bound unesterified fatty acids (FFA) of the plasma by inducing the hydrolysis of triglycerides in adipose tissue. Besides the pituitary peptide hormones, the catecholamines and their related analogues as a group exhibit potent lipolytic activity.

It is well established that tyramine can provoke a rise in plasma FFA in nearly every animal species so far investigated,[1, 2] and there is good evidence that it acts by mobilizing norepinephrine.[3] If the stores (including the adipose tissue) of the latter substance are depleted using reserpine or some related agent, then this lipolytic potency is impaired and even abolished which suggests that the lipolytic effect of tyramine is mediated through norepinephrine.

Metaraminol (1-α-methyl-β-hydroxy-metatyramine) has been shown to invoke a marked depletion of norepinephrine in the heart. At the same time it replaces this loss to a large extent.[4, 5] In adipose tissue metaraminol has also been detected after treatment with α-methyl-metatyrosine. Recently, Maître and Staehelin[6] described the appearance of α-methyl-norepinephrine in the hearts of guinea pigs after administration of metaraminol, while at the same time the norepinephrine level was reduced by 90 per cent.

During *in vitro* studies with rat epididymal fat pads metaraminol exhibited a negligible lipolytic potency, wheras the activity of α-methyl-norepinephrine was comparable to that of norepinephrine.[2, 7]

On the basis of the above mentioned observations, we conducted an investigation in order to compare the lipolytic effect of tyramine on metaraminol-treated guinea pigs with that of tyramine on similarly treated rats.

MATERIALS AND METHODS

Chemicals. L-Epinephrine from Siegfried AG, Zofingen, Switzerland; L-Norepinephrine and tyramine hydrochloride from Fluka AG, Buchs (SG), Switzerland; L-α-Methyl-norepinephrine hydrochloride (Corbasil®) from Farbwerke Hoechst AG, Frankfurt/M, Germany; *l*-Metaraminol bitarate (Aramine®) from Merck, Sharp & Dohme, Rahway, N.J., U.S.A.; D,L-α-Methyl-DOPA from Farbenfabriken Bayer AG, Leverkusen, Germany.

Animals. Male guinea pigs (250–300 g) and male albino rats (160–180 g) of the same breeding colony were used for *in vivo* studies. Heavy male guinea pigs (500–600 g) served as donors for *in vitro* experiments in order to ensure a sufficient amount of epididymal adipose tissue.

Determinations. Plasma FFA were measured colorimetrically according to Duncombe[8] for *in vivo* experiments, and titrimetrically by the method of Dole[9] for *in vitro*. studies.

The *in vitro* incubation of epididymal fat pads was essentially the same as the method of Jungas and Ball[10] with 4 per cent bovine albumin added. The glycerol content of the incubation medium was estimated by the method of Wieland.[11] Differential estimations of norepinephrine and α-methyl-norepinephrine were performed using the biological and fluorimetric assay procedures described previously.[12]

RESULTS

(a) In vitro *experiments*

The dose-response curves of α-methyl-norepinephrine, epinephrine, norepinephrine, and metaraminol obtained from guinea pig epididymal fat incubations *in vitro* are depicted in Fig. 1.

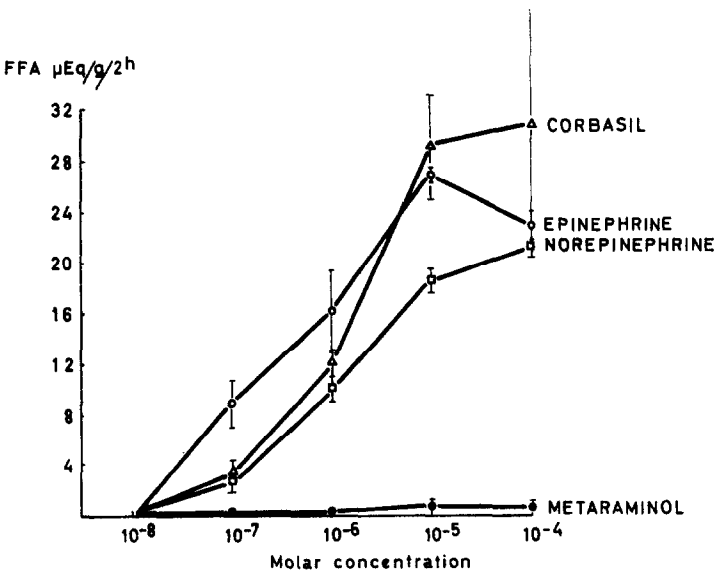

FIG. 1. Lipolytic activity of α-methyl-norepinephrine (Corbasil), epinephrine, norepinphrine, and metaraminol in pads of epididymal fat from guinea pigs incubated *in vitro*. Free fatty acids released into the medium during a 2 hr period are expressed as μEquiv per g of fat ± S.E.M.

The curves of α-methyl-norepinephrine, epinephrine, and norepinephrine show generally the same slope up to a molar concentration of 10^{-5}. α-Methyl-norepinephrine and epinephrine appear to reach a plateau at a concentration of 10^{-5} M at plasma FFA levels of 28–30 μEquiv/g fat after an incubation time of 2 hr. Norepinphrine, however, appears to be slightly less active. Half-maximal values were obtained with molar concentrations of about 10^{-6} for each amine. These values are in fair agreement with the results of Stock and Westermann[7] using the rat, with the exception that epinephrine was found to be less active than norepinephrine.[2] Metaraminol lacked lipolytic activity at all concentrations tested.

The response to metaraminol of rat adipose tissue seems to differ from that of guinea pig adipose tissue in that it exhibits a small but significant response at concentrations upwards from 10^{-4} M.[7] The glycerol, the albumin bound FFA values of the medium and the FFA content of the fat pads after termination of the incubation are listed in Table 1. The calculated ratio of total FFA (medium FFA plus tissue FFA)

TABLE 1. *In vitro* EFFECTS OF NOREPINEPHRINE, EPINEPHRINE, AND α-METHYL-NOREPINEPHRINE ON EPIDIDYMAL FAT OF GUINEA PIGS

	Norepinephrine molar concentration			Epinephrine molar concentration			α-Methyl-norepinephrine molar concentration		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
Glycerol	10·5	10·7	6·4	11·3	12·1	6·8	13·1	13·5	6·0
Medium free fatty acids (FFA)	21·6	19·0	10·2	23·1	27·0	16·4	30·9	29·4	12·4
Tissue free fatty acids (FFA)	7·4	7·7	3·0	5·3	7·0	2·5	3·9	4·9	1·1
Ratio: Total FFA to glycerol	2·8/1	2·5/1	2·1/1	2·5/1	2·8/1	2·8/1	2·7/1	2·5/1	2·3/1

Each value constitutes the mean of 3 experiments.

The response of metaraminol with respect to glycerol and FFA liberation was negligible within the limits of the accuracy of the method.

to glycerol is a measure of the release and re-esterification rate of FFA in adipose tissue. If only triglyceride molecules and no di- and monoglycerides are formed or cleaved during the action of the drugs, a ratio of 3 M of FFA to 1 M of glycerol results, smaller values indicate possible re-esterification.

From the data in Table 1 it can be deduced that none of the compounds has a pronounced influence on re-esterification under the conditions investigated.

(b) In vivo *experiments*

The results of the *in vivo* assays performed on guinea pigs and rats are summarized in Fig. 2. Metaraminol was injected subcutaneously and the animals were sacrificed after 16 hr, a suitable time during which to allow an ample depletion of norepinephrine, and after which a slightly elevated plasma FFA level was measured in both species.

In normal animals, tyramine induces a 2 to 3–fold increase in plasma FFA. This rise constitutes the well known tyramine effect.

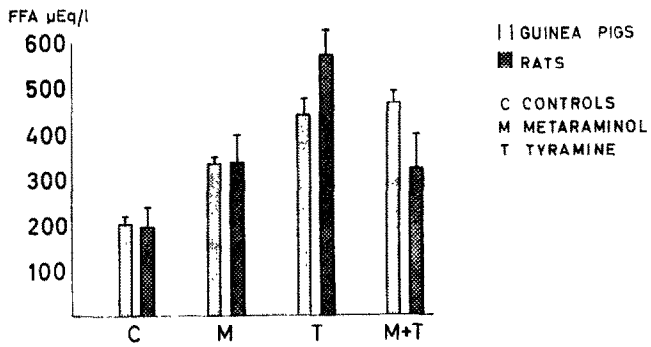

FIG. 2. 10 mg/kg L-metaraminol-bitartrate was applied subcutaneously 16 hr prior to a subcutaneous injection of 30 mg/kg tyramine-HCl. 20 min later the animals were exsanguinated under ether narcosis. Plasma free fatty acids are expressed as µEquiv/l. ± S.E.M. 21–25 guinea pigs and 7 rats were used per experiment.

In rats treated with metaraminol, tyramine had no additional lipolytic effect. However, in metaraminol-treated guineas pigs, tyramine induced an additional rise in plasma FFA.

In a second experiment, shown in Table 2, metaraminol and tyramine were injected i.p., and metaraminol caused the same slight elevation (about 50–130 µEquiv/l.) of plasma free fatty acids 16 hr after application. It was found that the animals consumed 60–90 per cent less food than the controls during the 16 hr. Untreated animals receiving over the same period of time the amount of food consumed by treated animals exhibited similarly increased plasma levels of FFA, thus proving that this effect can solely be attributed to reduced food intake.

Tyramine promotes a quick rise of fatty acids in both species to about equal levels. In the guinea pig the elevated level of fatty acids is maintained for at least 60 min, whereas in the rat it declines sharply after the maximum and reaches control values after 30 min. In rats, treatment with metaraminol completely abolished an additional lipolytic effect of tyramine, whereas in guinea pigs tyramine was still effective. These results are in agreement with our findings in the experiments using subcutaneous application.

The myocardial content of catecholamines of the same animals discussed above in Table 2, is shown in Table 3. The norepinephrine content in heart tissue of control guinea pigs was about two to three times higher than in hearts of rats. A single injection of metaraminol depleted, over a period of 16 hr, 90 per cent or more of the norepinephrine originally present. α-Methyl-norepinephrine was found in appreciable quantities in heart tissue of guinea pigs, whereas the same tissue in rats contained a negligible amount if any. Similarly α-methyl-norepinephrine was found in adipose tissue of guinea pigs (unpublished data).

DISCUSSION

Our results have shown that tyramine is *in vivo* lipolytically active only in guinea pigs but not in rats after pretreatment of both species with metaraminol. Since the epididymal fat tissue of guinea pigs responded *in vitro* to various lipolytic agents in a similar manner as that of rats,[7] the difference in response of the two species found *in vivo* might be due to a pharmakynetic activity.

TABLE 2. *In vivo* EFFECT OF METARAMINOL PRETREATMENT ON THE TYRAMINE INDUCED MOBILIZATION OF FATTY ACIDS ON GUINEA PIGS AND RATS

Species	Control	L-Metaraminol 5 mg/kg i.p. 16 h	Tyramine 10 mg/kg i.p.				L-Metaraminol 5 mg/kg i.p. 16 h plus Tyramine 10 mg/kg i.p.			
			5′	15′	30′	60′	5′	15′	30′	60′
Guinea pig	219 ± 17* (19)†	270 ± 23 (14)	499 ± 16 (14)	464 ± 20 (12)	433 ± 21 (12)	464 ± 22 (13)	463 ± 19 (13)	384 ± 19 (18)	446 ± 21 (24)	462 ± 26 (12)
Rat	183 ± 15 (14)	316 ± 18 (14)	489 ± 31 (14)	294 ± 17 (21)	177 ± 19 (14)	156 ± 18 (7)	343 ± 64 (7)	234 ± 17 (14)	230 ± 15 (14)	241 ± 16 (7)

* Free fatty acids as μEquiv/l ± S.E.M.
† Number of animals per experiment.

TABLE 3. EFFECTS OF L-METARAMINOL (5 MG/KG, I.P., 6–17 HR) ON MYOCARDIAL CATECHOLAMINE CONTENT OF GUINEA PIGS AND RATS

Species	n	Controls	Metaraminol-treated	
		Norepinephrine	Norepinephrine	α-Methyl-norepinephrine
Guinea pig	8	2·50 ± 0·11	0·27 ± 0·05	0·58 ± 0·07
Rat	6	1·01 ± 0·06	0·07 ± 0·004	< 0·02

Results are expressed in terms of μg/g (± S.E.M.) fresh tissue. n = number of extracts. 3–12 guinea pigs, resp. 5–20 rats were used for each extract.

In order to explain this discrepancy a number of factors must be taken in account, e.g. metaraminol as such, the remaining amount of norepinephrine, or the conversion of metaraminol into lipolytically more potent metabolites.

(1) *Metaraminol.* We have found metaraminol to possess acute lipolytic activity in rats, confirming the results of Stock and Westerman,[2] but contrasting with our *in vitro* experiments. The absolute amounts of incorporated metaraminol after treatment with α-methyl-meta-tyrosine or with metaraminol are larger in guinea pigs than in rats,[4] and it has been demonstrated in the cat that tyramine is able to release incorporated metaraminol.[13] Unfortunately, nothing is known about the FFA mobilizing activity of tyramine-released metaraminol.

(2) *Norepinephrine.* As to be seen in Table 3, the absolute content of the remaining norepinephrine after metaraminol treatment is considerably higher in guinea pigs than in rats. So far, nothing is known about the ability of tyramine to mobilize the remaining norepinephrine which could possibly account for the additional lipolytic effect of tyramine in guinea pigs.

(3) *Active metabolites.* A major difference between the two species investigated after treatment with metaraminol is the occurrence of α-methyl-norepinephrine in the hearts of guinea pigs but not in those of rats. Tyramine is likely to mobilize stored α-methyl-norepinephrine.[14] The *in vitro* experiments revealed a lipolytic activity for α-methyl-norepinephrine equal to that of epinephrine or norepinephrine.

We are inclined to believe that the α-methyl-norepinephrine stored in tissues of guinea pigs alone is responsible for the additional lipolytic effect of tyramine in this species. Moreover, the long-lasting lipolytic activity of tyramine in metaraminol-treated guinea pigs can be interpreted by a slow degradation of the active compound, α-methyl-norepinephrine, which is no substrate for monamine oxidase.[15]

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