Alcohol Inhibition of Cyclic AMP-induced Insulin Release

A. R. Colwell, Jr., M.D., M. Feinzimer, M.D., D. Cooper, and L. Zuckerman, M.S., Evanston and Chicago

SUMMARY

In order to define the role of alcohol in cAMP-induced insulin release, the two agents were infused into the pancreas or liver of normal dogs separately or in combination. Blood levels of insulin and glucose were measured in the portal vein. CAMP infusion in the pancreatic artery provoked a greater early insulin response than did a similar infusion into the portal vein when glycemic levels were comparable. The addition of alcohol suppressed insulin response to intrapancreatic cAMP. Alcohol in the absence of the stimulus failed to suppress insulin below basal levels. It is concluded that pancreatic alcohol directly inhibits cAMP-related insulin release and that this is the mechanism by which glucagon-induced insulin release is blunted by alcohol. Glucose-induced release, which is potentiated by intravenous alcohol, apparently involves another mechanism. DIABETES *22:* 854-57, November, 1973.

Cyclic AMP is thought to mediate glucagon-induced insulin secretion, and ethanol *blocks* this response.[1] In contrast, although cyclic AMP is also involved in insulin secretion provoked by glucose,[2] ethanol *potentiates* this effect.[3] In order to clarify the mechanisms involved, we investigated the influence of local alcohol on insulin release stimulated by pancreatic infusion of cyclic AMP.

In order to distinguish the cAMP-induced hyperglycemia from its insulinotropic effect, pancreatic and hepatic infusions were employed in dogs. For control purposes, alcohol was injected alone into the same sites. Infusion of the two agents concurrently permitted an evaluation of the alcohol effect on the stimulated islet response. Measurement of insulin efflux was accomplished by nonobstructive intraportal cannulation.

METHODS

Mongrel dogs weighing 16 to 28 kg. were fed a high carbohydrate ration for at least one week and fasted for sixteen hours before surgery. The dose of sodium Nembutal for intravenous anesthesia was 25 mg. per kg. of body weight. The pancreaticoduodenal artery was cannulated with an indwelling polyethylene catheter by the technic described previously.[4] A second tube was placed in the portal vein by threading it through a mesenteric vein so that it lay approximately one inch distal to the entrance of the pancreaticoduodenal vein. The procedure permitted determination of the effect of a five-minute infusion of the head and body of the pancreas on insulin efflux as reflected by the intraportal concentration without interfering with normal venous blood drainage. The portal tube was employed for hepatic infusions. In animals given hepatic infusions the pancreatic arterial circulation was dissected for sham cannulation in order to make the technic suitable for control purposes. Both the arterial and venous catheters were flushed with heparinized saline (1:10) before each infusion and sampling procedure.

Cyclic AMP* was dissolved and made up with saline to a concentration of 5 mg. per milliliter. The dose was 1.0 mg. per kilogram of body weight, and rate of infusion was set so that the total dose was administered in five minutes. Pure ethanol was diluted with saline to a concentration of 1.4 per cent and administered separately or concurrently with cyclic AMP in a mean dose of 40 mg. (2 mg. per kilogram of body weight) at the same rate. Although it was not measured, estimation of probable pancreatic arterial blood alcohol concentration from our dose suggests a similarity to that employed orally in humans to impair glucose tolerance.[5]

From the Departments of Medicine, Evanston Hospital, Evanston, and Northwestern University Medical School, Chicago, Illinois.

Accepted for publication May 21, 1973.

*Adenosine-3', 5'-cyclic phosphate (free acid) was purchased from Nutritional Biochemical Corporation, Cleveland, Ohio.

Assuming a blood flow of 10 to 20 ml. per minute, with an infusion rate of 8 mg. per minute, a pancreatic concentration of 40 to 80 mg. per cent was achieved. Although equivalent to peripheral blood levels in human beings having only mild alcohol intoxication, this amount will be shown to be sufficient to inhibit insulin response to a relatively large cAMP stimulus. Although intrapancreatic alcohol concentration was appreciable, portal and further systemic blood dilution probably preclude recirculation of an effective amount.

Heparinized portal venous and femoral arterial blood samples were collected fifteen and two minutes before and at intervals ranging from five to fifteen minutes after the infusions for two hours. Plasma was separated, frozen and stored at —10° C. for subsequent analysis. Immunoreactive insulin[6] and glucose[7] were estimated in duplicate. Femoral artery results that confirm the portal vein results are not shown but are referred to in the text when significantly different. The values for the later time periods are not included because of difficulties in interpretation of secondary responses.

Group means and their corresponding standard errors were compared using Student's *t* test, and the minimum level of acceptance for a significant difference is $p<0.05$. Values shown are the means ± S. E. M. Areas and group data are depicted as the means of the group of all the individual incremental values above the fasting value.

RESULTS

Pancreatic infusions. Cyclic AMP infusion into the pancreatic artery produces an immediate release of insulin (figure 1). This response is six times greater in the portal vein than in samplings made from the femoral artery (not shown). The maximal glycemia occurs ten minutes after infusion at both sampling sites. The glucose and insulin values both return toward basal levels at thirty minutes.

When alcohol is added to the pancreatic artery infusate of cAMP there is a marked diminution of the insulin released into the portal vein. The area under the portal venous insulin curve for the first thirty minutes due to this infusion is 574 ± 176 versus 2,413 ± 655 microunits per milliliter— minutes for the corresponding time without alcohol. The blood glucose response to the combination is essentially the same as that to cAMP alone (917 ± 28 to 821 ± 104 mg. per cent —minutes respectively).

Results from infusion of alcohol alone at this concentration are illustrated in this same figure. Although

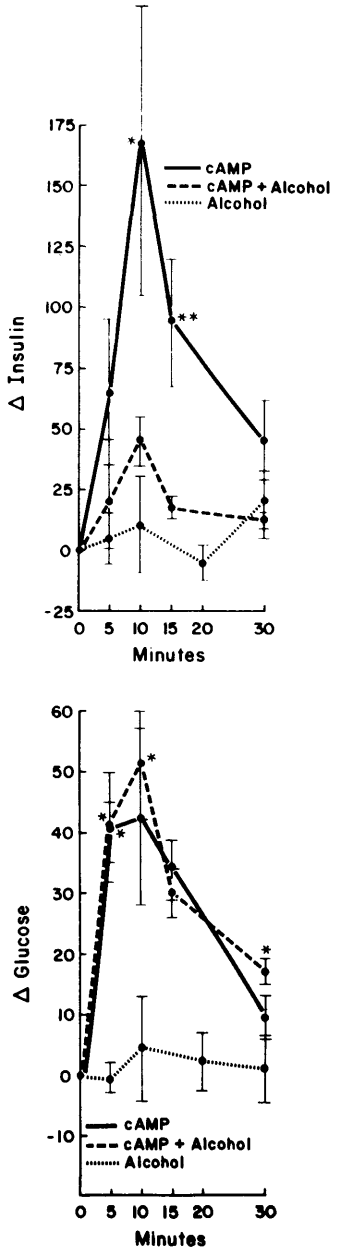

FIG. 1. Portal venous plasma insulin and glucose responses to intrapancreatic infusions of cAMP (five dogs), alcohol (six dogs), and the two substances combined (four dogs). Insulin and glucose values are plotted as the means (μU. per milliliter and mg. per cent, respectively) of the individual differences from their preinfusion levels. A single asterisk denotes a significant difference between the means ± S.E.M. for the cAMP versus the alcohol controls. A double asterisk refers to a significant difference ($p<0.05$) between cAMP and the combination of cAMP and alcohol.

a questionable early inhibition of basal insulin levels may be present, this is not statistically significant. When the alcohol-infused group areas are compared to the cAMP and cAMP plus alcohol groups for the first thirty minutes, the insulin and glucose values are significantly lower.

Hepatic infusions. The results of the three infusates when given to the dogs via the portal vein are shown in figure 2. As before, the cAMP infusion resulted in chronologically similar early glucose and insulin releases in the portal vein and femoral artery. The glucose curves are consistently depressed for the combination of cAMP and alcohol compared with cAMP alone. This is represented in the figures and also by comparison of the thirty minute areas (586 ± 126 and 796 ± 86 mg. per cent—minutes, respectively). Although this is not significant, the concomitant femoral artery samples are 475 ± 145 and 936 ± 67 mg. per cent—minutes respectively. Alcohol infusion into the portal vein was uneventful with respect to early glucose or insulin responses. The depressed portal vein insulin values during the first thirty minutes are not reflected in the femoral artery samples.

The fourfold reduction of the integrated insulin response found with the addition of alcohol to the pancreatic infusion of cAMP was not seen with the hepatic runs. Values for the hepatic group for cAMP were 1,049 ± 765 and 757 ± 490 microunit per milliliter—minutes for cAMP plus alcohol. The apparent earlier cAMP plus alcohol insulin response in this group compared with the pancreatic group was not significant. Similarly, the lower levels of cAMP-related insulin and glucose compared with the pancreatic group were not significant except for the integrated glucose response as measured in the femoral artery (475 ± 145 and 1,022 ± 169 mg. per cent—minutes respectively).

DISCUSSION

Our studies reveal that increasing the intrapancreatic concentration of cAMP well above physiologic levels produces an immediate rise in portal venous plasma insulin and significant hyperglycemia. Because of the similarity of this glycemia and that resulting from cAMP infused into the liver, the additional insulin release with the pancreatic infusion is directly attributable to the effect of cAMP on the islets. It is also demonstrated that the pancreatic cAMP stimulus actively induces hepatic glycogenolysis after perfusing the islets, and the resulting hyperglycemia is not suppressed by the released insulin.[8]

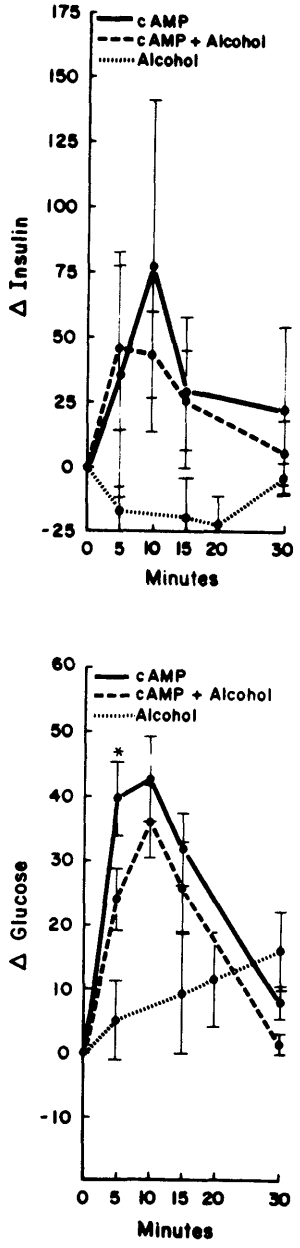

FIG. 2. Portal venous plasma insulin and glucose responses to intraportal infusions of cAMP (four dogs), alcohol (four dogs) and their combination (four dogs) are shown as in figure 1.

Previously, employing the same technic of intrapancreatic infusion of glucagon,[9] we showed a comparable but more rapid and sustained portal venous insulin response with greater peripheral arterial hyperglycemia. The resulting thirty minute areas under the curves demonstrate equivalent insulinemias for glucagon and cAMP (2,907 ± 654 and 2,413 ± 667

microunit per milliliter—minutes) with a fourfold larger glucagon-induced hyperglycemia (3,720 ± 439 and 936 ± 67 mg. per cent—minutes, respectively). Because cAMP was a more effective insulin secretogogue than glucagon for comparable glycemia, glucagon is not implicated in the response to a pancreatic cAMP infusion. However, if the insulin response has no direct relation to the degree of hyperglycemia, this conclusion may not be justified.

The rapid insulin response to intrapancreatic cAMP with a peak at ten minutes is compatible with release of stored granuoles occurring somewhat later than that found in the perfused rat pancreas.[10] With the high levels of cAMP used, it is possible that the stimulus could recirculate to the pancreas, but it is not likely that the concentration was sufficient to stimulate insulin secretion, since cAMP has a marked ability to permeate the liver cell, where it is metabolized.[11] Any recirculation of the portal infusate would only make the apparent beta cell stimulation by cAMP less effective in comparison to the pancreatic infusion.

The inhibition and potentiation of the insulin response to glucagon and glucose, respectively, by alcohol leads to the postulate of two separate mechanisms involved in insulin release. The response induced by glucagon has been shown to be modulated by cAMP[12] and independent of glucose.[13] However, recent evidence now links the glucose-induced release to increased levels of cAMP and implies a synergistic effect of glucose and cAMP.[2] This further speaks against hyperglycemia from hepatic glycogenolysis as the cause of cAMP-induced insulinemia. If cAMP is a mediator for both stimuli, how is a differential response to alcohol possible? Our experiments demonstrate that alcohol's main effect on the pancreas is to inhibit cAMP-mediated release. As opposed to studies employing intravenously injected alcohol,[1] these results and others from our laboratory[14] fail to show any potentiation of pancreatic alcohol on the glucose-induced release. Therefore, they support the idea of a single pathway for insulin release which can be stimulated by either glucose or cAMP.

ACKNOWLEDGMENT

Mrs. Betty Rutschmann analyzed blood insulin and glucose, Mrs. Betty Rachmeler drew the figures, and Mr. John Mitterling performed the dog surgery. Mrs. Nan McMillen performed the statistical analysis on the Northwestern Vogelback Computer, and Miss Grace A. Clarke typed the manuscript. Evanston Hospital supported these studies by donations from the Thomas J. Dee, George G. Moody, and Blunt families.

REFERENCES

[1]Friedenberg, R., Metz, R., Mako, M., and Surmaczynska, B.: Differential plasma insulin response to glucose and glucagon stimulation following ethanol priming. Diabetes *20*:397-403, 1971.

[2]Charles, M. A., Fanska, R., Schmid, F. G., Forsham, P. H., and Grodsky, G. M.: Adenosine 3', 5'-monophosphate in pancreatic islets. Glucose-induced insulin release. Science *179*:569-71, 1973.

[3]Siegal, A.M., Kreisberg, R.A., Owen, W.C., and Fox, O.J.. Some aspects of "acute phase" insulin release in healthy subjects. Diabetes *21*:157-62, 1972.

[4]Colwell, A.R., Jr., Zuckerman, L., and Berger, S.: Pancreatic insulin secretion following intrapancreatic infusion of amino acid. Diabetes *19*:217-27, 1970.

[5]Dornhorst, A., and Duyang, A.: Effect of alcohol on glucose tolerance. Lancet *2*:957-59, 1971.

[6]Morgan, C.R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115-26, 1963.

[7]Saifer, A., and Gerstenfeld, S.: The photometric microdetermination of blood glucose with glucose oxidase. J. Lab. Clin. Med. *51*:448-60, 1958.

[8]Williams, T.F., Exton, J.H., Friedmann, N., and Park, C.R.: Effects of insulin and adenosine 3', 5'-monophosphate on K+flux and glucose output in perfused rat liver. Am. J. Physiol. *221*:1645-51, 1971.

[9]Colwell, A.R., Jr., and Zuckerman, L.: Regulation of insulin release by pancreatic glucagon. Diabetes *19*:429-37, 1970.

[10]Basabe, J.C., Lopez, N.L., Viktora, J.K., and Wolff, F.W.: Insulin secretion studied in the perfused rat pancreas. II. Effect of glucose, glucagon, 3', 5' adenosine monophosphate, theophylline, imidazole and phenoxybenzamine; their interaction with diazoxide. Diabetes *20*:457-66, 1971.

[11]Villar-Palasi, C., Larner, J., and Shen, L.C.: Glycogen metabolism and the mechanism of action of cyclic AMP. Ann. N.Y. Acad. Sci. *185*:74-77, 1971.

[12]Malaisse, W.J., Malaisse-Lagae, F., and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. *46*:1724-34, 1967.

[13]Brisson, G.R., Malaisse-Lagae, F., and Malaisse, W.J.: The stimulus-secretion coupling of glucose-induced insulin release. IV. A proposed site of action for adenosine-3', 5'-cyclic monophosphate. J. Clin. Invest. *51*:232-40, 1972.

[14]Colwell, A.R., Jr., and Zuckerman, L.: Unpublished data.