ANNAL. BIOL. TIHANY | 35 | 163—171 | HUNGARIA 1969

EFFECT OF AGENTS MODIFYING THE LEVEL OF CYCLIC 3', 5'-ADENOSINE MONOPHOSPHATE IN ADIPOSE TISSUE ON MOBILISATION OF FATS IN FISH AND FROGS

TIBOR FARKAS

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

Received: January 20th, 1969.

It is accepted that catecholamines and some other lipolytic hormones stimulate the decomposition of triglycerides in adipose tissue of mammals by increasing the intracellular level of cyclic3', 5'-adenosine monophosphate(cAMP, SUTHERLAND et al. 1965, WEISS et al. 1966, BUTCHER et al. 1966). The effect of catecholamines is to first increase the cAMP content of adipose cells and this is followed by an increase in the lipolytic activity (BUTCHER et al. 1965). The cAMP enhances the activity of the adequately prepared adipose tissue homogenates too (RIZACK, 1964). It appears likely that the lipolytic activity of mammalian adipose tissue is partly a function of the level of cAMP in adipose cells.

Investigating the effect of catecholamines, adrenocorticotroph hormone and glucagon on the lipolytic activity of fish (FARKAS, 1967), green frog (FARKAS, 1966) and grass snake (FARKAS, 1968) adipose tissue under in vivo or in vitro conditions it was found that these hormones in contrast to those of mammals do not increase but actually decrease the free fatty acid production. From observations of the effect of the same hormones on frog adipose tissue phosphorylase (FARKAS, 1966), as well as from the observations that dichloroisoproterenol is able to antagonize the above effect of catecholamines (FARKAS, 1968) it may be inferred that there is also a biochemical system consisting of adenyl cyclase cAMP in the adipose tissue of lower vertebrates. It is certainly clear that the catecholamines at least decrease the free fatty acid production through this mechanism. It is possible that catecholamines enhance the formation of α-glycerophosphate by stimulating the decomposition of glycogen and that this in turn leads to an increase in the reesterification processes. On the other hand it is questionable whether the cAMP plays such a role in the maintenance of the lipolytic activity in the adipose tissue of lower vertebrates as it was supposed to do in mammals.

In the present study the effect of two agents is investigated on the mobilisation of fats in fish and frogs which were shown to modify the lipolytic activity of catecholamines i.e. to influence the cAMP level in mammalian adipose tissue. Theophylline and nicotinic acid were selected for this purpose, the former affects the mobilisation of fats in the same way as the catecholamines, the latter in the opposite direction (TRINER and NAHAS, 1966, CARLSON, 1963, FARKAS et al. 1964, BOMBELLI et al. 1965, BJORNTROP, 1965).

Material and methods

The experiments were carried out on the bream *(Abramis brama* L.), adult male marsh frogs *(Rana ridibunda* L.*)* and on grass snakes *(Natrix natrix* L.) weighing 200—250, 60—70 and 80—100 grams respectively. The fish were collected from Lake Balaton and kept in suitable aquaria, the frogs were purchased and the grass snakes collected. The animals were brought into the institute one week before the experiment. No food was given to the animals in captivity.

The drugs, dissolved in physiological saline, were injected intraperitoneally into the fish, and into the ventral lymph sac of the frogs. The control animals received physiological saline only.

Blood was withdrawn by cutting the caudal vein from the fish and by decapitation from the frogs and collected into prechilled heparinized centrifuge tubes containing 0.1 ml of 2% heparin solution.

The in vitro experiments were carried out on adipose tissues taken from freshly killed animals. The adipose tissues were cut up in 5—10 mg pieces and incubated at room temperature and at pH 7.4 in albumin free frog-Ringer solution. The free fatty acid content of the adipose tissues were determined at the start and at the end of the experiments, the difference giving the amount of fatty acids produced during the experiment.

Results

After injection of theophylline into fish and frogs —in contrast to the mammals (Hynie et al. 1966, Triner and Nahas, 1966) — no increase was obtained in the plasma free fatty acid levels *(fig. 1.)*. Both fish and frogs responded to the drug with a decrease in the plasma free fatty acid level but as the *fig. 1.* shows the fish reacted more sensitively than did the frogs. 40 mg/kg theophylline evoked a maximal plasma free fatty acid response in the fish, but in the frog a dosage of over 100 mg/kg was needed to produce the same effect. The blood sugar level was increased in both animals. This effect of theophylline is the same in fish and frogs as in mammals.

Under in vitro conditions in the presence of theophylline the free fatty acid production is diminished *(fig. 2.)*. Incubating the adipose tissue of the fish pike perch *(Lucioperca lucioperca* L.) in the presence of both catecholamine and theophylline the production of fatty acids was further diminished, but the effect was only additive.

The in vivo administration of nicotinic acid into untreated rats results in a decrease of plasma free fatty acids (Farkas et al. 1964, Bombelli et al. 1965). When nicotinic acid was injected into fish no decrease was obtained in the plasma free fatty acid level within 6 hours after the injection even when the dose was increased ten fold *(fig. 3.)*. In the experiments presented in the *fig. 3.* the animals were killed 3 hours after the administration of nicotinic acid. After nicotinic acid treatment the plasma free fatty acid level has increased in the frog. In both species blood glucose was decreased by nicotinic acid.

As shown above and presented earlier, both theophylline and epinephrine decreases the level of free fatty acids in the blood of lower vertebrates. *Fig. 4.*

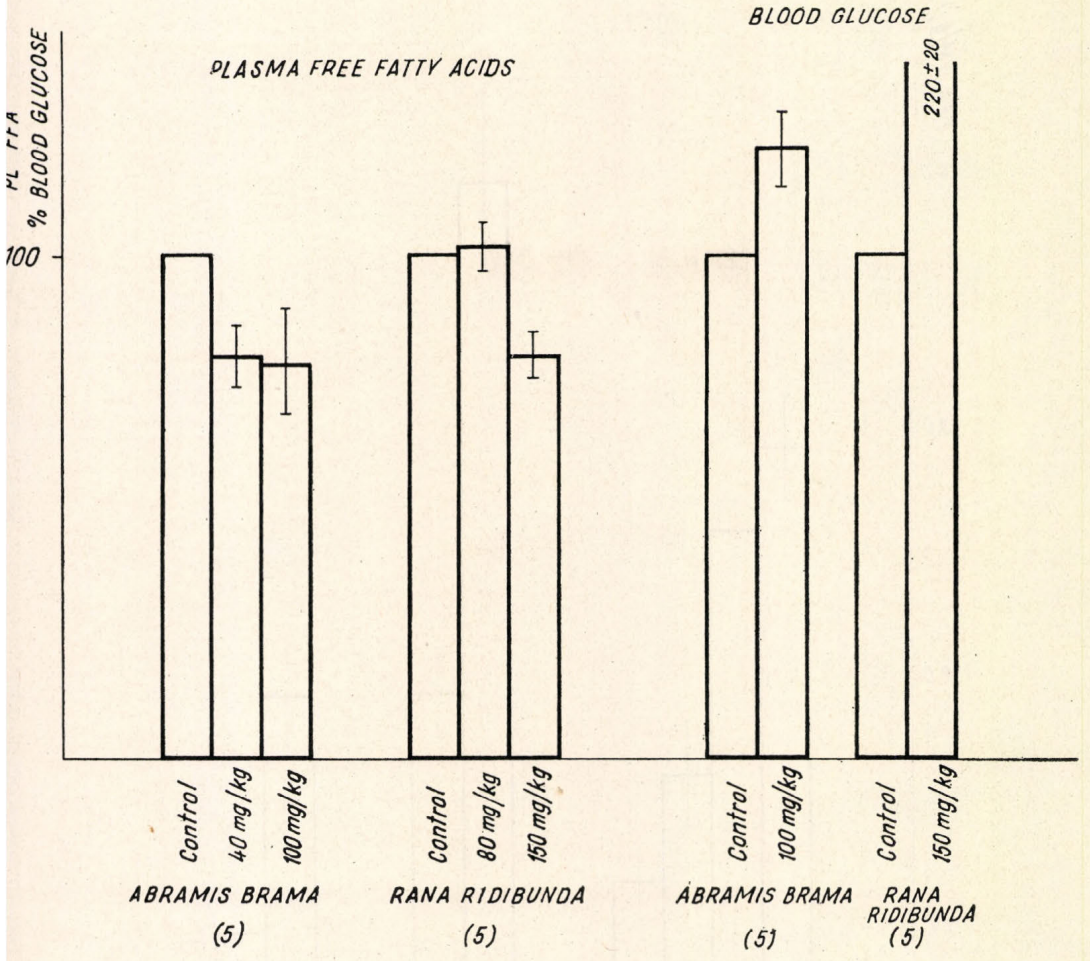

Fig. 1. Effect of theophylline on the mobilisation of fats and glycogen in fish and frog. The animals were killed two hours after injecting the drog. The results are expressed in the per cent of the control.

demonstrates that nicotinic acid is able to antagonize this effect of both agents. Thus the effect of nicotinic acid is essentially the same in the lower and the higher vertebrates except that in mammals it antagonises the lipolytic activity of catecholamines. Injecting both nicotinic acid and epinephrine in the fish produced an increase in the plasma free fatty acid level. A similar result was obtained when dichloroisoproterenol was used to antagonise the catecholamines in fish (FARKAS, 1958).

Discussion

The intracellular level of cAMP in the adipose tissue of mammals is regulated by the relation of two processes: the formation and decomposition of the nucleotide. Adenyl cyclase is responsible for its synthesis and a specific

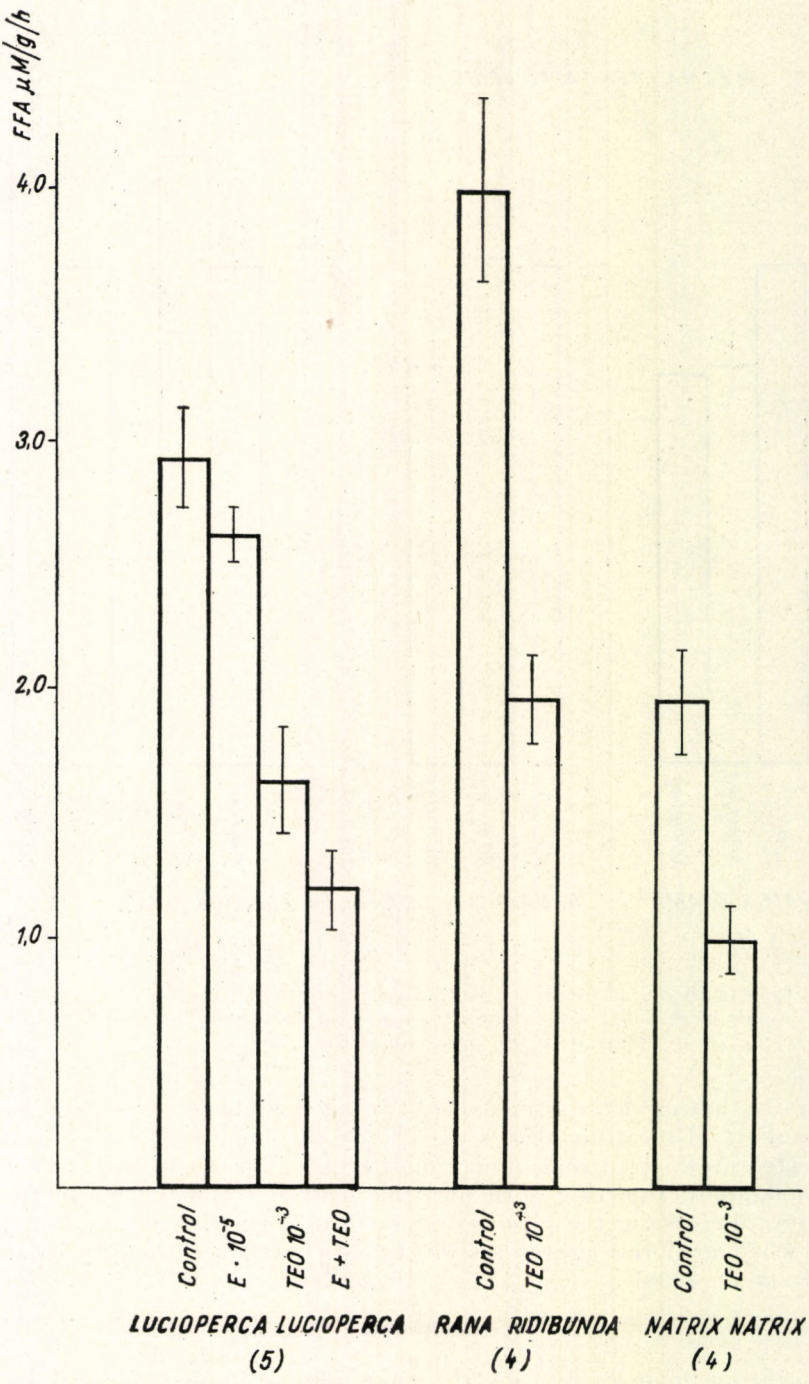

Fig. 2. Effect of theophylline on the free fatty acid production in vitro. The adipose tissues were incubated 60 minutes in albumin free frog Ringer solution at room temperature and at pH 7.4.

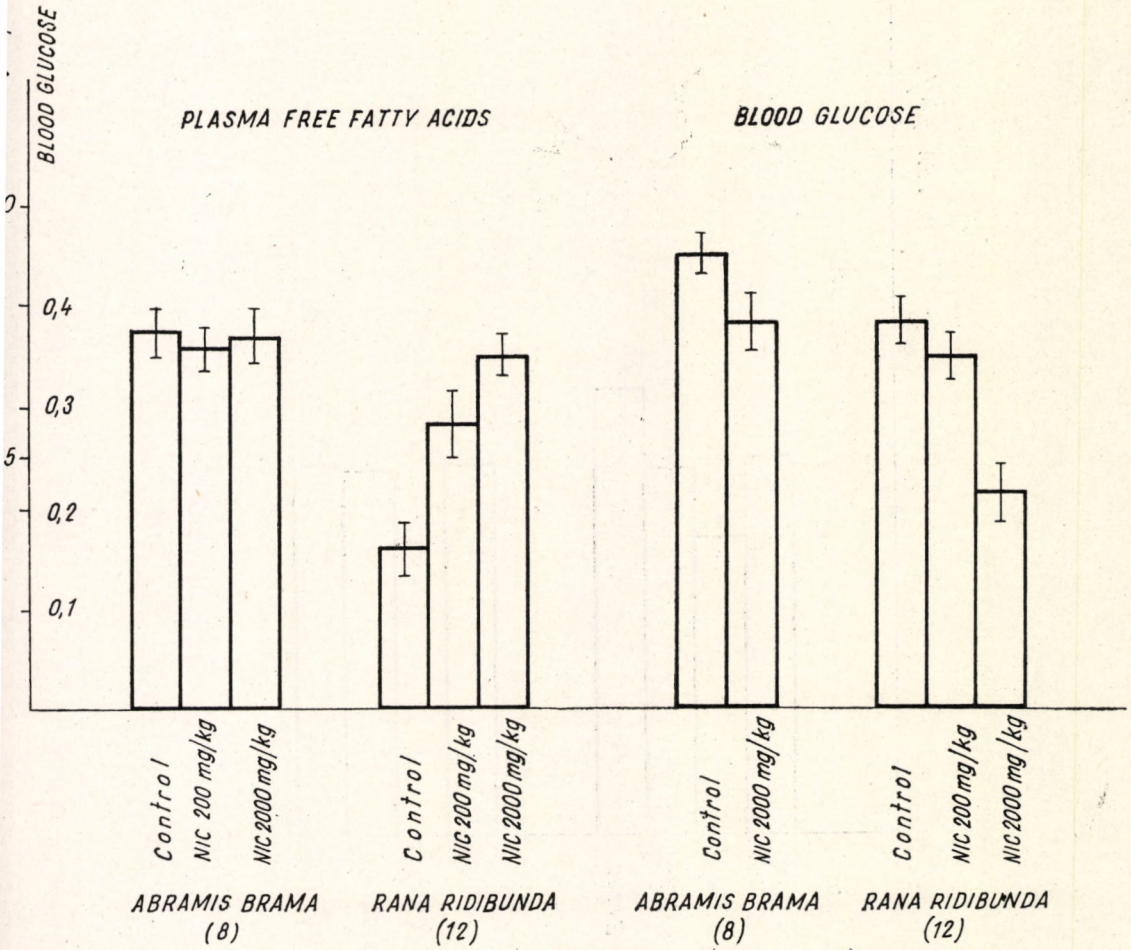

Fig. 3. Effect of nicotinic acid on plasma free fatty acid level and blood glucose in fish and frog.
The animals were killed 3 hours after administration of nicotinic acid.

phosphodiesterase for its decomposition. The former, in the presence of Mg^{++} ions, removes two phosphate moieties from ATP and joins the remainder to the 3rd carbonic atom of the ribose the latter splits the ring formed by the cyclase reaction and forms 5AMP.

The drugs employed in the present study affect the intracellular level of cAMP in different ways. Methylxantines (theophylline, cafeine) are able to block the phosphodiesterase and in this way to increase the cAMP level in the adipose tissue. Theophylline increases the level of free fatty acids in the blood of rats and potentiates the lipolytic effect of catecholamines both under in vivo and in vitro conditions (Hynie et al. 1966, Triner and Nahas, 1966). Nicotinic acid by stimulating the above enzyme (Krishna et al. 1966) may decrease the production of free fatty acids and antagonize the lipolytic effect

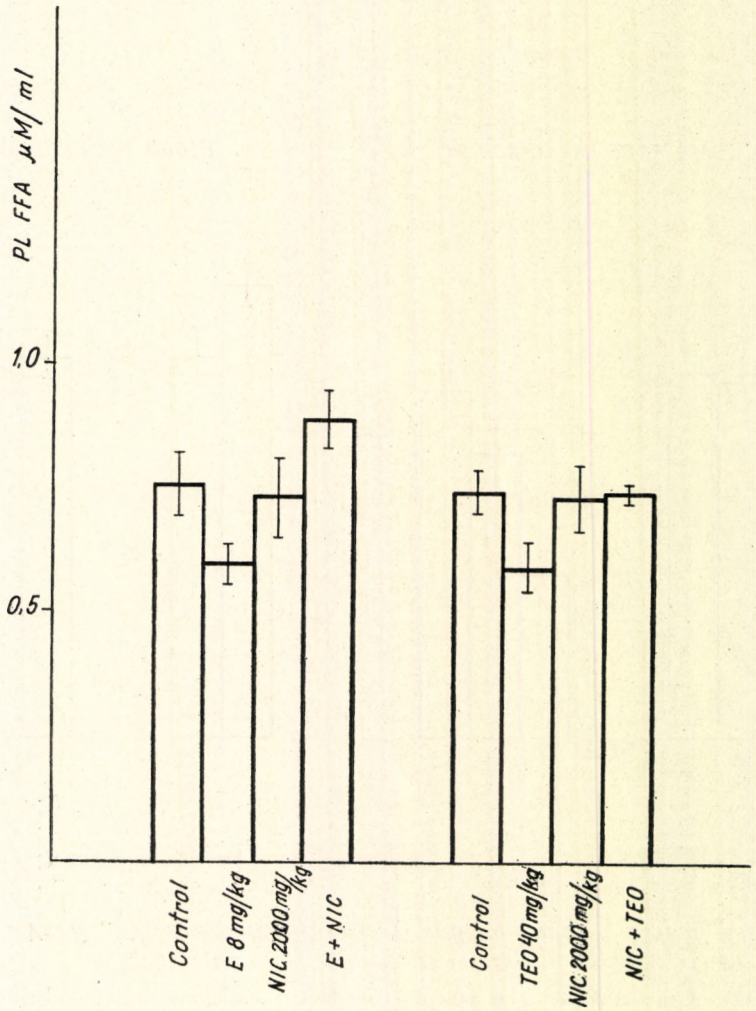

Fig. 4. The reversal of epinephrine and theophylline induced decrease of plasma free fatty acid level in fish by nicotinic acid.

of catecholamines (CARLSON, 1963, VERTUA et al., 1964, BJORTROP, 1965) and dibuthyryl cAMP (MELVIN et al. 1968) under in vitro and in vivo conditions.

If cAMP played any role in the maintenance of the lipolytic activity in fish and frog adipose tissue theophylline should increase and nicotinic acid decrease the plasma free fatty acid level. If, however, the catecholamines decreased the free fatty acid production in fish and frog adipose tissue by increasing the intracellular level of cAMP, it could be expected that:

1. theophylline would affect the free fatty acid production in the same and nicotinic acid in the opposite direction as the catecholamines and

2. nicotinic acid would antagonise the effect of catecholamines and theophylline on the mobilisation of fatty acids.

The experiments have shown, that theophylline decreases the production of free fatty acids in adipose tissue in vitro and the level of plasma free fatty acids in vivo. The adipose tissues incubated in the presence of both catecholamines and theophylline produced less free fatty acids than if they had been incubated only in the presence of epinephrine or theophylline.

In vivo administration of nicotinic acid did not lead to the expected decrease in the plasma free fatty acid levels. It left unchanged the plasma free fatty acids in the fish and inreased their level in the frogs. The blood glucose level was decreased in both cases.

The hypoglycemic activity of nicotinic acid has been described in the literature and evidence has been presented that-like insulin-it stimulates the uptake of glucose into the adipose tissue (LEE et al. 1961, BJORNTROP, 1965). Its effect on the plasma free fatty acid level was opposite to that which the hypoglycemic activity would have suggested, however. Increased uptake of glucose results in an increase in reesterification of the liberated fatty acids and this in turn leads to a decrease in the plasma free fatty acids. No final explanation can be offered at present as to why nicotinic acid does not lead to a decrease in the plasma free fatty acid level. It is possible that this may be related to its effect on the direction of glucose decomposition. Nicotinic acid-like insulin-directs the glucose into the pentose phosphate shunt (LYNN et al. 1960, LEE et al. 1961) which results in the formation of free fatty acids and not α-glycerophosphate.

The observation that nicotinic acid antagonised the effect of epinephrine and theophylline on the free fatty acid level furnishes further evidence that catecholamines, and probably the other lipolytic hormones too, decrease the plasma free fatty acid level in lower vertebrates by increasing the intracellular level of cAMP in the adipose tissue.

It may be inferred from the above work that cAMP formed by the lipolytic hormones does not play any role in the maintenance of the lipolytic activity in the adipose tissue of lower vertebrates. How this nucleotide stimulates the triglyceride lipase in the adipose tissue of mammals is not clear. It is possible that the enzyme, like the phosphorylase, has an active and an inactive form (HYNIE et al. 1966) and that it affects the conversion of the inactive lipase to an active form. On the other hand, it is not clear whether this is the only lipase in the adipose tissue of the mammals. The presence of a lipase independent of catecholamines supports the results of RUBINSTEIN et al. (1964) showing that besides the lipoprotein lipase and hormone sensitive lipase there also exists a lipase in the adipose tissue of the rat which does not require catecholamines for its activity. The effect of nicotinic acid on the in vitro free fatty acid production of rat adipose tissue suggests the presence of such a catecholamine-independent lipolytic activity as well. Under in vitro conditions, nicotinic acid is not able to antagonise the free fatty acid production of the untreated tissues, only that of the catecholamine treated tissues (CARLSON, 1963). From the observation that under in vitro conditions maximally only a 70% decrease was obtained in the plasma free fatty acids of rats treated with nicotinic acid (BOMBELLI et al. 1965) it may be inferred that about 30% of the fatty acids is the product of such a catecholamine-independent lipolytic activity. Because the catecholamines did not increase the rate of decomposi-

tion of triglycerides in the adipose tissue of fish, frog and grass snake, it is thought that this catecholamine independent lipolytic activity is the only one ensuring the decomposition of triglycerides in these animals.

Summary

Theophylline under in vivo and in vitro conditions decreased the plasma free fatty acid level and adipose tissue free fatty acid production in fish *(Abramis brama* L., *Lucioperca lucioperca* L.), frog *(Rana ridibunda* L.*)* and grass snake *(Natrix natrix* L.*)* After in vivo administration of nicotinic acid, the plasma free fatty acids remained unchanged in the fish, while in the frog, their level was raised. Nicotinic acid antagonised the effect of epinephrine and theophylline on the free fatty acid level in the fish. Because theophylline is known to increase and nicotinic acid to decrease the intracellular level of cyclic 3', 5'-adenosine monophosphate in the adipose tissue it is supposed that this nucleotide does not play role in the control of the lipolytic activity in the adipose tissue of lower vertebrates.

REFERENCES

Bombelli R., T. Farkas, R. Vertua (1965): Investigations on the effect of nicotinic acid on plasma free fatty acid levels. — *Med. Pharmacol. exp.* **12,** 8-14.

Bjorntrop P. (1965): Effect of nicotinic acid on adipose tissue metabolism in vitro. — *Metab. Clin. Exp.* **14,** 836—840.

Butcher R. W., R. J. Ho, M. C. Meng, E. W. Sutherland (1965): Adenosine 3', 5' monophosphate in biological materials, II. The measurement of adenosine 3', 5' monophosphate in tissues and the role of the cyclic nucleotida in the lipolytic response of fat to epinephrine. — *J. Biol. Chem.* **240,** 4515—4523.

Butcher R. W. (1966): Cyclic 3', 5'-AMP and the lipolytic effects of hormones on adipose tissue. — *Pharmacol. Rev.* **18,** 237—241.

Carlson L. A. (1963): Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. — *Acta Med. Scand.* **173,** 719—722.

Dole V. P. (1956): A relation between non esterified fatty acids in plasma and the metabolism of glucose. — *J. Clin. Invest.* **35,** 150—154.

Farkas T., R. Vertua, M. M. Usardi, R. Paoletti (1964): Investigationson the mechanism of action of nicotinic acid. — *Life Sci.* **3,** 821—827.

Farkas T. (1966): Effect of norepinephrine on adipose tissue lipase and phosphorylase activity of the frog *Rana esculenta* L. — *Annal. Biol. Tihany* **33,** 159—166.

Farkas T. (1967): The effect of catecholamines and adrenocorticotrophic hormone on blood and adipose tissue FFA levels in the fish, *Cyprinus carpio* L. — *Progr. Biochem. Pharmacol.* **3,** 314—319.

Farkas T. (1968): The effects of some adrenergic blocking agents on the mobilisation of fatty acids in lower vertebrates. — *Annal. Biol. Tihany* **35,** 127—133.

Hynie S., A. Krishna, B. B. Brodie (1966): Theophylline as a tool in studies of the role of cyclic adenosine 3', 5'-monophosphate in hormone-induced lipolysis. — *J. Pharmacol. Exptl. Ther.* **153,** 90—96.

Hyvärinen D., E. A. Nikkila (1962): Specific determination of blood glucose with o-toluidine. — *Clin. Chim. Acta* **7,** 140—143.

Krishna G., B. Weiss, I. J. Davies, S. Hynie (1966): Mechanism of nicotinic acid inhibition of hormone-induced lipolysis. — *Fed. Proc.* **25,** 719.

Lee H. M., R. M. Ellis, M. V. Sigal, Jr. (1961): Some insulin-like effects of nicotinic acid observed with isolated rat epididimal adipose tissue. — *Biochem. Biophys. Acta* **49,** 408—410.

Lynn W. S., R. M. MacLeod, R. H. Brown (1960): Effects of epinephrine, insulin and corticotropin on the metabolism of rat adipose tissue. — *J. Biol. Chem.* **235,** 1904—1911.
Melvin B., N. S. Merlind, J. T. Roave (1968): Control of the metabolism and lipolytic effects of cyclic 3', 5'-adenosine monophosphate in adipose tissue by insulin, methylxanthines and nicotinic acid. — *J. Biol. Chem.* **243,** 3973—3977.
Rizack M. A. (1964): Activation of an epinephrine sensitive lipolytic activity from adipose tissue by adenosine 3', 5'-phosphate. — *J. Biol. Chem.* **229,** 392—395.
Rubinstein D., S. Chik, J. Naylor, J. C. Beck (1964): Lipolytic action of epinephrine in adipose tissue homogenates. — *Amer. J. Physiol.* **206,** 149—152.
Triner L., G. G. Nahas (1966): Effects of teophylline and catecholamines on lipolysis and glycogenolysis in vivo. — *J. Pharmacol. Exptl .Ther.* **153,** 569—572.
Sutherland E. W., L. Ove, R. W. Butcher (1965): The action of epinephrine and the role of the adenyl cyclase system in hormone action. — *Rec. Prog. in Hormone Res.* **21,** 623—646.
Weiss B., J. I. Davies, B. B. Brodie (1966): Evidence for a role of adenosine 3', 5'-monophosphate in adipose tissue lipolysis. — *Biochem. Pharmacol.* **15,** 1553—1562.

A ZSÍRSZÖVET cAMP SZINTJÉT BEFOLYÁSOLÓ ÁGENSEK HATÁSA ZSÍRMOZGÓSÍTÁSRA HALAKBAN ÉS KÉTÉLTŰEKBEN

Farkas Tibor

Összefoglalás

Teophyllin hatására in vivo vagy in vitro körülmények között csökken a plazma szabad zsírsavak szintje, ill. a zsírszövet szabad zsírsav termelése halban *(Abramis brama)* és kétéltűben *(Rana ridibunda)*. Nikotinsav in vivo adagolása után a halban változatlan maradt, kétéltűben pedig emelkedett a plazma szabad zsírsavak szintje. Nikotinsav antagonizálta adrenalin és teophyllin hatását a plazma szabad zsírsavszintre halban. Minthogy teophyllin növeli, nikotinsav pedig csökkenti a zsírszövet cAMP szintjét emlősökben, jelen és korábbi eredményeink alapján feltételezzük, hogy ez a nucleotida nem játszik szerepet a lipolytikus aktivitás fenntartásában alacsonyabbrendű gerincesekben.

ВОЗДЕЙСТВИЕ ВЕЩЕСТВ, ИЗМЕНЯЮЩИХ УРОВЕНЬ ЦИКЛИЧЕСКОЙ АМФ ЖИРОВОЙ ТКАНИ, НА МОБИЛИЗАЦИЮ ЖИРА РЫБ И АМФИБИЙ

Т. Фаркаш

Под влиянием теофиллина in vivo и in vitro снижается уровень свободных жирных кислот плазмы и синтез свободной жирной кислоты в жировой ткани рыб *(Abramis brama)* и амфибий *(Rana ridibunda)*. После введения никотиновой кислоты in vivo уровень свободных жирных кислот плазмы у рыб останется без изменения, а у амфибий — увеличивается. Никотиновая кислота оказала антагонистическое адреналину и теофиллину действие в изменении уровня свободных кислот плазмы рыб. Так как теофиллин увеличивает, а никотиновая кислота снижает уровень циклической АМФ жировой ткани млекопитающих, на основе этих и предыдущих данных автор предполагает, что этот циклический нуклеотид не играет роли в сохранении липолитической активности низщих позвоночных.