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3′, 5′-AMP-induced hyperglycemia in intact rats and in the isolated perfused rat liver*

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PREVIOUS reports have shown that the hyperglycemic response to epinephrine is mediated by a cyclic nucleotide, 3′,5′-AMP,[1] which acts as a cofactor for dephosphophosphorylase kinase, the enzyme that catalyzes the formation of the active form of phosphorylase.[2] Several papers recently have appeared in which 3′,5′-AMP has been shown to exert a physiological response in organs both *in situ* and when completely isolated. Hilton *et al.*[3] have shown that infusions of either ACTH or 3′,5′-AMP into the adrenal artery of the dog result in elevation of hydrocortisone concentration in the adrenal vein. Orloff and Handler[4] demonstrated a vasopressin-like effect by painting the serosal surface of the toad bladder with 3′,5′-AMP.

Unpublished data have shown that infusion of epinephrine into the portal cannula of the isolated artificially perfused rat liver causes hyperglycemia, glycogenolysis, and activation of phosphorylase.[5] Perske *et al.*[6] and Niemeyer *et al.*[7] in studies employing the intact rat, were unable to demonstrate epinephrine-induced phosphorylase activation. On the other hand, intravenous injections of 3′,5′-AMP (4 mg/kg) into dogs showed slight increases in blood glucose concentration.[2]

This communication is concerned with the effect of intraperitoneal administration of 3′,5′-AMP into the intact rat and the effect of infusion of the cyclic nucleotide into the isolated perfused liver preparation.

METHODS

The liver perfusion method used is a modification by Miller† of the method reported by Miller *et al.*[8] and further modified for these studies by Northrop and Parks.[5] By the use of this technique it is possible to determine blood glucose concentrations immediately before and after passage of blood through the liver and to remove representative samples of liver tissue at selected times before and after infusion of an agent such as 3′,5′-AMP. Thus rapid changes in the concentration of metabolites and the activities of various enzymes can be determined in the tissue. Blood glucose concentrations

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† J. A. Miller, personal communication.

were determined by the glucose oxidase procedure[9] after deproteinization,[10] on both "portal" and "caval" samples obtained from blood circulating in the perfusion apparatus and in tail-vein blood samples obtained from intact rats. Glycogen determinations were made on alkaline-digested liver samples in which glycogen was precipitated by alcohol,[11] and measured as glucose.[12] Phosphorylase activity was determined on liver samples which had been frozen in a liquid nitrogen-cooled isopentane bath immediately after removal from the perfusion apparatus. The frozen samples then were pulverized in a stainless steel mortar with a stainless steel pestle, maintained about $-70°$ in an ethanol–dry ice bath. Aliquots of pulverized frozen liver were homogenized in a solution containing 0·15 M potassium chloride and 0·01 M sodium fluoride and brought to a final dilution of 1:10. The phosphorylase activities of the homogenates were assayed in the direction of glycogen formation by the method of Cori *et al.*[13] Male rats of the Holtzman strain, weighing about 300 g, served as the liver donors for the perfusion experiments. Male rats weighing about 100 g were employed in the *in-vivo* studies.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the results obtained from intraperitoneal injection of 3′,5′-AMP into intact rats and infusion of the nucleotide into the isolated liver preparation.

Table 1 clearly demonstrates that the intraperitoneal administration of 10 mg of 3′,5′-AMP/kg into the intact rat causes hyperglycemia. Since with broken cell preparations activation of hepatic

TABLE 1. EFFECT OF 3′,5′-AMP ON INTACT RATS

No. of rats	Before treatment blood glucose* (mg/100 ml)	Treatment 3′,5′-AMP (mg/kg)	After treatment blood glucose* (mg/100 ml)
5	76 ± 5·6	0·00	78 ± 3·4
5	68 ± 1·1	0·10	73 ± 5·1
5	74 ± 2·0	1·00	74 ± 2·4
5	65 ± 3·4	10·00	120 ± 3·3
5	71 ± 2·5	100·00	118 ± 6·8

Male rats (100 g) were anesthesized with intraperitoneal administration of sodium pentobarbital (40 mg/kg). Thirty minutes later control blood samples were obtained followed by the intraperitoneal administration of the appropriate dose of 3′,5′-AMP contained in 1 ml of 0·15 M (potassium) phosphate buffer, pH 7·3; 15 min after injection of the nucleotide a second blood sample was obtained.

* Values are expressed as means ± standard error.

phosphorylase is effected by 3′,5′-AMP concentrations in the range of 10^{-6} to 10^{-7} M, the dosage employed in this study (10 mg/kg) appears to be well above the physiologic level.[14] However, factors such as the rates of absorption, distribution, and metabolism have profound influences on the actions of all drugs, and without further study one cannot estimate the actual concentration of cyclic nucleotide that will be attained in a tissue after the injection of a given dose. Several suggested explanations of the cyclic nucleotide-induced hyperglycemia are: (1) that it acts through the nervous system or on some other organ or tissue to release glycogenolytic agents such as catecholamines or by preventing the inactivation of intrinsic catecholamines; (2) that it acts directly in the liver cell after penetration of the cell membrane; or (3) that it acts on receptors in the liver cell membrane without penetration into the intracellular space. Hilton *et al.*[3] were unable to detect a change in catecholamine content of adrenal vein blood after infusion of 3′,5′-AMP into the adrenal artery of the dog. To rule out the possibility of 3′,5′-AMP-induced release of glycogenolytic agents or stimulation of other extrahepatic factors, it was decided to employ the isolated liver perfusion technique.

Table 2 shows that hyperglycemia was elicited in the isolated perfused liver by 3′,5′-AMP concentrations as low as 0·002 mg/ml of blood. The rate of increase of hyperglycemia was similar above cyclic nucleotide doses of 0·02 mg/ml of blood, whereas the response to 0·002 mg/ml was definitely

suboptimal and perhaps in the threshold range for these experimental conditions. The dosage, 0·002 mg/ml, is a concentration of about 6×10^{-6} M and approaches the concentration reported to activate phosphorylase in broken cell preparations. Furthermore, the present studies were performed without the addition of agents such as theophylline which inhibit the hydrolysis of 3′,5′-AMP by phosphodiesterase. Infusion of 5′-AMP (2·0 mg/ml blood) did not elicit a hyperglycemic response. The perfusion study does not distinguish between 3′,5′-AMP action at the intra- or extracellular level

TABLE 2. EFFECT OF 3′,5′-AMP ON THE ISOLATED PERFUSED RAT LIVER

Nucleotide in blood (mg/ml)	Blood glucose (mg/100 ml)		Liver phosphorylase specific activity (20 mg liver/assay tube)		Blood flow (ml/min)	
	Control	Nucleotide	Control	Nucleotide	Control	Nucleotide
2·3*	233	330	3·4	8·2	4·7	4·3
2·5	94	143	2·4	9·7	6·5	5·5
1·8*	260	360	2·4	9·4	6·3	5·5
1·0	197	293			7·1	7·8
0·2	290	373	3·1	8·5	4·8	6·1
0·02	167	213	2·8	8·1	9·6	8·0
0·002	283	303			7·3	6·8
0·0002	143	140			6·8	6·5
0·0†	187	183	3·0	2·5	9·0	9·0

Control samples of blood and tissue were taken after a 60-min equilibration period. Nucleotide-treated samples of blood and tissue were taken after a 7-min period of nucleotide infusion at the stated concentrations. Phosphorylase activity was assayed in the direction of glycogen formation and expressed in micromoles of product formed per minute per gram of wet weight of liver at 30°. Each dose was tested in a different liver preparation.

* Comparison of analysis made on control and treated liver samples demonstrated approximately 1·0 g/100 g decrease in glycogen concentrations after cyclic nucleotide infusion.

Nucleotide (mg/ml blood)	Glycogen (g/100 g)	
	Control	Nucleotide
2·3	2·98	1·89
1·8	2·45	1·47

† Results obtained in control experiments showed that glycogen levels remained virtually unchanged; e.g. in this experiment a difference in glycogen of 0·04 g/100 ml was seen between the first and second liver samples.

of the liver cell, but does indicate that the hyperglycemic response *in vivo* is the result of a direct effect of 3′,5′-AMP on the liver and is not due to the release of humoral agents from other organs or to the mediation of neurogenic stimuli.

In experiments where glycogen concentrations were determined in hepatic samples before and after a 5-min nucleotide infusion a decrease of about 1 g per cent was found in most cases. In similar experiments in which equilibrated blood containing no drug was infused for 60 minutes, a glycogen decrease of about 0·04 g/100 ml per 10 min was demonstrated. No changes in blood flow or bile flow rates were observed throughout the cyclic nucleotide infusion period.

Phosphorylase activity was ascertained before and after 3′,5′-AMP infusions. From data presented in Table 2 it can be seen that marked increases in enzyme activity were observed after the infusion of the nucleotide. It is assumed that the phosphorylase activation mechanism proposed by Sutherland[15] accounts for this increase in enzyme activity.

The effect of theophylline and adrenergic blocking agents on 3′,5′-AMP-induced hyperglycemia in intact animals and in the isolated liver preparation is currently under investigation.

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Morphine inhibition of drug metabolism in the rat

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CHRONIC morphine administration to rats is known to produce inhibition of morphine metabolism by the pathways of N-demethylation and glucuronide formation, coincident with the development of morphine tolerance.[1, 2] Axelrod[1] has suggested that morphine tolerance may be a biochemical correlate of the decreased ability of tolerant animals to N-demethylate morphine. This has been disputed most recently by Elison *et al.*[3] upon the basis of enzyme kinetic studies relative to rates of demethylation of several narcotic drugs. Recent studies in our laboratories,[4] in which we have used a four-day pretreatment of rats with morphine to block pituitary ACTH release caused by 'stress' situations, have suggested that morphine may be a nonspecific inhibitor of drug metabolism. The morphine inhibition can, in the intact animal, be overcome with ACTH and is not noted with compounds (barbital) that do not undergo extensive metabolism. Results of studies *in vitro* and *in vivo* bearing on this question are presented.

Male Holtzman rats weighing between 190 and 220 g were used in all experiments and were maintained on commercial laboratory chow with free acess to water. Animals were treated daily for four days with morphine sulfate (20 mg/kg) or saline (1 ml/kg) by intraperitoneal injection. This pretreatment regimen with morphine has been shown by Munson and Briggs[5] to block pituitary activation (ACTH release) by 'stress' situations, whereas the adrenal remains responsive to exogenous ACTH. Groups of control and morphine-pretreated animals were taken 24 hr after the last dose of morphine and either sacrificed for determination *in vitro* of hexobarbital and morphine metabolism, or intact animals from these groups were given hexobarbital (100 mg/kg), meprobamate (300 mg/kg), or barbital (250 mg/kg) by intraperitoneal injection for determination of duration of drug response. In another experiment morphine-pretreated animals received ACTH (100 mU/animal) by intravenous injection 2·5 hr before receiving hexobarbital; controls received an equal volume of saline by injection before hexobarbital. Hexobarbital and morphine metabolism was studied in liver slice preparations from control and morphine-pretreated rats.

Morphine-pretreated animals sleep for significantly longer intervals than do control animals after hexobarbital or meprobamate, whereas barbital sleeping time is not affected. Further, it is possible to overcome the effect of morphine pretreatment by the administration of ACTH prior to hexobarbital.