GLUCAGON-LIKE ACTION OF N^6, 2'-O-DIBUTYRYL CYCLIC 3',5'-AMP ON PERFUSED RAT LIVER

Lawrence A. Menahan* and Otto Wieland

Klinisch-Chemisches Institut
Krankenhaus München - Schwabing
8 München 23, Germany

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In addition to its well-known glycogenolytic effect, glucagon has been shown to increase ketogenesis, gluconeogenesis and ureogenesis in the perfused livers of fasted rats (1,2). Cyclic 3',5'-AMP (cAMP) has been postulated as the mediator or "second messenger" of glucagon action on liver, with particular reference to its glycogenolytic effect (3). The present report indicates that, following N^6,2'-O-dibutyryl cyclic 3',5'-AMP (DBcAMP) addition to the perfusion medium, an increase in endogenous urea, glucose and ketone body production occurs in the isolated, perfused liver from fasted rats. This would add substantial support to the hypothesis that cyclic 3',5'-AMP is the mediator of the effects of glucagon on gluconeogenesis, ketogenesis and ureogenesis.

Materials and Methods

Details concerning the technique of liver perfusion, sampling and analyses of the perfusate have been described previously (1,2). In the present work, the omission of added glucose from the medium enabled smaller changes in medium glucose to be detected. Sodium 5'-adenosine monophosphate or sodium N^6-2'-O-dibutyryl-3',5'-AMP

*NATO Post-doctoral Fellow 1966-1967.

(a gift from Dr. Bergmeyer, C.F. Boehringer Soehne, Biochemical Department, Tutzing, West Germany) were added as a single dose in neutral solution, after 40 min. of perfusion time, to give the medium concentrations desired. The rate of production of various metabolites was calculated as μmoles/g fresh weight/min from the 5 medium samples taken during the time period 40-80 min. Medium urea was determined on protein-free filtrates with urease (Biochemical Test Combination, Boehringer, TC-UR-1). Liver glycogen was isolated by alcohol precipitation from an alkaline digest of freeze-stopped liver tissue as modified by W. Kleinow (personal communication). After enzymatic hydrolysis, glycogen was determined and expressed as μmoles glucose/g wet weight (4). The results were tested for statistical significance by the method of "paired data" comparison (5).

Results and Discussion

Dibutyryl-3',5'-cyclic AMP in concentrations of 10^{-4} and 10^{-5}M increased significantly ($P < 0.05$) both the rate of gluconeogenesis and ureogenesis by approximately 100% when compared to the perfusions with no additions or with comparable concentrations of 5'-AMP (Table 1). The perfused liver from 24-hr fasted rats derives most, if not all, of its carbon for glucose production from endogenous protein sources as indicated by the constant one to one relationship of urea production to glucose production (Fig. 1). Lactate utilization is extremely low (0.10 μmole/g/min) and remains constant under all conditions. Likewise, a glyco-

Table 1

Effect of Dibutyryl-3′,5′-AMP and 5′-AMP on Metabolism of Perfused Liver from 24-hr Fasted Rats

Additions were made in neutral solution after 40 min of pre-perfusion. The rates of the various syntheses were calculated for the period 40-80 min and expressed as μmoles/g/min ± S.E.M.

Conc	No. of Exp	Gluconeogenesis		Ketogenesis		Urea	
		DBcAMP	5′-AMP	DBcAMP	5′-AMP	DBcAMP	5′-AMP
10^{-3}M	3	0.16±0.05	0.32±0.03	0.69±0.04	0.42±0.06	0.17±0.01	0.33±0.04
10^{-4}M	4	0.26±0.07	0.14±0.03	1.04±0.16	0.52±0.13	0.24±0.05	0.15±0.01
10^{-5}M	6	0.26±0.05	0.14±0.02	0.82±0.06	0.42±0.04	0.26±0.02	0.14±0.01
10^{-6}M	2	0.18	0.14	0.61	0.51	0.16	0.16
Nil	4	0.12±0.06		0.40±0.04		0.12±0.02	

genolytic effect of DBcAMP can be excluded for the observed glucose production as biopsy studies indicated that glycogen expressed as μmoles glucose/g wet weight decreases from 0.8 to 0.3 following DBcAMP administration. This could account for only 2 min of glucose production at the observed rate. All these results would indicate that the increased gluconeogenesis following DBcAMP is derived from endogenous liver protein.

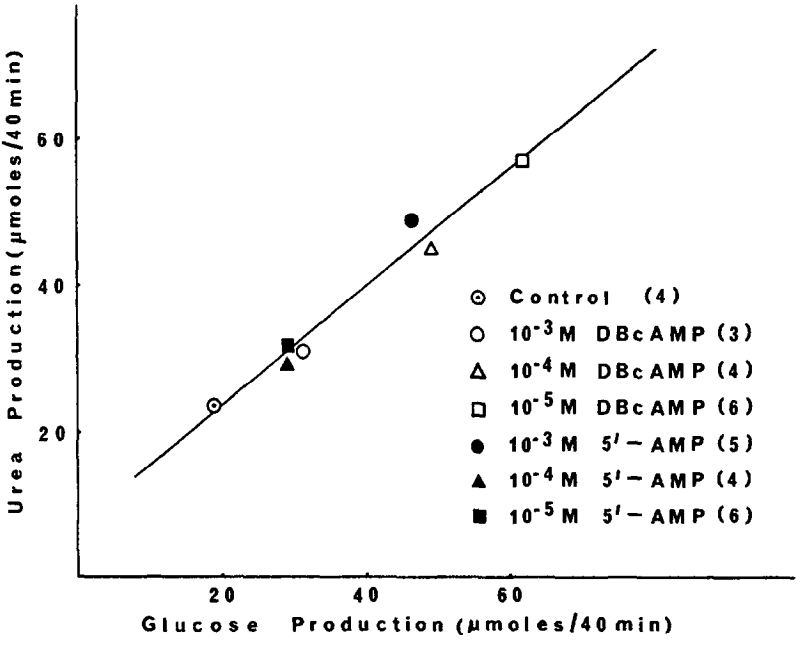

Fig. 1. Relationship Between Gluconeogenesis and Ureogenesis in Liver Perfusion Following DBcAMP or 5'-AMP

Numbers in parentheses indicate the number of perfusions represented by the respective symbols

Similarly, the significantly increased ($P < 0.05$) glucose production (100%) following the addition of 10^{-3} M 5'-AMP seems to be the result of increased protein catabolism.

Hunter and Jefferson (6) have recently reported in a short communication that the addition of 3 mM 5'-AMP to the perfusate results in a 30% increase in glucose and urea production from livers from 24-hr fasted rats.

At the same concentrations of DBcAMP, namely 10^{-4} and 10^{-5} M, which increased glucose and urea production, there was a significant doubling ($P < 0.05$) of ketone production. A considerably higher concentration (10^{-3} M) of cAMP was required to increase ketone body production in mouse liver homogenates (7). Although DBcAMP has been demonstrated to increase lipolysis in adipose tissue (8) and the increased ketogenesis in liver following glucagon and cAMP addition has been discussed as being due to stimulation of a triglyceride lipase (1,7), an inhibition of re-esterification and/or the TCA cycle or other unknown mechanisms cannot be excluded for the observed stimulation of ketogenesis by DBcAMP.

The lack of stimulation of ketogenesis, ureogenesis and gluconeogenesis in liver by the higher concentration (10^{-3} M) of DBcAMP is similar to the effect observed by Rizack (9) in adipose tissue with cAMP. He found that concentrations of cAMP higher than 2×10^{-5} M inhibited lipolytic activity in cell-free extracts.

Summary

Concentrations of DBcAMP at 10^{-4} or 10^{-5} M approximately doubled endogenous glucose, ketone and urea production by the perfused livers from 24-hr fasted rats. 5'-AMP stimulated glucose and urea production at higher concentrations (10^{-3} M). The response of endogenous liver metabolism to DBcAMP is quite similar to that following

glucagon addition.

Acknowledgments

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