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EFFECT OF NOREPINEPHRINE ON ADIPOSE TISSUE LIPSAE AND PHOSPHORYLASE ACTIVITY OF THE FROG *RANA ESCULENTA L.*

TIBOR FARKAS

Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

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Catecholamines have different effects on the adipose tissue of mammals. Beside their effect on the uptake of glucose in the adipose tissue (CAHILL et al. 1960) they stimulate the phosphorylase enzyme and through this the decomposition of the glycogen stored in the adipose tissue (VAUGHAN 1960, FRERICHS et al. 1962). Their other important effect is to stimulate the hydrolysis of the neutral triglycerides into fatty acids and glycerine (WHITE et al. 1958). The catecholamines exercise this effect through the "hormon sesitive" lipase (RIZACK, 1960), which in several respects differs from the triglyceride lipase present in the adipose tissue (RUBINSTEIN et al. 1964).

In examinations conducted on a fresh water fish, *Cyprinus carpio L.* it was demonstrated that in the adipose tissue of fish — similary to that of mammals — intesive lipolysis takes place. Several lipolytic hormones very effective in mammals (catecholamines and adreno-corticotroph hormone) have, however, no effect on the extent of the hydrolysis of glycerides under in vivo or in vitro conditions. (FARKAS, in press). It was concluded from these results that the hormone sensitive lipase is absent in the adipose tissue of fish and it appears only later in the phylogeny.

For further elucidation of the problem investigations were started on other lower vertebrate organisms. The present paper describes the effect of norepinephrine on the lipase and phosphorylase activity of the adipose tissue of the frog, *Rana esculenta L.*

Material and method

Adult male frogs *(Rana esculenta L.)* weighing 50—80 g were used for the experiments. The frogs were purchased from the official firm MAVAD and kept in aquarium containing a few cm of water. The animals were used up within a week from the purchase. Food was denied from the animals during that time.

The frogs were brought into the laboratory the evening before the experiment, to adapt them to the experimental temperature. Norepinephrine diluted in physiological saline (0.48 per cent NaCl) was injected into the ventral

lymph sac. The animals were killed by decapitation two hours after the injection and the blood was collected in prechilled heparinized tubes. The adipose tissues were rapidly removed and placed into physiological saline.

In vitro experiments.

The finger-like branching parts of the adipose tissues were cut into 100—150 mg portions. From one piece the free fatty acid content of the adipose tissue was determined while the other parts were incubated in 3 ml physiological solution in the presence or absence of norepinephrine (2 μg/ml) for 60 minutes at room temperature. The composition of the physiological solution was the following: NaCl:0.083 M, $NaHCo_3$:0.025 M, KCl:0.002 M, KH_2PO_4:0.012 M, CaCl:0.025 M, $MgCl_2$:0.003 M. (FENN, 1936). The pH of the solution was adjusted to 7.4. After the incubation period the tissues were removed from the medium, blotted gently and the free fatty acid content or the phosphorylase activity were determined.

Analytical procedures

The free fatty acid level of the blood plasma was determined according to DOLE (DOLE, 1956) from 0.5 ml blood plasma. The blood sugar was measured colorimetrically according to HYVÄRINEN (HYVÄRINEN et al. 1962). For determination of free fatty acid content of the adipose tissue the tissues were homogenized in POTTER homogenizator in the presence of n-heptane. The homogenizate was filled up to 5 ml. 1 ml of aliquots were titrated with 0.01 N NaOH in the presence of bromthymol blue indicator. The results are given in μM free fatty acid/g adipose tissue.

For the assaying the phosphorylase activity the SUTHERLAND'S method was chosen (SUTHERLAND 1955). The adipose tissues were homogenized in the presence of 0.1 M NaF. The end volume of the homogenizate was 2.5 ml. Then the homogenizate was centrifuged at 4°C for 15 minutes at 1500 r. p. m. and the separated lipoid layer removed. 0.5 ml of the supernatant was incubated in the presence of 4.0 mg glycogen, 31.5 μmoles glucose — 1 — phosphate, 1.4 μmoles 5 — adenosine monophosphate and 85 μmoles NaF for 60 minutes at pH 6.3 and room temperature. End volume of the reaction mixture was 1.3 ml. The reaction was stopped in the 0 and 60 minute by the addition of 0.25 ml 0.3 M $HClO_4$. The precipitated proteins were removed with centrifuging for 15 minutes at 6000 r. p. m. Phosphorylase was measured into the direction of glycogen synthesis. The glycogen was determined according to VAN der VIES (1954). Incubations and glycogen measurements were conducted in 3 parallels.

Results

The adipose tissue of the frog contains a substantial amount of free fatty acid. The amount of the free fatty acid in frog adipose tissue seems to be higher than in mammals. Free fatty acid contents of the right and left side adipose tissue are different. From determinations made on 5 animals the following mean values were obtained: right side adipose tissue: 3.969 ± 0.52 μM/g, left side adipose tissue: 2.648 ± 0.26 μM/g. The difference is significant ($p < 0.05$).

For the experiments the left side adipose tissues were used which were larger than the pair on the opposite side.

Fig. 1. shows that in the adipose tissue an intensive lipolysis takes place under in vitro conditions. The fatty acids produced accumulate in the adipose tissues. The accumulation is linear with time in the first 90—120 minutes.

Effect of norepinephrine on the free fatty acid production of the adipose tissue.

Norepinephrine significantly increases the intensity of the lipolytic processes in the adipose tissue of mammals under in vivo or in vitro conditions. To study its effect on the fatty acid production of the adipose tissue of the frog 2 series of experiments were conducted. In the first series of experiments the hormone was administered in vivo (500 μg/animal) and both the plasma and adipose tissue free fatty acid levels were determined. The adipose tissues of the animals treated with norepinephrine in vivo were incubated in vitro to establish whether the adipose tissue of the treated animals produces more free fatty acid than that of the control animals. In the other esxperimental series the adipose tissue of untreated animals were incubated in vitro in the presence or absence of norepinephrine (2 μg/ml incubation medium) and the free fatty acid production of the adipose tissues was determined.

Fig. 2. shows that the in vivo administration of norepinephrine did not lead to the increase of the free fatty acid level of the blood plasma. In fish, after the administration of the hormone both the plasma free fatty acid level and the adipose tissue free fatty acid content were significantly decreased. These effects of the hormone could not be observed in frogs. It is evident from both the increased blood glucose levels and from its effect on the chromatophores that the hormone entered the blood circulatory system. 20—25 minutes after the injection of norepinephrine the animals have lost their brownish green colour and became pale green. This effect of the hormone subsisted for hours.

From *fig. 2.* it appears also that norepinephrine under in vitro conditions did not stimulate the production of the free fatty acids in the frog adipose tissue. The presence of the hormone leads to a decreased free fatty acid production, the difference is however not significant as compared with the control. The adipose tissue of the animals treated with norepinephrine in vivo does not produce significantly more free fatty acid than that of the untreated animals. These experiments suggest that norepinephrine one of the most effective lipid mobilizing hormone in the mammals is inactiv in stimulating the breakdown of triglycerides in the adipose tissue in frogs.

The effect of norepinephrine on phosphorylase acivity.

Fig. 3. demonstrates the presence of phosphorylase acivity in the adipose tissue of frog. The hormones stimulating the breakdown of triglycerides in mammalian adipose tissue increase also the activity of the adipose tissue phosphorylase. Since norepinephrine has no effect on the lipolytic activity of the adipose tissue of frog, it seemed interesting to examine whether the phosphorylase of the adipose tissue is sensitive to the hormone.

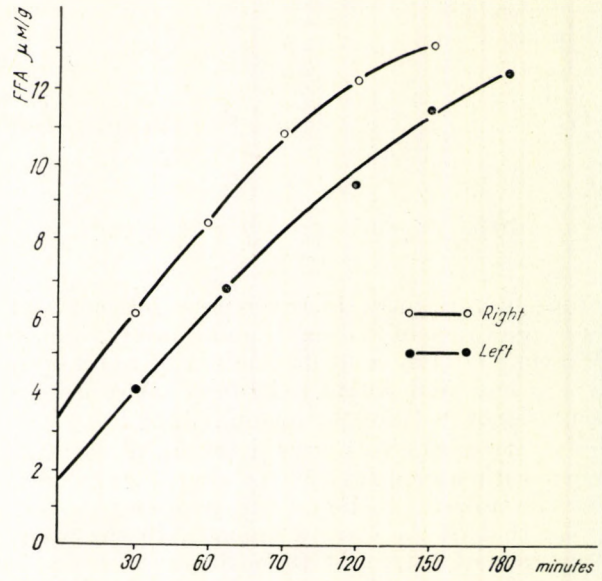

Fig. 1. Free fatty acid production in the adipose tissue of frog. The points are the mean values of measurement obtained from 3 animals

1. ábra Szabadzsírsav termelés kecskebéka zsírszövetében. A pontok 3 állatból kapott mérési eredmények átlagértékei.

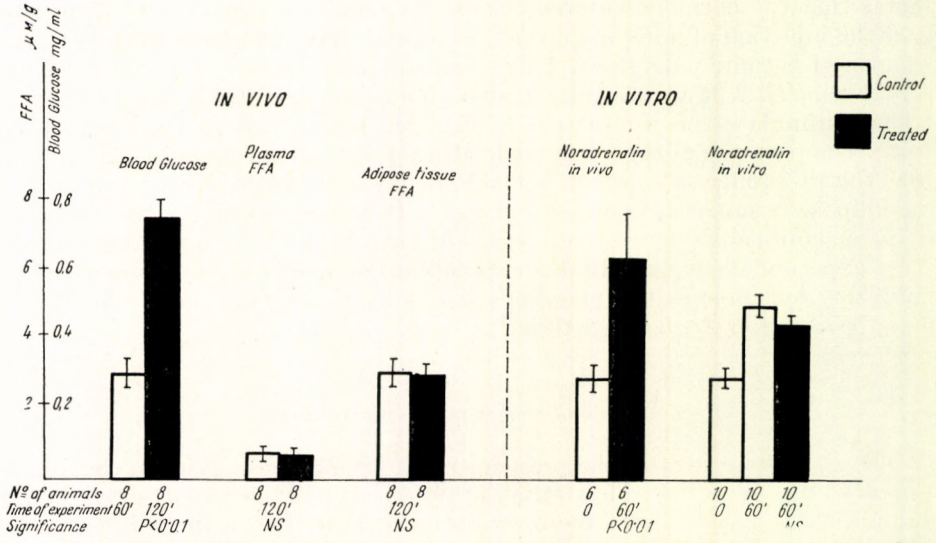

Fig. 2. The effect of noradrenaline on plasma free fatty acid level and on the free fatty acid production of the adipose tissue

2. ábra Noradrenalin hatása vérplazma szabad zsírsav szintjére és a zsírszövet szabad zsírsav termelésére.

To decide this question two experiments were conducted. In the first one norepinephrine (500 μg/animal) was administered in vivo 2 hours before the death of the animals. The adipose tissues were rapidly removed and their phosphorylase activity was compared with that of the adipose tissue of the

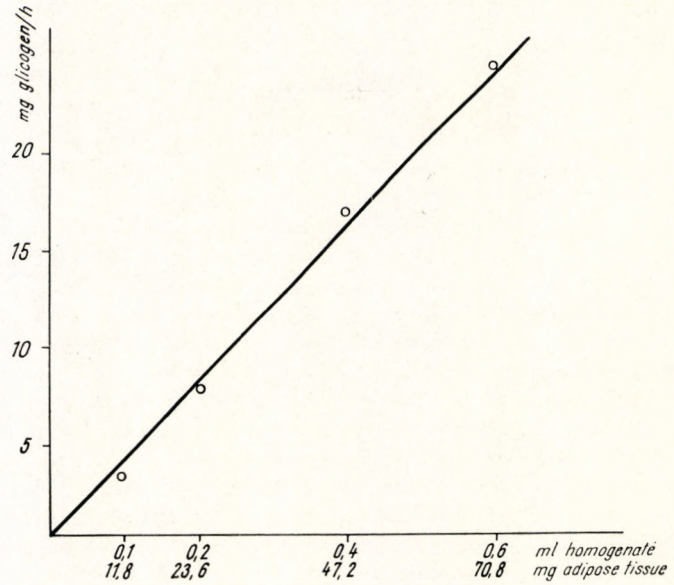

Fig. 3. Phosphorylase activity of the frog adipose tissue. The homogenizate was prepared from the right side adipose tissue of 3 frogs. Every point is the mean result of 6 measurements

3. ábra Béka zsírszövet foszforilase aktivitása. A homogenizátumot 3 béka jobb oldali zsírszövetéből készítettük. Minden pont 6 mérés átlageredménye.

untreated animals. In the second experiment the adipose tissue of untreated animals was incubated both in the presence or absence of norepinephrine (2 μg/ml incubation medium) and their phosphorylase activity has been determined.

Table 1.

The effect of noradrenaline on the phosphorylase activity of frog adipose tissue

1. táblázat

Noradrenalin hatása béka-zsírszövet foszforilase aktivitására

In vivo			In vitro		
Control	Noradrenalin	Note	Control	Noradrenalin	Note
glycogen mg/g/h	glycogen mg/g/h	Megjegyzés	glycogen/mg/g/h	glycogen mg/g/h	Megjegyzés
21.10	117.5		14.62 ± 0.25	26.24 ± 0.15	$P < 0.01$
16.15	79.0		51.94 ± 0.24	73.90 ± 0.40	$P < 0.01$
48.25	143.0		1.98 ± 0.35	8.11 ± 0.41	$P < 0.01$
18.15	92.5		4.41 ± 0.26	8.20 ± 0.14	$P < 0.01$
22.50	83.5		42.81 ± 0.30	64.59 ± 0.25	$P < 0.01$
Átlag 26.3	103.1	$P < 0.01$			

Table 1. demonstrates that after in vivo or in vitro administration of norepinephrine the phosphorylase activity is increased. This observation indicates that the hormone under the experimental conditions employed reached the adipose cells and proves that the lipolytic system of the adipose tissue of frogs is insensible to norepinephrine.

Discussion

The mammals mobilize the triglycerides stored in their fat depot in the form of free fatty acids which entering the blood circulatory system bound to protein are transported to the site of their utilization. The mobilization of fats is under a complicated metabolic, endocrine and nervous control. The facts that in both fish and amphibians an intensive lipolysis takes place in the adipose tissue and their blood contains also a considerable amount of free fatty acids suggest that the mobilization of triglycerides in the form of free fatty acids is general in vertebrates. It is also common in all groups of vertebrates that the plasma free fatty acid level is controlled by the carbohydrate metabolism of the adipose cells. Peroral administration of glucose to carp (FARKAS, in press) and to frog (study in progress) has the same effect on the plasma free fatty acid level as in mammals.

Catecholamines and probably all other adipokinetic hormones exercise their effect on the free fatty acid production of the mammalian adipose tissue through the stimulation of the "hormone-sensitive" lipase described by RIZACK (RIZACK 1964). It seems very probable that the hormones regulate the breakdown both of glycogen and triglycerides in the adipose tissue through the same mediator substance. KLAINER (1962) demonstrated that epinephrine enhances the tissue level of cyclic 3',5'-adenosine monophosphate which through a mechanism already known increases the phosphorylase activity of various tissues including the adipose tissue. It has been also demonstrated that cyclic 3',5'- adenosine monophosphate increases the free fatty acid production of the rat adipose tissue homogenizates (RIZACK 1964). BUTCHER (1965) found that the amount of this nucleotide in the adipose cells between certain limits is proportional to the lipolytic activity of the adipose tissue and that the inhibition of its formation influences the free fatty acid production.

The formation of the cyclic 3', 5'-adenosine monophosphate under the influence of various hormones was described in several animal species (SUTHERLAND 1962) and the presence of this nucleotide has been demonstrated in many animals. There is no doubt that its norepinephrine stimulated formation was involved also in the increase of the phosphorylase activity of the frog adipose tissue. On the other hand the fact that norepinephrine in frogs, norepinephrine and other catecholamines in fish were ineffective on the lipolytic activity of the adipose tissue, supports our earlier assumption i. e. the "hormone sensitive" lipase is absent from the adipose tissue of lower vertebrates.

The hormone-sensitive lipotlytic system has an importance in mammals in "emergency conditions" as with its aid it is possible to mobilize a great amount of energy within a short time. The absence of the "hormone-sensitive" lipase from the adipose tissue of lower vertebrates points to the fact that this form of gaining energy appears only later in the vertebrate phylogeny. Our present investigations do not exclude the possibility that in emergency condi-

tions the energy stored in the adipose tissue of lower vertebrates could be mobilized in some other forms than free fatty acids but they also raise the possibility that in such circumstances these organisms may mobilize other compounds (carbohydrates). The latter assumption may be supported by the observation that — as far as it can be concluded from the hyperglycemic effect of norepinephrine — the hepatic phosphorylase of fish and amphibans seems to be more sensitive to norepinephrine than that of mammals.

Summary

Norepinephrine administered in vivo (500 μg/animal) increased the blood sugar level in adult male frogs, but it had no demonstrable effect on the free fatty acid level of the plasma. The adipose tissue of frogs treated with norepinephrine in vivo did not contain more free fatty acid than that of the untreated animals and such adipose tissues did not produce more free fatty acid in vitro. Norepinephrine in vitro (2μg/ml incubation medium) did not increase the lipolytic activity of the adipose tissue of the frog. The in vivo or in vitro administration of the hormone increased significantly the phosphorylase activity of the adipose tissue. The results seem to indicate that the "hormone-sensitive" lipase is absent in the frog adipose tissue.

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NORADRENALIN HATÁSA KECSKEBÉKA *(RANA ESCULENTA L.)* ZSÍRSZÖVETÉNEK LIPASE ÉS FOSZFORILASE AKTIVITÁSÁRA

Farkas Tibor

Összefoglalás

In vivo adagolt noradrenalin erősen megemelte a kecskebéka *(Rana esuclenta L.)* vércukorszintjét, de nem volt kimutatható hatással a plasma szabad zsírsav szintjére. Noradrenalinnal in vivo kezelt békák zsírszövete nem tartalmazott több szabad zsírsavat, mint a kezeletlen állatoké, és az ilyen zsírszövetek in vitro sem termeltek több szabad zsírsavat. Noradrenalin in vitro sem növelte kecskebéka zsírszövetének lipolytikus aktivitását. In vivo, vagy in vitro adagolása után lényegesen megnövekedett a zsírszövet foszforilase aktivitása. Az eredmények arra utalnak, hogy a béka zsírszövetéből hiányzik a ,,hormon- érzékeny'' lipase.

ВЛИЯНИЕ НОРАДРЕНАЛИНА НА АКТИВНОСТЬ ФОСФОРИЛАЗЫ И ЛИПАЗЫ ЖИРОВОЙ ТКАНИ *RANA ESCULANTA L.*

Тибор Фаркаш

Норадреналин *in vivo* значительно повышает уровень сахара в крови лягущки, но не влияет на уровень свободных жирных кислот в сыворотке. Жировая ткань лягушки, получившей *in vitro* норадреналин, не содержит больше свободных жирных кислот, чем в контроле. Нет повышения уровня жирных кислот и при обработке такой ткани *in vitro*. Норадреналин *in vitro* не увеличивает липолитической активности жировой ткани. После дачи норадреналина *in vivo* или *in vitro* значительно увеличивается фосфорилазная активность жировой ткани. Полученные данные указывают на отсутствие гормончувствительной липазы в жировой ткани лягушки.