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GLYCOGENOLYTIC EFFECT OF DIBUTYRYL CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE IN PERFUSED RAT LIVER

ROBERT A. LEVINE and SUSAN E. LEWIS

Department of Medicine,
The Brooklyn-Cumberland Medical Center and State University of New York,
Downstate Medical Center, Brooklyn, N.Y., U.S.A.

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Abstract—The glycogenolytic action of the N^6-2′-*O*-dibutyryl derivative of cyclic adenosine 3′,5′-monophosphate was demonstrated in isolated perfused rat liver. The initial hyperglycemic response induced by the dibutyryl derivative exceeded that of the monobutyryl derivative and the parent compound in equimolar doses. The dibutyryl derivative was especially potent during the first 30 min of infusion and produced a 70 per cent greater glycemic response compared to the monobutyryl derivative. Near maximal glycogenolysis was demonstrated as early as 2 hr. The findings support the hypothesis that the dibutyryl derivative penetrates the liver cell more readily, or is less susceptible to hydrolysis by phosphodiesterase, than the monobutyrl derivative or the parent compound.

THE GLYCOGENOLYTIC action of cyclic adenosine 3′,5′-monophosphate (3′,5′-AMP) has been well documented *in vivo* and in isolated perfused rat liver.[1–6] Recent studies in this laboratory have demonstrated the increased hepatic glycogenolytic activity of the 2′-*O*-monobutyryl derivative of 3′,5′-AMP (MBC).[6] The present study was undertaken to compare the glycogenolytic and hyperglycemic actions of N^6-2′-*O*-dibutyryl 3′,5′-AMP (DBC) with 3′,5′-AMP and MBC in isolated perfused rat liver.

MATERIALS AND METHODS

The details of the perfusion technique have been reported previously and remain unchanged.[6] Only those livers with a similar initial glycogen value from animals within the same weight range as in the previous study[6] were used to compare the glycogenolytic action of an equimolar amount of DBC* with MBC† and 3′,5′-AMP.† All data were analyzed by the Student *t*-test.[7] The nucleotide content of the DBC was 98 per cent by chromatographic analysis using methods previously described.[6]

After a 30-min control period, employing Ringer infusion alone, 7·8 m-mole DBC was added initially to the perfusate reservoir, which contained 85 ml of heparinized, diluted rat blood. For the remainder of the perfusion, the nucleotide was infused at the rate of 7·8 m-mole/hr in ten experiments. Infusion was by endoportal route into the liver, as previously described.[6]

* Obtained from Calbiochem, Los Angeles, Calif., as the monopotassium salt (Lot 70146).
† Obtained from Schwarz BioResearch, Inc., Orangeburg, N.Y.

In order to obtain comparative data testing DBC, MBC and 3′,5′-AMP simultaneously, acute injections of nucleotides in concentrations of 0·2 m-mole, which would induce glycemia without maximal glycogenolysis, were rapidly administered sequentially in three perfusion experiments. The periodic addition of DBC, MBC and 3′,5′-AMP was varied so that their administration was alternated equally between first second and third dosage positions.

Perfusate samples were taken at 0·5- or 1-hr intervals in the infusion studies and at 0·25-hr intervals during the acute injection experiments. Glucose was determined by the glucose oxidation procedure of Cawley *et al.*[8] One of the four caudate lobes was partially removed after 30 min of perfusion and a second caudate lobe was removed after 2·5 hr of liver perfusion. A final sample of the median lobe was removed at the termination of the experiment. Analyses were made for glycogen content of liver samples by using the anthrone technique, as previously described.[6] Adequate bile production, rate of blood flow and normal appearance of the liver at the end of perfusion were used as criteria of successful perfusion.

Infusion of butyric acid* in the dose of 1 mg/hr was employed as a control study, because this compound was a possible contaminant of the DBC.

RESULTS

The mean initial blood glucose concentration after 30 min of infusion with Ringer solution alone (zero time) was 268 ± 38 mg/100 ml for DBC, 338 ± 53 mg/100 ml

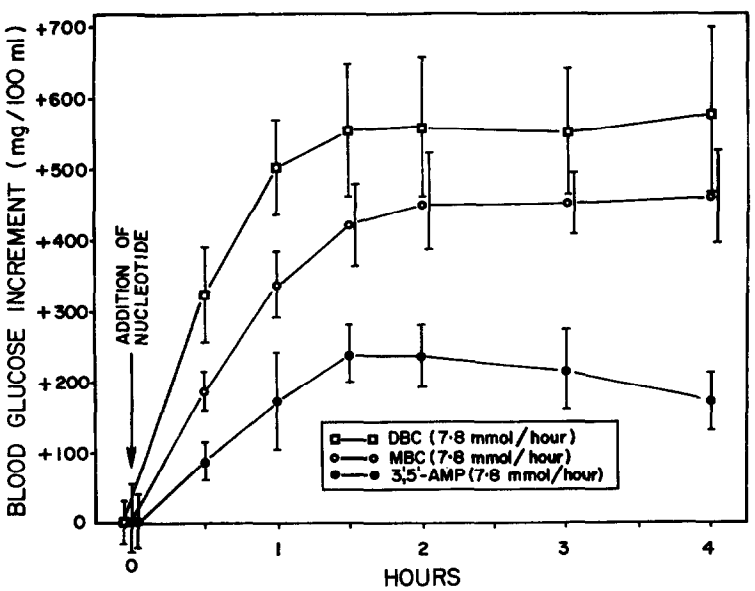

FIG. 1. Changes in blood glucose after addition of DBC in 4-hr perfusions of isolated livers from fed donor rats. Ten liver perfusions were performed with DBC (7·8 m-mole/hr) and compared to 7 perfusions each with the MBC and cyclic nucleotide compounds at equimolar doses. Lines extending from each point represent ±S.D.; the abscissa represents increment change over the control level. The data for MBC and 3′,5′-AMP have been published elsewhere.[6]

* Obtained from J. T. Baker Co., Phillipsburgh, N.J.

for MBC and 322 ± 107 mg/100 ml for 3′,5′-AMP. The blood glucose values were significantly different between DBC and MBC only ($P < 0.01$). There was a highly significant increase in blood glucose ($P < 0.001$) as early as 0·5 hr after administration of DBC, which persisted throughout 4 hr of infusion ($P < 0.001$) as shown in Fig. 1. The hyperglycemic response of DBC always exceeded that of 3′,5′-AMP at the equimolar dose ($P < 0.001$). The increase in blood glucose induced by DBC was significantly greater than that of MBC at 0·5 hr ($P < 0.001$), at 1 hr ($P < 0.001$), at 1·5 hr ($P < 0.005$), at 2 hr ($P < 0.05$) and at 3 hr ($P < 0.025$). There was no significant difference between the two butyryl derivatives at the 4-hr blood glucose level. The greatest difference between DBC and MBC occurred during the first 30 min, when there was a 70 per cent increase in glycemic activity of DBC compared to MBC. During the ensuing periods of infusion, DBC exceeded the glycemic potency of MBC by 49 per cent at 1 hr, 40 per cent at 1·5 hr, 24 per cent at 2 hr, 12 per cent at 3 hr and 26 per cent at 4 hr.

After 2 hr of infusion, the hepatic glycogen concentration had decreased in 9 of 10 experiments to levels below 1 mg/g (range 0·27–0·66 mg/g). In the other experiment, the 2-hr glycogen concentration was 12·3 mg/g and subsequently decreased to 1·91 mg/g after 4 hr of perfusion. The mean glycogen concentration for all ten samples at 2 hr was 1·6 ± 3·7 mg/g, which was not significantly different from the final glycogen values (Table 1). As shown in Table 1, the pretreatment levels of heptic glycogen were

TABLE 1. EFFECTS OF DBC, MBC AND 3′,5′-AMP ON HEPATIC GLYCOGEN CONCENTRATIONS*

Compound	Expt. No.	Hepatic glycogen (mg/g)		
		Pretreatment	Post-treatment	Mean difference
DBC	10	32·8 ± 7·5	0·6 ± 0·5*†	32·2 ± 7·5‡
MBC	7	32·2 ± 7·6	0·8 ± 0·5*†	31·5 ± 7·7‡
3′,5′-AMP	7	31·4 ± 7·4	9·1 ± 6·9	22·2 ± 3·0

* Values are means ±S.D. Pretreatment samples were taken after a 30-min period of infusion with Ringer solution, in addition to perfusion with diluted blood. Post-treatment values represent final determinations 4 hr after infusion with the compounds shown. Probability of chance difference ($P < 0.001$†; $P < 0.005$‡) between values for the DBC and MBC groups and 3′,5′-AMP group. No significant differences were found between the pretreatment glycogen content of all three groups of nucleotides or between the DBC and MBC post-treatment and mean difference values. The data for MBC and 3′,5′-AMP have been published elsewhere.[6]

not significantly different for DBC, MBC or 3′,5′-AMP. The post-treatment values of the DBC derivative were significantly lower than the 3′,5′-AMP values ($P < 0.001$), and the mean difference values of the DBC were also significantly higher than those of the parent compound ($P < 0.005$). There was no significant difference between DBC and MBC in either the post-treatment or mean difference values.

In the acute injection studies, responses to all three nucleotides were more variable than in the constant infusion experiments. Nevertheless, both DBC and MBC significantly exceeded the glycemic potency of 3′,5′-AMP, as shown in Table 2. DBC also produced a greater hyperglycemic response than MBC, although this

was not statistically significant. A representative perfusion study is shown in Fig. 2. Only those experiments in which the final glycogen content exceeded 3·0 mg/g were included. In other experiments, not shown, larger concentrations of nucleotides (0·8–2·0 m-mole) produced maximal glycogenolysis during the course of the first or second injection, preventing any further evaluation of a hyperglycemic response after subsequent addition of another nucleotide. However, in these studies DBC was found to be more potent than MBC or 3′,5′-AMP, although all three compounds were not tested simultaneously.

TABLE 2. MAXIMAL GLYCEMIC RESPONSES AFTER ACUTE ADMINISTRATION OF DBC, MBC AND 3′,5′-AMP (3 EXPERIMENTS)*

Compound	Blood glucose increment (mg/100 ml)
DBC	165 ± 14†
MBC	139 ± 20†
3′,5′-AMP	82 ± 34

* Values represent increment change (means ±S.D.) over glucose concentration immediately preceding acute injection of nucleotides (0·2 m-mole). Initial control glucose concentration was 288 ± 44 mg/100 ml. Initial glycogen value was 28 ± 8 mg/g.

† Probability of chance difference between values for DBC and MBC groups and 3′,5′-AMP group ($P < 0.005$). No significant differences were found between DBC and MBC groups.

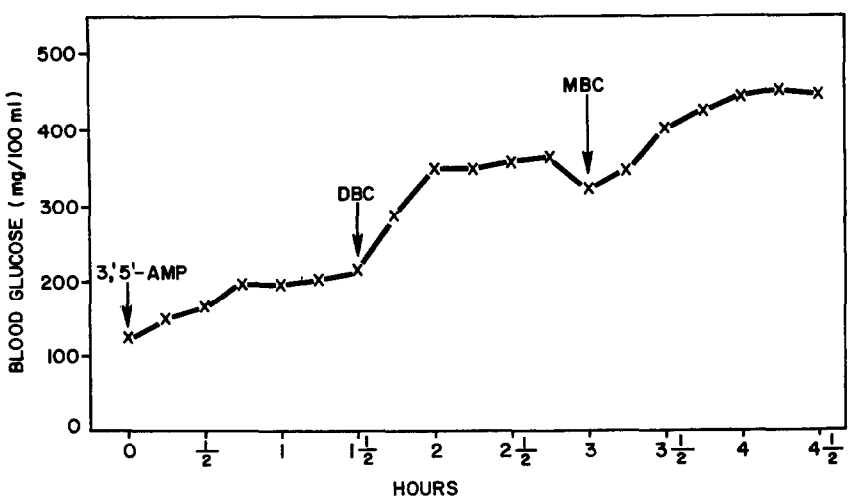

FIG. 2. Representative study showing comparative changes in blood glucose concentration after sequential addition of 3′,5′-AMP, DBC and MBC. Nucleotides were administered rapidly by endoportal route in equimolar does (0·2 m-mole). Initial blood glucose (zero time) taken after 30 min of perfusion with heparinized, diluted rat blood.

The control infusion of butyric acid failed to induce any glycemic or glycogenolytic responses or to produce any deleterious effects during liver perfusion.

DISCUSSION

Previous studies employing the monobutyryl derivative of 3′,5′-AMP demonstrated its increased glycemic and glycogenolytic activity compared to the parent compound.[6] The current experiments indicate that the dibutyryl derivative further enhances the glycemic effect of 3′,5′-AMP. This was particularly evident during the initial 30 min of infusion. Presumably, glycogenolysis occurred more rapidly with DBC than with MBC. It is possible that the addition of a single butyryl group to the cyclic nucleotide increased its hydrophobic tendency, and thus its ability to penetrate the liver cell, or increased its resistance to hydrolysis by phosphodiesterase. The addition of a second butyryl group to the nucleotide could have further increased its lipid solubility or its resistance to inactivation, thereby promoting even greater physiological responses.

Although DBC induced more hyperglycemia than MBC during the first hour, after 2 hr of infusion both butyryl derivatives produced near-maximal glycogenolysis in comparison to the inability of the parent compound to cause similar glycogenolysis. In the case of DBC, a marked decrease in glycogen occurred as early as 2 hr after its administration (1·6 $\pm$ 3·7 mg/g), which was similar to the 2-hr glycogen values found after MBC infusion (1·6 $\pm$ 1·1 mg/g) but not previously reported. At the termination of the perfusion experiments, an equimolar dose of 3′,5′-AMP failed to produce maximal glycogenolysis, although a 3-fold larger dose of 3′,5′-AMP had previously been shown to induce a prominent hepatic glycogenolysis.[6]

The differences in the hyperglycemic effects of the three nucleotides during constant infusion studies are well documented, but it is not clear whether increased glycogenolysis is the exclusive or even the major cause of this effect. The initial hyperglycemia was greater with DBC than with MBC or 3′,5′-AMP, especially after 30 min, indicating greater glycogenolysis through the mechanism of phosphorylase activation. Presumably, after 2 hr further phosphorylase stimulation, if possible, would not cause greater glycemia if the glycogen content was already so depleted. Since the glycogen levels in the liver after infusion with MBC and DBC reached minimal detectable concentrations after 2 hr, the contribution of glycogenolysis during the last 2-hr period to the blood sugar levels cannot be evaluated and probably did not play a significant role. Prior studies with a variety of glycogenolytic agents in perfused rat livers similarly have shown that prolonged maintenance of hyperglycemia may follow maximal depletion of glycogen.[2, 9, 10] Therefore, it is possible that the latent hyperglycemic effects may be due in a large degree to increased gluconeogenesis or other factors not related to glycogenolysis. Gluconeogenesis has been shown to be produced in the perfused liver after 3′,5′-AMP administration.[3]

In view of their potent action on hepatic glycogenolysis, it is not surprising that the butyryl derivates would activate phosphorylase to a greater degree than 3′,5′-AMP. Henion *et al.* showed that DBC had a relative potency of 91·2 per cent compared to those of MBC at 34·5 per cent and of 3′,5′-AMP at 1·9 per cent in relation to activation of hepatic phosphorylase in dog liver slices.[11] They further demonstrated the increased glycemic activity of the dibutyryl derivative in two intact anesthetized dogs who showed a maximum 33 per cent increase in blood glucose compared to MBC.[11] A recent preliminary report similarly documented the hyperglycemic action of DBC

in perfused rat livers, although no comparisons were made with MBC or 3′,5′-AMP and the donor rats, unlike those in our study,[12] had been fasted. The findings in our constant infusion and acute injection experiments in isolated perfused rat liver would therefore confirm the increased glycemic potency of the dibutyryl derivative in a variety of species, including the rat and the dog. Our studies demonstrated that butyric acid had no glycogenolytic or toxic effect in perfused rat liver, and therefore had no pharmacologic action as a contaminating agent in DBC.

Previous studies have indicated a greater physiologic effect of the dibutyryl derivative compared to the parent compound in isolated fat cells,[13] adrenal slices,[14] parotid[15] and thyroid.[16] However, it is of interest that studies by Robison *et al.* in perfused nonworking rat hearts failed to demonstrate any effect of the dibutyryl derivative or the parent compound, and provided evidence that the dibutyryl compound may permeate the heart cell even less readily than the parent compound in that isolated system.[17] Similarly, one of us (Robert A. Levine) has recently observed that at equimolar concentrations the dibutyryl derivative had practically no relaxant effect on the isolated rat ileum, while the parent compound had a dramatic inhibitory action, mimicking the effects of epinephrine.[18] These findings would indicate that the potency of butyryl derivates are greater in some but less in other tissues. Studies on the specificity of butyryl derivatives in a variety of tissues and species will be necessary to document the relative roles of increased cellular permeability or inactivation by phosphodiesterase in explaining the activity of these compounds.

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