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CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND ADENYLCYCLASE IN LIVER, ADIPOSE TISSUE AND BRAIN OF RATS TREATED WITH β-PYRIDYLCARBINOL

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Abstract—In tissues of starved rats cyclic adenosine 3′,5′-monophosphate (cyclic AMP) was determined by an enzymatic isotope dilution method. A single per os application of 1 m-mole/kg β-pyridylcarbinol (or nicotinic acid) caused a bi-phasic change of cyclic AMP simultaneously in epididymal fat, liver and brain: initially a transient decrease of about 20–40 per cent occurred for 30 min followed by an increase of up to 150 per cent of controls after 1 hr. The levels returned to normal after 1 hr in liver and brain and after 5 hr in adipose tissue. The increase of cyclic AMP was not related to an increased activity of adenylcyclase in liver homogenate. Cyclic AMP decreased simultaneously with the free fatty acids (FFA) in plasma, but the subsequent increase of cyclic AMP did not parallel any further change in blood plasma, such as an increase of FFA (rebound) and of urea or decrease of cholesterol and triglycerides. It is therefore unlikely that the known changes of plasma metabolites due to β-pyridylcarbinol or nicotinic acid depend directly on variations of total cyclic AMP in the tissues studied.

CYCLIC adenosine 3′,5′-monophosphate (cyclic AMP) has been implicated as an intracellular messenger in the lipolytic action of epinephrine (for review see ref. 1) as well as in the antilipolytic action of insulin[2] and nicotinic acid.[3] Most studies in connection with cyclic AMP have, however, been performed only *in vitro*.

The present investigation deals with the level of cyclic AMP and the activity of adenylcyclase in tissues of rats given a single large dose of antilipolytic agents, i.e. β-pyridylcarbinol or nicotinic acid respectively.

EXPERIMENTAL PROCEDURE

Animals and preparation of tissues. Male albino rats weighing 140–160 g from a closed randomized colony (stock Füllinsdorf) were starved for 24 hr (drinking water *ad lib.*) and then given per stomach tube (at 8.00 a.m.) a single dose of 1·0 m-mole/kg nicotinic acid or β-pyridylcarbinol tartrate suspended in 5 per cent gum arabic. Controls received the vehicle only.

The rats were decapitated for the measurement of various plasma metabolites and adenylcyclase activity in liver. For the determination of cyclic AMP the tissues were rapidly removed from stretched animals, i.e. a liver lobe within 15 sec, brain and epididymal fat pads in less than 30 sec. Liver and brain were directly put into liquid nitrogen, the fat pads after a quick rinsing into Krebs–Ringer-bicarbonate, pH = 7·4. The previously reported[4] post-mortal increase of cerebral cyclic AMP in mice did not influence the present results since it occurred after 30 sec only. The level of cyclic

AMP in liver lobes removed by freezing clamps (of −190°) was the same as that in liver obtained as described above.

Isolation and determination of the cyclic AMP. At +2° the frozen tissue was homogenized in 5–10 vol. 5% trichloracetic acid, centrifuged for 30 min with 30,000 *g* and the supernatant washed three times with ether.[5] After addition of 0·2 ml Tris-buffer 0·5 M, pH = 8·0, to 3 ml supernatant, all the nucleotides except cyclic AMP[6] were removed by a $ZnSO_4$-$Ba(OH)_2$-precipitation[7] and put on a column of 0·5 × 5·5 cm AG 50 W — X 4 (200–400 mesh) to remove traces of cations. The column was washed with 1 ml bidest. water and eluted with 6 ml water. The eluate was supplemented with 0·2 ml Tris-buffer and put on a column of AG 1 — X 2 (200–400

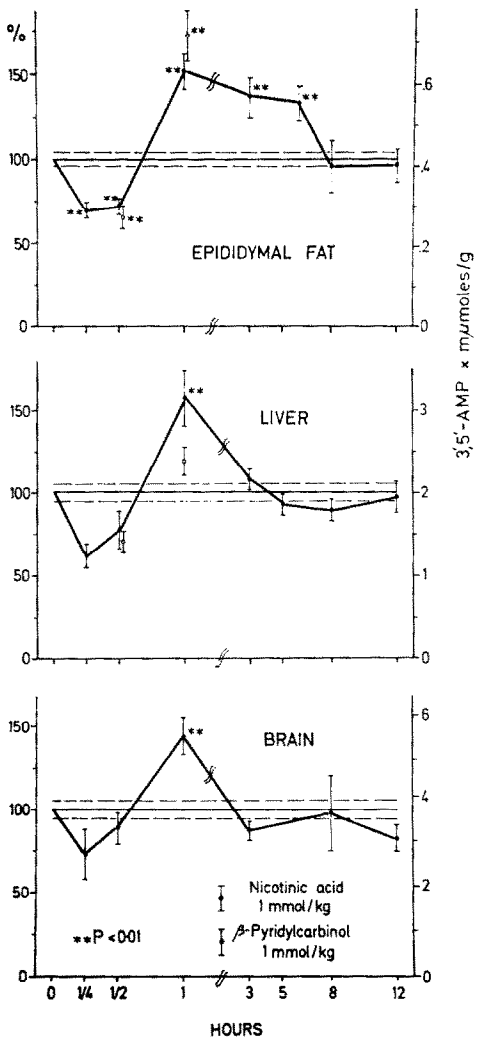

FIG. 1. Changes of total 3',5'-AMP in various tissues after a single dose of 1 m-mole/kg of β-pyridylcarbinol or nicotinic acid. At time 0 the drug was given per os to starved rats. Each point represents a mean ± standard error of 3–15 determinations.

mesh) which was subsequently washed with 1 ml water and eluted with 4 ml 0·1 N HCl. After evaporation under reduced pressure the residue of the eluate was taken up in 0·2 ml Tris-buffer. An aliquot (20 μl) was used for the isotope dilution method with phosphodiesterase according to Brooker *et al.*,[8] with the difference that 150,000 counts/min [^{3}H]3′,5′-AMP (1·3c m-mole, Amersham) were used instead of 10,000 counts/min.[9] During the incubation the K_m of the purified phosphodiesterase ($0{\cdot}15 \pm 0{\cdot}03 \times 10^{-6}$ M as calculated by a computer program[10]) was lower than the concentration of cyclic AMP (1×10^{-6} M radioactive cyclic AMP mixed with about 2×10^{-6} M inactive one) as postulated.[11] Triplicate assays were run on each tissue and the results were averaged and calculated as one determination. The recovery of the extraction procedure varied between 60–70 per cent (in normal and β-pyridylcarbinol-pretreated rats) and the results were corrected correspondingly.

The specificity of the present method seems to be fair because of two facts:

(a) After preincubation of deproteinized ether-extracted samples with purified phosphodiesterase no cyclic AMP could be recovered.

(b) In rats treated with glucagon cyclic AMP was increased several fold in the liver[5,12] but not in brain[13] (results not shown).

In the present experiments the absolute levels of cyclic AMP (Fig. 1) had the same order as reported in the literature.[5,4,14–19] As these controls never showed significant diurnal changes they were averaged for each organ and taken as 100 per cent.

Adenylcyclase activity. The enzyme activity was measured in total homogenates of livers in 5 vol. 0·32 M sucrose. The rats (female, 60–80 g) were treated as for cyclic AMP determination. The total homogenates were incubated by a modification (Burkard and Gey 1970, in preparation) of the procedure of Rodbell,[7] i.e. for 10 min at 37° with 1·0 mM ATP including 15 μC α-^{32}P and 0·07 μC cyclic AMP-^{3}H (for recovery), 20 mM proxyphylline/purophyllin; Siegfried S.A., Zofingen, Switzerland 40 mM Tris HCl buffer, pH = 7·3, ATP-regenerating system[20] without or with sodium fluoride 1×10^{-2} M in a total volume of 0·45 ml. The newly formed cyclic AMP was isolated according to Krishna *et al.*[21] The overall recovery was about 35 per cent and was corrected in the results.

Determination of the plasma metabolites. Approved methods were used for the assay of free fatty acids (FFA),[22,23] glucose,[24,25] urea nitrogen,[26] cholesterol[27] and triglycerides.[28]

RESULTS AND DISCUSSION

A single large dose of β-pyridylcarbinol had a bi-phasic effect on total *cyclic AMP* which was almost identical in epididymal fat, liver and brain (Fig. 1): there was an initial decrease of total cyclic AMP of about 20–40 per cent ($P < 0{\cdot}01$ for epididymal fat) at 15 and 30 min, followed by an increase up to 150 per cent of controls ($P < 0{\cdot}01$ for all organs). The increase lasted in the adipose tissue for at least 5 hr but in liver and brain only for about 1 hr. According to pilot studies nicotinic acid gave similar results at least in liver and adipose tissue.

The *adenylcyclase activity* of liver (as measured without fluoride) showed a tendency to increase 4 hr after β-pyridylcarbinol, i.e. much later than the rise of the cyclic AMP levels. A small decrease would hardly have been detected since the enzyme activity in the liver was very small (at the limit of the method, i.e. only 0·04 per cent turnover/10 min incubation). When, however, the enzyme was maximally activated by fluoride no

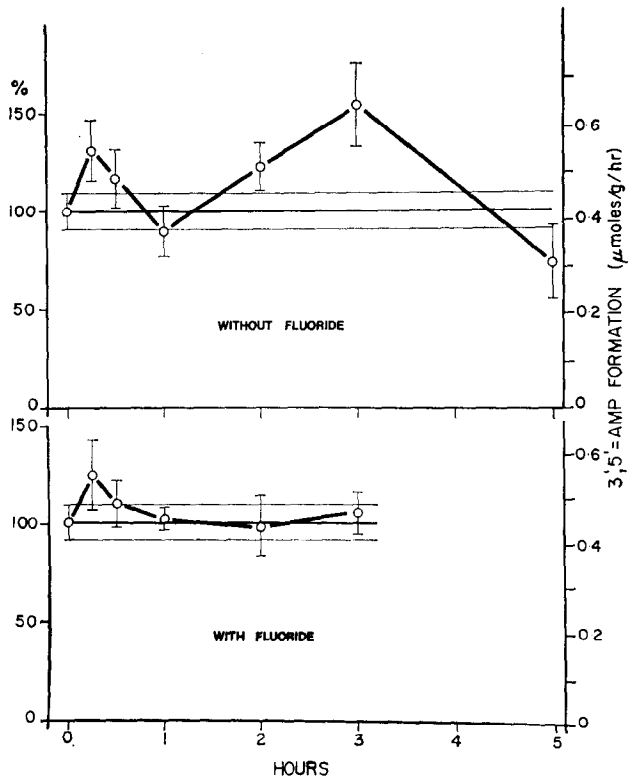

FIG. 2. Influence of pretreatment with 1 m-mole/kg of β-pyridylcarbinol on the activity of adenylcyclase in rat liver. Rats were treated as in Fig. 1 and the total homogenate of livers incubated as described under methods. Each point represents a mean of 6–30 determinations ± standard error.

effect could be seen (Fig. 2). Since the alterations of cyclic AMP in liver are obviously not directly related to changes of adenylcyclase activity they might rather depend on changes of the phosphodiesterase activity[29–36] and/or other phenomena like storage or binding.

FFA. The initial decrease of cyclic AMP occurs about the same time as the decrease of FFA in blood plasma but the latter lasts much longer, i.e. for about 6 hr (Fig. 3). This means that the FFA are still depressed while the cyclic AMP showed an increase. There is no significant change of cyclic AMP during the "rebound" phase of FFA, e.g. at 12 hr.

It has been stated by Butcher *et al.*[3] that *in vitro* $3{\cdot}3 \times 10^{-5}$ M nicotinic acid almost completely suppresses the formation of cyclic AMP as induced by epinephrine and caffeine in isolated fat cells.[37] The present experiments allow the conclusion that nicotinic acid and β-pyridylcarbinol respectively are able to decrease the total cyclic AMP even in intact rats. But the initial decrease of total cyclic AMP after a large dose of β-pyridylcarbinol or nicotinic acid is rather small and very transient. Therefore, it can at most initiate the depression of plasma FFA but can hardly be responsible for the

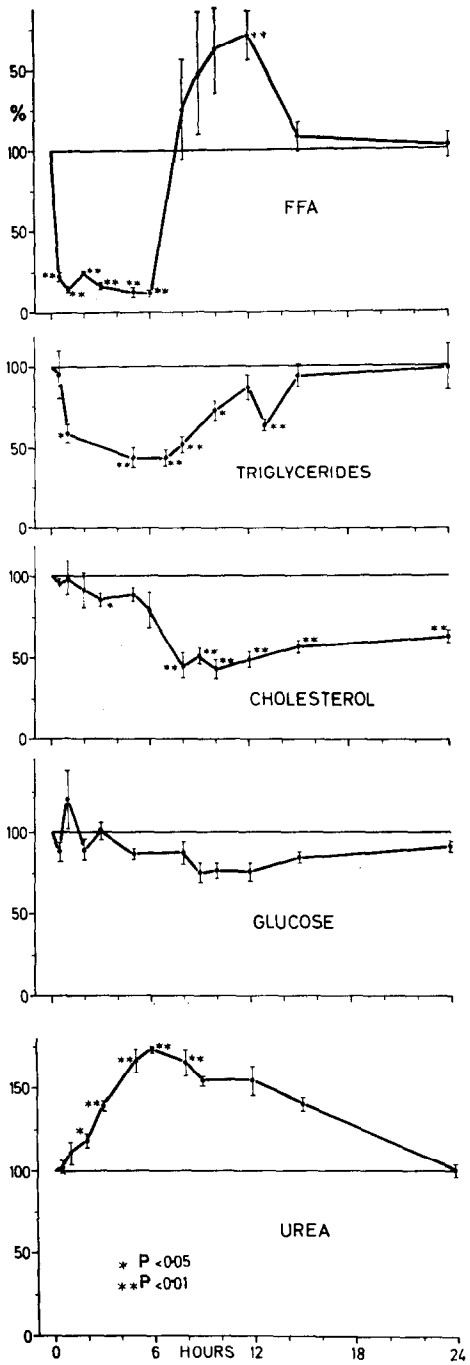

FIG. 3. Changes of plasma values after a single per os dose of 1 m-mole/kg of β-pyridylcarbinol. The animals were treated as in Fig. 1. The calculation of percentage values was done in comparison to controls killed at the same time interval (100 per cent). The average ± standard error of absolute values was for FFA 690 ± 45 μequiv./l., triglycerides 54 ± 3 mg/100 ml, cholesterol 58 ± 2 mg/100 ml, glucose 102 ± 3 mg/100 ml and urea 48 ± 1 mg/100 ml. Each point represents a mean of at least five determinations ± standard error.

inhibition of lipolysis during the whole experimental period. Furthermore, no correlation seems to exist between the total level of cyclic AMP in tissues and the rebound phenomenon of FFA.

Other plasma metabolites. Changes of total level of cyclic AMP in the tissues tested lack also a time correlation to plasma levels of glucose, urea, triglycerices and cholesterol (Fig. 3). This does, however, not exclude that particular, possibly secondary effects of β-pyridylcarbinol are mediated through the ulterior rise of cyclic AMP, e.g. enhancement of glycogenolysis, gluconeogenesis and lipolysis in liver. Both, the rapid decrease of cyclic AMP and the subsequent overshoot reaction occur in a rather parallel manner in liver, adipose tissue and brain. Since these changes seem to lack a distinct connection to the most direct effects of pyridine derivatives they could be related to circulatory alterations.

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