THE EFFECT OF VARIOUS 3'5' CYCLIC NUCLEOTIDES ON GLUCONEOGENESIS AND GLYCOGENOLYSIS IN THE PERFUSED RAT LIVER*

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Summary

The effects of various 3'5' cyclic nucleotides on hepatic gluconeogenesis and glycogenolysis were examined in the isolated perfused rat liver. Each of the 3'5' purine nucleotides (cAMP, cGMP and cIMP) increased gluconeogenesis equally and to the same degree as maximal doses of glucagon. The 3'5'-pyrimidine nucleotides cUMP and cCMP caused modest increments in gluconeogenesis and cTMP had no gluconeogenic effect. A similar pattern of stimulation by the various cyclic nucleotides was observed on hepatic glycogenolysis. The 3'5' purine nucleotides were equally potent stimulators of glycogenolysis and produced effects comparable to glucagon. cUMP and cCMP were less effective and cTMP had no glycogenolytic activity.

Glucagon, epinephrine and norepinephrine have potent stimulatory effects on both hepatic glycogenolysis and gluconeogenesis (1-4). Cyclic 3'5'-adenosine monophosphate (cAMP), which also stimulates hepatic glycogenolysis and gluconeogenesis, has been assumed to be the common mediator - the "secondary messenger" - for the action of each of these hormones at the cellular level (4-6). Preliminary reports have suggested that other

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cyclic nucleotides may affect hepatic glycogenolysis. Levine has reported that 3'5'-inosine monophosphate (cIMP) stimulates glycogenolysis in the perfused rat liver more potently than cAMP, but that 3'5'-guanine monophosphate (cGMP) has only a minimal effect (7). Glinsmann et al, however, found that cAMP and cGMP stimulate glycogenolysis equally (8). No studies of the effects of various cyclic nucleotides on hepatic gluconeogenesis have yet been reported. Using rat renal cortical slices, Pagliara and Goodman have shown that cAMP and cIMP stimulate whereas cGMP inhibits renal gluconeogenesis (9). Other investigators have recently reported that cAMP and cGMP differentially affect sodium and water transport by the toad bladder (10) and the release of growth hormone by the pituitary (11).

The possibility that different cyclic nucleotides may serve as specific secondary messengers for individual hormones and/or may mediate different, perhaps opposing, actions suggests a new and attractive concept for the mechanism of hormone action at the cellular level. The present studies were undertaken to study the effects of a series of 3'5' purine and pyrimidine cyclic nucleotides on gluconeogenesis and on glycogenolysis in the isolated perfused rat liver.

Methods and Materials

Liver perfusions were performed using a modification of the recirculation technique described by Williamson et al (12). Livers were obtained from Sprague-Dawley male rats weighing 250-300 g and fed Purina rat chow. Studies of gluconeogenesis were carried out with livers obtained from rats fasted for 24 hours. Livers were perfused with Krebs-Ringer bicarbonate buffer, pH 7.3 - 7.4 containing 3 gm% Fraction V bovine albumin for three consecutive 45 minute periods: first, a control period without gluconeogenic substrate; second, with sodium lactate (20 mM); and third, with sodium lactate (20 mM) and the test substance. Studies of glycogenolysis were carried with livers obtained from fed rats using two consecutive 45 minute perfusion periods: first, with Krebs-

Ringer bicarbonate albumin buffer containing glucose (5 mM) and second, with the same buffer and the test substance. Hepatic glucose production was calculated from the glucose concentration of perfusate samples obtained at 15 minute intervals and measured by the Technicon ferricyanide micromethod.

Glucagon was used at a concentration of 3.3 X 10^{-8}M; cAMP*, cGMP, cIMP and the 3'5'-cyclic nucleotides of uridine (cUMP), cytidine (cCMP) and thymidine (cTMP) were present at a perfusate concentration of 1 X 10^{-3}M; N^6, 2'-O-dibutyryl cyclic 3'5' adenylic acid (DBcAMP) was used at concentrations ranging from 1 X 10^{-7}M to 1 X 10^{-4}M.

Results

Gluconeogenesis - Preliminary studies confirmed the findings of Exton and Park (13) that, after a brief transient period of glycogenolysis, the rate of net hepatic glucose production in livers from fasted rats in the absence of added substrate continued at a constant rate (20-28 μmoles/100 gm body wt/hr) for 135 minutes. The addition of Na lactate (20 mM) sharply increased this rate about 400 percent for a period of at least 90 minutes and glucagon, added either at the same time as lactate or subsequently, induced a further increase of 60-90 percent (Figure 1).

DBcAMP (1 X 10^{-6}M) increased glucose production 95% above the rate with lactate alone (Table I). No detectable effect was observed with this nucleotide at concentrations less than 1 X 10^{-7} M or, paradoxically, at concentrations greater than 1 X 10^{-4} M.

Each of the purine 3'5' cyclic nucleotides stimulated glucose production nearly equally, increasing the rate observed with lactate alone approximately 75 percent (Table I). The pyrimidine 3'5' cyclic nucleotides cUMP and cCMP caused moderate increments ($\sim$ 48 percent) in glucose production (Table I), but no effect was observed with cTMP.

*cAMP (free acid, Sigma Chemical Co.); DBcAMP (Na^+ salt, Schwarz Bio-Research, Inc.); cGMP (K^+ salt, CalBioChemical, NH_4^+ salt, Sigma, Na^+ salt, Boehringer-Mannheim); cIMP (Na^+ salt, Sigma); cTMP (free acid, Sigma); cCMP (free acid, Sigma); cUMP (NH_4^+ salt, Sigma); Glucagon (insulin free) was a gift of Dr. D. K. Behrens, Lilly Research Laboratories.

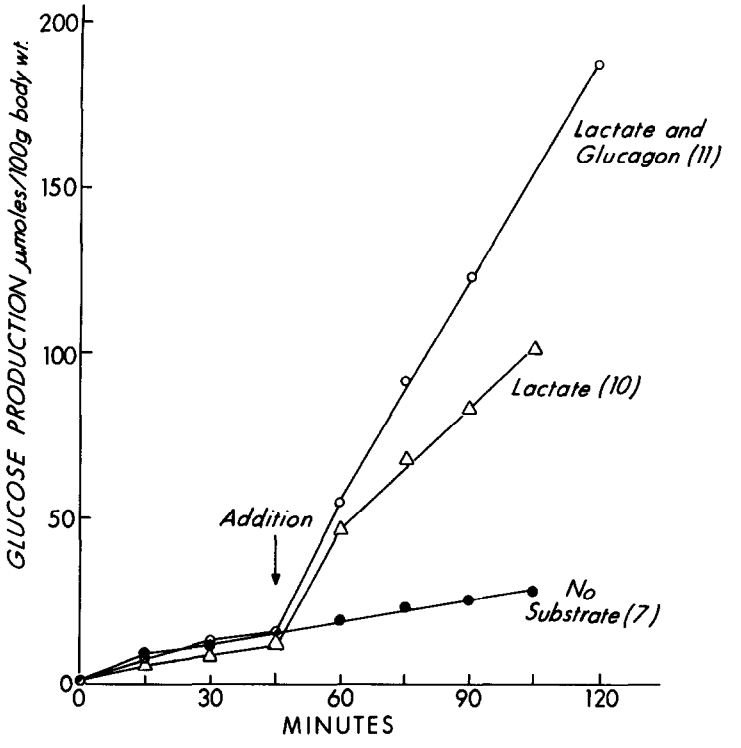

Figure 1 - Cumulative glucose production by isolated perfused livers in the absence of substrate, with Na lactate (20 mM) and with Na lactate (20 mM) plus glucagon (3.3×10^{-8}M). Increments in glucose production are all statistically significant after 60 minutes of perfusion ($p < 0.01$).

Glycogenolysis - A brief but rapid release of glucose, much greater than that seen with livers of fasted animals, was observed immediately after perfusion was begun in livers of fed rats. Thereafter, glucose release remained constant at a rate (50-100 μmoles/100 gm body wt/hr) two to four times greater than that observed in fasted rats (Figure 2). Glucagon produced a rapid and marked increase in glycogenolysis (Table II). A comparable degree of stimulation was also caused by each of the purine cyclic nucleotides (Figure 2, Table II). Smaller increments were observed with cUMP and cCMP but no detectable effect was noted with cTMP.

TABLE I

EFFECT OF CYCLIC NUCLEOTIDES ON HEPATIC GLUCONEOGENESIS

GLUCOSE PRODUCTION

Nucleotide*		Control	Lactate	Lactate Plus Nucleotide	Stimulation by Nucleotide
		μmoles per 100 g body weight per hour			percent
Glucagon	(12)†	24	105	181‡	72
DBcAMP	(5)	21	111	216‡	95
cAMP	(8)	18	116	209‡	80
cGMP	(10)	21	98	173‡	77
cIMP	(6)	20	87	154‡	77
cUMP	(7)	19	92	136‡	48
cCMP	(6)	18	77	113‡	47
cTMP	(5)	22	89	95	7

* Perfusate conc. DBcAMP - 1×10^{-6}; all other cyclic nucleotides - 1×10^{-3}M.

†Number of perfusions

‡Incremental stimulation significant at $p < 0.01$

TABLE II

EFFECT OF 3'5' CYCLIC NUCLEOTIDES ON HEPATIC GLYCOGENOLYSIS

GLUCOSE PRODUCTION

NUCLEOTIDE*		Unstimulated	With Nucleotide
		μmoles per 100 g body weight per hour	
Glucagon	(4)†	62	581
cAMP	(5)	77	498
cGMP	(4)	95	480
cIMP	(5)	68	499
cUMP	(5)	61	320
cCMP	(6)	117	241
cTMP	(6)	97	73

*Nucleotide concentrations same as in Table I.

†Number of perfusions

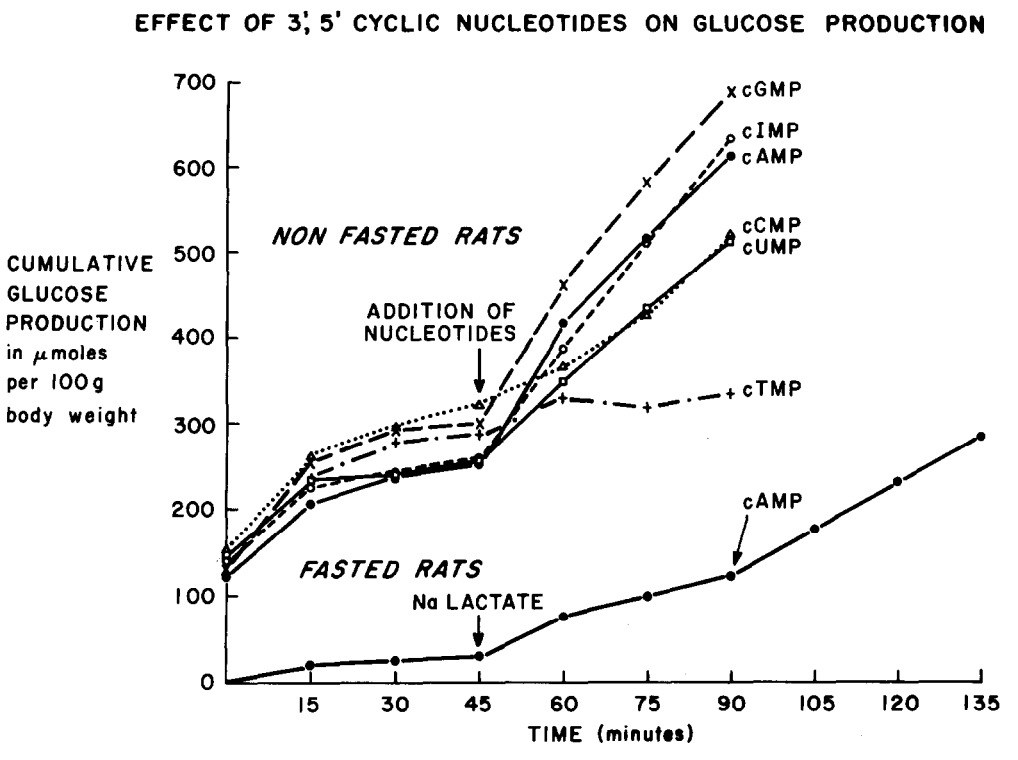

Figure 2 - Effect of 3'5' cyclic nucleotides on glycogenolysis. The purine cyclic 3'5' nucleotides (cAMP, cGMP, cIMP) stimulated glucose release comparably; cUMP and cCMP were less potent stimuli and cTMP had no effect. For comparison of relative rates of glucose production by gluconeogenesis and glycogenolysis, cumulative glucose production by fasted rat livers perfused with sodium lactate (20 mM) and cAMP (3 mM) is shown at the bottom of the figure.

Discussion

These studies demonstrate that 3'5' cyclic nucleotides other than cAMP are potent stimulators of both hepatic glycogenolysis and gluconeogenesis and, therefore, may act as "secondary messengers". Although several lines of evidence suggest that cGMP may be physiologically important (9-11, 14-16), there is no previous data to suggest that other purine and pyrimidine 3'5' cyclic nucleotides are either physiologically active or naturally occurring metabolites.

Stimulation of both gluconeogenesis and glycogenolysis was equivalent for each of the purine cyclic nucleotides and was comparable to that obtained with glucagon. The pyrimidine cyclic nucleotides cCMP and cUMP were considerably

less effective than the purine derivatives and cTMP caused no stimulation of either gluconeogenesis or glycogenolysis. Differences in cellular permeability and rates of hydrolysis as well as differential effectiveness as moderators of enzyme activity may be factors contributing to the variable potencies exhibited by this series of cyclic nucleotides.

The failure to observe increased intracellular cAMP levels in livers perfused with cGMP (8) and other purine and pyrimidine nucleotides [1] would exclude variations in the inhibition of cAMP phosphodiesterase activity by other cyclic nucleotides as an explanation for their variable potencies.

The paradoxical failure of DBcAMP at high concentrations (1×10^{-4} M) to stimulate gluconeogenesis confirms the findings of Menaham and Wieland (17). These results raise the possibility that the effects obtained with high concentrations of DBcAMP may not always represent a true index of the biological activity of cAMP; a conclusion consistent with the recent report of Goodman on the differential effect of insulin on the lipolytic activity of cAMP and DBcAMP in adipose tissue (18).

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