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# MECHANISMS RESPONSIBLE FOR THE HYPERGLYCEMIA PRODUCED BY THE DIBUTYRYL DERIVATIVE OF CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE*

N. ALTSZULER, I. RATHGEB and R. STEELE

Department of Pharmacology, New York University School of Medicine, New York, N.Y. 10016, and Biology Department, Brookhaven National Laboratory, Upton, N.Y. 11973, U.S.A.

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**Abstract**—Glucose release by the liver and glucose uptake from the blood were measured in intact unanesthetized dogs in the postabsorptive state by the use of $^{14}$C-glucose. Plasma insulin concentrations were monitored by radioimmunoassay. It was found that the hyperglycemia resulting from the infusion (at 5 mg/kg/hr, i.v.) of $N^6$-2′-*O*-dibutyryl cyclic 3′,5′-adenosine monophosphate (dibutyryl cAMP) was due for the most part to increased glucose release. Plasma insulin concentration rose to the same level during dibutyryl cAMP infusion as during a comparable hyperglycemia produced by intravenous infusion of glucose. It is concluded that dibutyryl cAMP, at a concentration sufficient for a pronounced hepatic response, exerts no direct action on the β cells *in vivo* to enhance or inhibit glucose-stimulated insulin release. At the same elevated plasma insulin level, glucose uptake was increased less during dibutyryl cAMP hyperglycemia than during the comparable hyperglycemia caused by glucose infusion. It is concluded that a small part of the hyperglycemia maintained by dibutyryl cAMP is due to resistance on the part of the tissues to insulin-stimulated glucose uptake. When epinephrine was infused to yield a comparable hyperglycemia, glucose release from the liver increased but the plasma insulin concentration and the uptake of glucose by the tissues did not increase.

THE HYPERGLYCEMIC action of intravenously administered dibutyryl cAMP† was reported by Posternak *et al.*[1,2] in their pioneering description of the biological activity of derivatives of cyclic AMP. These investigators attributed the hyperglycemic action to an enhancement of hepatic glycogenolysis; in support of this, they demonstrated that the compound causes an increased activation of phosphorylase by a liver tissue extract system. Dibutyryl cAMP was found to be more active as a hyperglycemic agent *in vivo* then cyclic AMP, which they suggested may be due to the greater resistance of the butyryl derivatives than unsubstituted cyclic AMP to the action of phosphodiesterase.

Our own previous work[3] on the mechanism of epinephrine-induced hyperglycemia has shown that only a part of the hyperglycemic effect is due to increased release of glucose by the liver. Another part of the effect, one which is responsible for the maintenance of hyperglycemia once it has been established, is due to a relative inhibition of

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† Abbreviations used are dibutyryl cAMP for $N^6$-2′-*O*-dibutyryl cyclic 3′,5′-adenosine monophosphate, and cyclic AMP for cyclic 3′,5′-adenosine monophosphate.

glucose uptake. This is called a relative inhibition because the glucose uptake does not decrease below baseline values, but rather fails to increase in the usual way in the presence of hyperglycemia. The infused epinephrine prevents the extra insulin secretion which the hyperglycemia would otherwise evoke and, in addition, the increased glucose concentration fails to increase glucose uptake at the unchanged plasma insulin concentration.

It then became of interest to investigate the changes in plasma insulin concentration and in glucose release and uptake during the hyperglycemia brought about by the infusion of dibutyryl cAMP.

## MATERIALS AND METHODS

All experiments were carried out on normal adult male and female dogs about 18 hr after feeding. The dogs were unanesthetized and trained to lie quietly with their heads enclosed in a plastic cylinder. Glucose-$^{14}$C (uniformly or C-6 labeled) was administered through a saphenous vein as a priming injection followed immediately by a continuous infusion at a constant rate. Serial blood samples were drawn from the jugular vein through an indwelling polyethylene catheter into heparinized syringes. The blood samples were centrifuged and the plasma deproteinized according to Somogyi.[4] The plasma glucose concentration was determined by the glucose oxidase method.[5] The glucose in the plasma filtrates was isolated as the glucosotriazole derivative for the determination of its specific activity by liquid scintillation counting as described previously.[6] Calculations of the rates of glucose production by the liver and overall uptake of glucose by the tissues in the steady state[7] and during periods of changing plasma glucose concentrations[8] have been described.

Plasma insulin concentrations were determined by the radioimmunoassay technique, using talcum powder to separate free insulin from the insulin–antibody complex.[9]

Cyclic AMP and the sodium salt of dibutyryl cAMP were purchased from commercial sources and were infused intravenously at rates of 10 and 5 mg/kg/hr respectively.

## RESULTS

The effects of infusion of dibutyryl cAMP (5 mg/kg/hr) for 2 hr on the concentrations of plasma glucose and insulin and on the rates of hepatic glucose release and glucose uptake by tissues are shown in Table 1. One dog (AL 76) received an infusion of cyclic AMP (10 mg/kg/hr for 2 hr), but since these specific effects did not differ from those produced by the lesser dose of the dibutyryl derivative, the data are included here. The dibutyryl cAMP infusion resulted in a moderate elevation of the plasma glucose concentrations; the mean of the differences from control values is significantly ($P < 0{\cdot}05$) above zero at 15, 30, 45 and 60 min. Plasma insulin concentration was also increased significantly ($P < 0{\cdot}001$–$0{\cdot}02$) during the first hour of infusion and declined thereafter as the hyperglycemia diminished.

Hepatic glucose output increased promptly and remained significantly ($P < 0{\cdot}01$–$0{\cdot}05$) elevated during the infusion period. Similarly, the overall uptake of plasma glucose by the tissues was increased significantly ($P < 0{\cdot}01$–$0{\cdot}05$) after 15 min of infusion and remained so during the remainder of the infusion period; both glucose output and uptake returned rapidly to control values at termination of the infusion.

A comparison of these effects of dibutyryl cAMP with those obtained by infusion of

TABLE 1. EFFECT OF INFUSION OF DIBUTYRYL cAMP (5 mg/kg/hr) FOR 120 min ON PLASMA CONCENTRATION OF GLUCOSE AND INSULIN AND ON GLUCOSE PRODUCTION AND UPTAKE

| | Minutes after start of dibutyryl cAMP infusion | | | | | | | Minutes after end of infusion | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dog | 0 | 0–15 | 15–30 | 30–45 | 45–60 | 60–90 | 90–120 | 0–15 | 15–30 | 30–60 |
| Plasma glucose concentration (mg/100 ml) | | | | | | | | | | |
| AL 76 | 99 | 115 | 115 | 114 | 113 | 110 | 110 | 100 | 98 | 99 |
| AL 76 | 95 | 111 | 123 | 119 | 114 | 115 | 113 | 103 | 95 | 93 |
| AL 64 | 103 | 121 | 140 | 153 | 140 | 126 | 115 | 101 | 105 | 103 |
| AL 79 | 99 | 112 | 115 | 114 | 108 | 102 | 109 | 91 | 91 | 92 |
| AL 74 | 124 | 156 | 168 | 164 | 146 | 134 | 136 | 126 | 117 | 111 |
| Mean | 104 ± 5 | 123 ± 8 | 127 ± 6 | 132 ± 10 | 124 ± 8 | 117 ± 6 | 117 ± 5 | 104 ± 6 | 101 ± 5 | 100 ± 3 |
| Plasma insulin concentration ($\mu$U/ml) | | | | | | | | | | |
| AL 76 | 17 | 40 | 35 | 38 | 34 | 36 | 56 | 28 | 18 | 20 |
| AL 76 | 20 | 39 | 55 | 31 | 39 | 33 | 36 | 24 | | 18 |
| AL 64 | 16 | 28 | 58 | 63 | 64 | 50 | 20 | 21 | 41 | 45 |
| AL 79 | 21 | 35 | 44 | 41 | 35 | 16 | 24 | 10 | 11 | 13 |
| Mean | 19 ± 1 | 36 ± 3 | 48 ± 5 | 43 ± 7 | 43 ± 7 | 34 ± 7 | 34 ± 8 | 21 ± 4 | 23 ± 9 | 24 ± 7 |
| Glucose production (g/m²/hr) | | | | | | | | | | |
| AL 76 | 3·31 | 5·24 | 5·02 | 4·60 | 4·03 | 4·45 | 4·09 | 2·37 | 2·48 | 2·99 |
| AL 76 | 3·11 | 5·02 | 5·78 | 5·46 | 4·19 | 4·92 | 4·19 | 2·67 | 2·43 | 2·54 |
| AL 64 | 2·89 | 4·95 | 6·28 | 6·71 | 4·45 | 5·12 | 4·70 | 2·30 | 4·13 | 2·52 |
| AL 79 | 5·63 | 9·75 | 8·81 | 8·83 | 7·76 | 8·03 | 7·32 | 4·79 | 4·82 | 5·17 |
| AL 74 | 3·13 | 7·62 | 7·43 | 7·41 | 6·12 | 6·30 | 5·26 | 3·11 | 3·05 | 3·23 |
| Mean | 3·61 ± 0·51 | 6·52 ± 0·95 | 6·66 ± 0·66 | 6·60 ± 0·74 | 5·31 ± 0·72 | 5·76 ± 0·64 | 5·11 ± 0·59 | 3·05 ± 0·46 | 3·38 ± 0·47 | 3·29 ± 0·40 |
| Glucose uptake (g/m²/hr) | | | | | | | | | | |
| AL 76 | 3·31 | 3·14 | 5·02 | 4·73 | 4·16 | 4·64 | 4·09 | 3·69 | 2·82 | 2·93 |
| AL 76 | 3·11 | 3·02 | 4·25 | 5·97 | 4·78 | 5·15 | 4·32 | 3·97 | 3·49 | 2·80 |
| AL 64 | 2·89 | 2·87 | 4·08 | 5·21 | 5·95 | 5·92 | 5·32 | 3·90 | 3·69 | 2·61 |
| AL 79 | 5·63 | 6·89 | 8·32 | 9·00 | 8·77 | 8·52 | 7·16 | 6·81 | 4·82 | 5·09 |
| AL 74 | 3·12 | 4·09 | 6·03 | 7·91 | 8·08 | 6·93 | 5·18 | 4·17 | 4·11 | 3·53 |
| Mean | 3·61 ± 0·51 | 4·06 ± 0·75 | 5·54 ± 0·78 | 6·56 ± 0·82 | 6·35 ± 0·90 | 6·23 ± 0·69 | 5·21 ± 0·54 | 4·51 ± 0·58 | 3·79 ± 0·33 | 3·39 ± 0·45 |

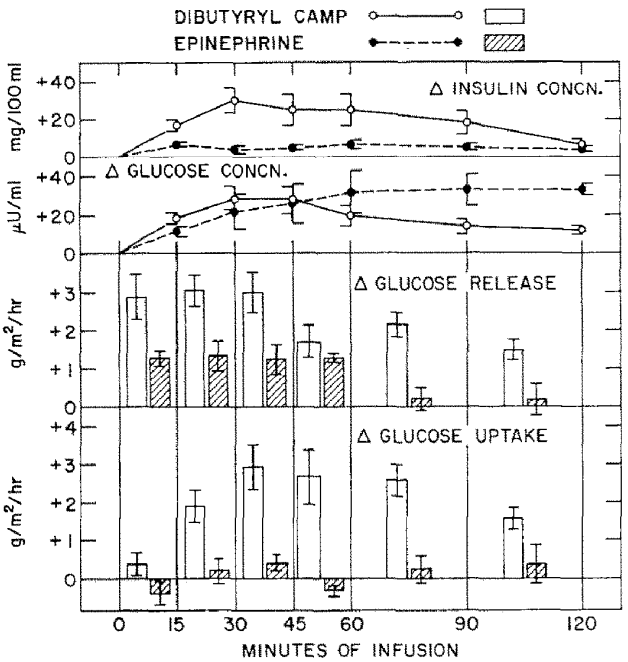


FIG. 1. Effects of infusion of epinephrine (6·0 μg/kg/hr) and dibutyryl cAMP (5 mg/kg/hr) for 2 hr into normal dogs on plasma concentrations of glucose and insulin and on rates of hepatic glucose release and overall uptake by tissues. All values are given as changes from control values obtained in the same animals immediately prior to the infusions. The vertical lines or brackets denote twice the standard error of the mean. Further discussion and statistical evaluation of the differences are given in the text.

epinephrine is shown in Fig. 1. The increment in plasma glucose concentration over the control value is similar in both cases during the first 60 min; it is higher for epinephrine at 90 min ($P < 0{\cdot}05$) and 120 min ($P < 0{\cdot}001$). The increase in plasma insulin concentration is significantly ($P < 0{\cdot}00001$–$0{\cdot}05$) greater during dibutyryl cAMP infusion than during epinephrine infusion during this first hour. The lack of the usual increase in plasma insulin levels in the presence of hyperglycemia during epinephrine infusion in the dog has been reported by us previously[3] from this laboratory.

The hepatic glucose output was increased by epinephrine infusion during the first hour of infusion and returned to control values thereafter, whereas the hyperglycemia persisted. Infusion of dibutyryl cAMP resulted in a significant ($P < 0{\cdot}01$–$0{\cdot}05$) increase in hepatic glucose output at all periods up to 120 min.

A striking difference is seen during the infusion of the two substances on overall glucose uptake by the tissues. Whereas epinephrine hyperglycemia produced very little change, dibutyryl cAMP hyperglycemia increased glucose uptake over the control values at all periods except the initial 15-min period. These changes differ significantly ($P < 0{\cdot}01$–$0{\cdot}05$) from control values and from the minimal changes due to epinephrine infusion.

To further evaluate the effects on glucose uptake, glucose loads were administered at rates which produced hyperglycemia similar to that observed during infusion of

TABLE 2. PLASMA GLUCOSE AND INSULIN CONCENTRATIONS DURING INFUSION OF EPINEPHRINE, DIBUTYRYL cAMP AND GLUCOSE IN NORMAL DOGS

| Treatment | Glucose (mg/100 ml) | Insulin ($\mu$U/ml) |
|---|---|---|
| None* | 99 ± 2† | 16 ± 2 |
| Epinephrine† | 125 ± 5 | 24 ± 2 |
| Dibutyryl cAMP§ | 128 ± 4 | 42 ± 3 |
| Glucose‖ | 125 ± 1 | 40 ± 3 |

* Control values obtained in 15 dogs.

† Mean ± standard error of mean.

‡ Epinephrine 6 $\mu$g/kg/hr; mean values based on 16 values obtained during 60-min infusion in four dogs.

§ Dibutyryl cAMP, 5 mg/kg/hr; mean values based on 16 values obtained during 60-min infusion in four dogs.

‖ Glucose, 300 mg/kg/hr; mean values based on 30 values obtained during a 60-min infusion in six dogs.

dibutyryl cAMP and epinephrine. $^{14}$C-glucose was added to each load to attain a specific activity close to that already existing in the plasma glucose, in order to minimize errors due to the delayed mixing of the load with the body glucose. These glucose loads evoke increases in plasma insulin concentration, as shown in Table 2, which are similar to those observed during the infusion of dibutyryl cAMP. Figure 2 presents the changes in glucose uptake above control values during infusion of epinephrine,

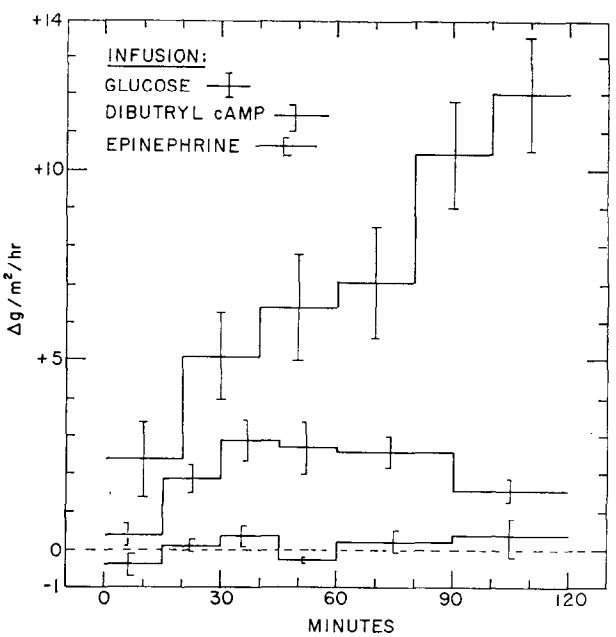


FIG. 2. Changes from control values in overall tissue uptake of plasma glucose in normal dogs during a 2 hr infusion of epinephrine (6·0 $\mu$g/kg/hr), dibutyryl cAMP (5 mg/kg/hr) or glucose (222–360 mg/kg/hr). The vertical lines or brackets represent twice the standard error of the mean. Further details and statistical evaluation are given in the text.

dibutyryl cAMP or glucose. It can be seen that while, in contrast to epinephrine, dibutyryl cAMP results in a significant increase in glucose uptake, this increase nevertheless is less than that obtained during the infusion of glucose. Treating several periods collectively to simplify comparisons reveals that the increase in glucose uptake in the 20–80 min period of glucose infusion is significantly greater ($P < 0{\cdot}001$) than the increase in glucose uptake in the 30–90 min period of dibutyryl cAMP infusion, despite the fact that plasma glucose and plasma insulin concentrations are comparable. The difference is more marked beyond these periods, but at glucose and insulin levels which are no longer similar. Thus it would appear that, while dibutyryl cAMP infusion results in an increased glucose uptake, there is also a relative inhibition of glucose uptake which cannot be attributed to a failure of the plasma insulin concentration to increase in response to hyperglycemia.

## DISCUSSION

The hyperglycemia produced by the infusion of dibutyryl cAMP is in large part a consequence of increased glucose release by the liver. About twice as much extra glucose (see Fig. 1) is released during the first 60 min of dibutyryl cAMP infusion as during the first 60 min of an epinephrine infusion which produces the same level of hyperglycemia.

In these experiments, carried out in the postabsorptive state, increased hepatic glycogenolysis undoubtedly is responsible for much of the increase in glucose release by the liver. Increased gluconeogenesis may also supply some of the extra glucose. In this regard, dibutyryl cAMP[10] and cyclic AMP[11] have been found to increase gluconeogenesis in perfused liver systems. In separate experiments in this laboratory,[12] it has been shown that dibutyryl cAMP infusion does not increase plasma lactate concentration. Thus an increased amount of plasma lactate, derived from muscle glycogenolysis, is not available as substrate for gluconeogenesis as is the case in epinephrine-induced hyperglycemia.

A separate influence which contributes to the dibutyryl cAMP hyperglycemia is revealed by experiments in which a comparable hyperglycemia is induced by the intravenous infusion of glucose. The uptake of glucose from the blood by the tissues is less during the dibutyryl cAMP hyperglycemia (see Fig. 2). This difference exists in spite of the fact that the plasma insulin concentration is elevated to the same extent in the two kinds of hyperglycemia (see Table 2). In other experiments in this laboratory,[12] it has been demonstrated that infusion of dibutyryl cAMP causes an overall inhibition of triglyceride lipolysis as demonstrated by lowered plasma free fatty acid and glycerol concentrations. Similarly, intravenous injection of cAMP into normal dogs has been found to lower plasma free fatty acids concentration[13].* Thus there is no indication that the observed inhibition of glucose uptake can be ascribed to increased intracellular free fatty acid concentration in those tissues which participate in glucose uptake. It would appear that a separate and unknown effect of dibutyryl cAMP is involved which directly or indirectly opposes the influence of insulin to increase glucose uptake.

In the case of epinephrine infusion, the usual increase in plasma insulin concentration is not evoked by the hyperglycemia (see Table 2), and the relative inhibition of glucose uptake is greater than with dibutyryl cAMP (see Fig. 2). The impairment of

* N. Altszuler, I. Rathgeb and R. Steele, unpublished observations.

glucose uptake is greater than that which can be explained by inhibition of extra insulin secretion, since the increased glucose concentration fails to increase glucose uptake at the normal or even slightly elevated plasma insulin concentration which prevails during epinephrine infusion. Epinephrine infusion does elevate triglyceride lipolysis, so the possibility that an increased intracellular free fatty acid concentration may be inhibiting glucose uptake cannot be ruled out as an explanation in this instance. However, it should be noted that norepinephrine infusion, which also increases plasma free fatty acid concentration, has no influence on glucose uptake.[3]

The participation of intracellular cyclic AMP in the stimulation of insulin secretion by the $\beta$ cell has been deduced from experiments *in vivo*[14] in which the phosphodiesterase inhibitor, theophylline, was found capable of enhancing insulin secretion when cyclic AMP was being generated within the $\beta$ cells. Also the rat pancreas *in vitro* was found to secrete extra insulin in response to high concentrations of cyclic AMP in the perfusion fluid.[15] The intervention of epinephrine in inhibiting extra insulin secretion in response to hyperglycemia[16,17] is thought to occur in man by way of alpha adrenergic receptors in the $\beta$ cell which operate to inhibit the generation of cyclic AMP. An opposing effect of epinephrine to stimulate cyclic AMP generation and insulin secretion is unmasked when the alpha adrenergic receptors are blocked.[14,18]

In the present experiments, the similar hyperglycemia obtained by infusion of dibutyryl cAMP and by glucose also evokes a similar elevation in plasma insulin concentration. The latter finding suggests that dibutyryl cAMP does not *per se* stimulate insulin secretion; however, unlike epinephrine, dibutyryl cAMP does not prevent the usual increment in insulin secretion in response to the hyperglycemia.

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