Effect of Ethanol on the Glucose-mediated Insulin Release in Triply Catheterized Anesthetized Pigs

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SUMMARY

In order to further elucidate the potentiating effect of ethanol on the glucose-mediated insulin response, triply catheterized anesthetized pigs were submitted to an intravenous glucose infusion test after a four-hour preinfusion with ethanol (11 pigs) or saline (six pigs, control experiment). During the tests portal, hepatic, and peripheral venous blood was drawn simultaneously. Two series of ethanol-preinfusion experiments were carried out: in one series the serum ethanol concentration was maintained at approximately 10 mM and in the other at 19 mM. Compared with saline, 10 mM of serum ethanol induced a more than threefold increment in the insulin secretory response to glucose as estimated in the portal blood ($p < 0.01$). Likewise, hepatic and peripheral venous blood insulin levels were enhanced ($p < 0.01$). In contrast, 19 mM of serum ethanol did not elevate serum insulin levels above those found in the control experiments. When individual incremental portal insulin areas were plotted against the corresponding average value of serum ethanol in the preinfusion period, a significant inverse relationship was found ($p < 0.02$), indicating a decrease in the potentiating effect of ethanol on the glucose-mediated insulin response with increasing levels of serum ethanol. Comparison of portal and hepatic incremental insulin areas revealed that ethanol did not, in the concentration range investigated, influence the hepatic insulin degradation rate. In conclusion, ethanol seems to potentiate, in an inverse concentration-dependent manner, the glucose-mediated insulin response through an action directly on the pancreas. DIABETES *25*:752-57, September, 1976.

Recent investigations in normal humans have shown that intravenous infusion of ethanol induced enhanced serum insulin concentrations in peripheral venous blood during a subsequent intravenous glucose tolerance test[1,2] or intravenous tolbutamide test.[2] Even though peripheral vein insulin concentration may be used as a rough index of pancreatic insulin secretion,[3] it is difficult to draw any conclusion regarding a direct effect of ethanol on insulin secretion, since insulin is secreted into the portal system and transverses the hepatic bed before it reaches the periphery. Ethanol metabolism in the liver has been reported to diminish degradation of some other substances (e.g., galactose) by the liver.[4] Therefore, the possibility that insulin levels in peripheral blood after glucose or tolbutamide administration were increased as a result of an ethanol-induced diminished hepatic insulin degradation also has to be considered.

Consequently, the present investigation was designed to compare, in anesthetized pigs, the effect of ethanol on insulin concentrations in simultaneously obtained samples of portal, hepatic, and peripheral venous blood.

MATERIALS AND METHODS

Experimental Procedure

Pigs weighing 20 to 38 kg. were anesthetized with halothane-nitrous-oxide-oxygen after a 12-hour fast but free access to drinking water. The abdomen was

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opened by the midline incision and Teflon catheters were inserted into the portal vein and inferior vena cava, the tips of the catheters being placed approximately 2 cm. from the hepatic hilus and at the entrance of the hepatic veins, respectively. Catheters were also placed in a jugular vein and a hindleg vein. Portal, hepatic, and peripheral venous blood was drawn simultaneously and collected in plain tubes on ice. After clotting, the serum was separated and stored in plastic tubes at −25° C. until analyzed. Between drawings of samples the catheters were kept patent by instillation of 0.9 per cent sodium chloride. Ethanol was administered intravenously to 11 pigs as a 5 per cent solution in 0.9 per cent sodium chloride. This solution was given simultaneously in the jugular vein by means of a drip and in the hindleg vein by means of a pump. After 50 minutes the ethanol infusion in the jugular vein was completed, at which time the serum ethanol concentration had been brought to the desired level: between 5 and 20 mM (23-92 mg. per 100 ml.). Ethanol was infused at a constant rate by the pump in order to keep the serum ethanol concentration constant. Administration of ethanol started four hours before an intravenous glucose infusion test was initiated by injecting 0.5 gm. of 50 per cent glucose per kilogram body weight over four minutes, time 0 being the middle of the injection. During the four-hour preinfusion period blood glucose concentration was determined frequently by means of the Dextrostix-Eyetone-System (Miles Laboratories, Elkhart, Indiana)[5] and normoglycemia maintained, if necessary, by infusing small amounts of a 5.5 per cent glucose solution. In all experiments blood samples were drawn 10 and five minutes before and 5, 10, 20, 30, 40, 50, and 60 minutes after infusion of glucose. Blood samples for serum ethanol determination were drawn from the jugular vein at hourly intervals during the preinfusion period and at the termination of the experiment. Control experiments with administration of identical amounts of 0.9 per cent sodium chloride without ethanol were performed in six pigs.

Laboratory Investigations and Calculations

Serum samples were assayed for glucose on a Technicon AutoAnalyzer by a hexokinase method[6] and for insulin content by a sensitive radioimmunoassay.[7] Standards were highly purified porcine insulin (monocomponent insulin) from Novo Research Institute, Copenhagen. Detection limit, precision, accuracy, and specificity of the assay have been given in detail elsewhere.[8] Serum ethanol was measured by a gas-chromatography method.[9]

The net increase in the amount of insulin secreted in a given experiment was calculated by integration of the insulin concentration curve during the intravenous glucose infusion test, the basic level being used as baseline (i.e., the incremental insulin area).

The hepatic insulin extraction ratio of a given experiment was calculated by dividing the difference between the portal and hepatic incremental insulin areas of the experiment by the incremental portal insulin area.

Results were evaluated statistically by means of analysis of variance followed with the Dunnett's two-tailed multiple comparison test. P-values less than 0.05 were considered significant. For linear regression analysis the least-squares method was used.

RESULTS

Preliminary results indicated that low and high serum ethanol concentrations might influence glucose-mediated insulin release differently. Therefore, two series of ethanol preinfusion experiments were performed. In the first series, comprising six pigs with a mean body weight of 29.8 ± 6.3 (S.D.) kg., the mean serum ethanol concentration was 10.0 ± 3.0 (S.D.) mM. The second series comprised five pigs with a mean body weight of 24.2 ± 2.4 (S.D.) kg. In this series the mean serum ethanol concentration was 19.0 ± 1.8 mM. Within the individual experiments fluctuations in serum ethanol concentration never exceeded ± 15 per cent of the mean value. The groups of pigs will be designated the 10-mM-ethanol group and the 19-mM-ethanol group, respectively. Six pigs formed the control group (mean weight: 26.0 ± 5.6 (S.D.) kg.). The mean body weight of the groups did not differ significantly.

Effect of Intravenous Ethanol Alone on Serum Glucose and Insulin Concentration

During the four-hour preinfusion period serum glucose concentration tended to decline gradually but to a slight degree in most experiments. At the same time, serum insulin concentration declined (data not shown). Among the three series of experiments serum glucose and insulin concentrations were not significantly different, and in all instances normoglycemia was quickly achieved by the intravenous infusion of small amounts of glucose.

Effect of Intravenous Ethanol on Serum Glucose Concentration During the Intravenous Glucose Infusion Test (Figure 1)

Serum glucose concentration was measured in the jugular vein only (figure 1). The mean glucose concentrations of the ethanol experiments did not differ significantly from those of the control experiment either before the start of the glucose infusion or during the glucose infusion test.

Effect of Intravenous Ethanol on Serum Insulin Concentration During the Intravenous Glucose Infusion Test (Table 1, Figure 2)

Figure 2 shows mean values ± S.E.M. for serum insulin concentration in portal, hepatic, and jugular venous blood during the glucose infusion experiments following preinfusion with ethanol or saline. Table 1 gives the figures and pertinent statistical data.

1. 10-mM-ethanol group: Following glucose infusion, serum insulin concentration increased significantly above fasting levels in portal as well as hepatic and peripheral venous blood, and at all three sampling sites the increment was significantly above that observed in the control experiment. Peak insulin concentrations were reached after approximately 30 minutes.

2. 19-mM-ethanol group: The mean insulin concentration curve obtained in these experiments did not differ significantly from that of the control experiments. In fact, serum insulin concentrations were

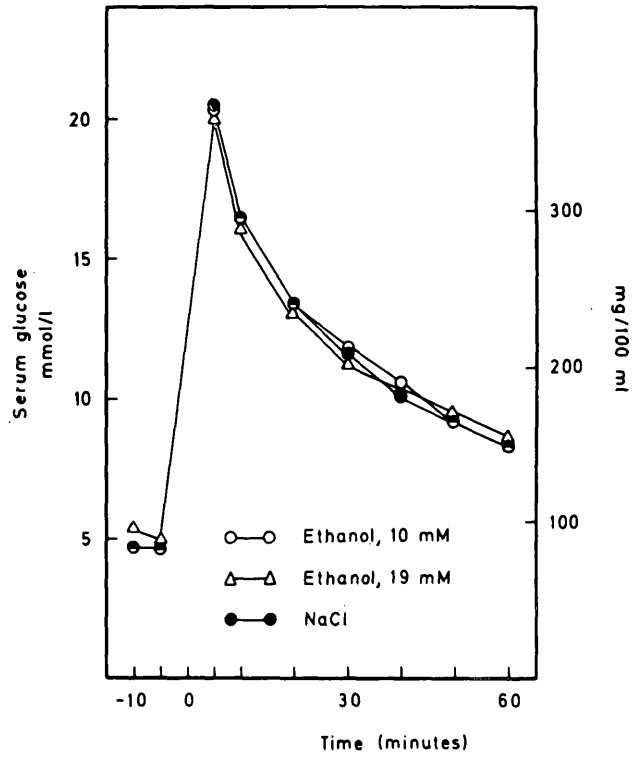

FIG. 1. Mean serum glucose concentration in peripheral venous blood during intravenous glucose infusion tests following preinfusion with ethanol or saline for four hours. Two series of ethanol preinfusion experiments were performed, one with an average serum ethanol concentration of 10 mM (six pigs), the other with an average serum ethanol concentration of 19 mM (five pigs). Six pigs were preinfused with saline. S.E.M.s were too small to be indicated.

TABLE 1

Corresponding concentrations (mean ± S.E.M.) of serum insulin (μU. per ml.) determined in portal, hepatic, and jugular venous blood in anesthetized pigs submitted to intravenous glucose tolerance tests following preinfusion with ethanol or saline for four hours.

	Minutes		0*	5	10	20	30	40	50	60
	1. NaCl	(6)	6.7±1.2	9.5± 1.6	12.3± 1.9	17.0±2.2	23.0±7.8	16.5±2.6	16.3±2.9	14.2±2.1
	2. Ethanol, 10 mM	(6)	8.3±1.5	35.3±11.2	40.2±10.7	43.3±8.4	46.8±9.1	44.7±7.4	40.5±8.5	31.5±5.1
Portal	3. Ethanol, 19 mM	(5)	6.6±0.7	7.8± 1.9	9.6± 1.2	14.0±2.1	13.4±2.1	13.2±3.4	12.8±4.2	16.6±7.2
Vein	p (2 vs. 1)		N.S.	<0.05	<0.05	<0.01	N.S.	<0.01	<0.05	<0.05
	p (3 vs. 1)		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	1. NaCl	(6)	5.3±1.0	8.0± 1.4	9.2± 1.7	11.2±2.2	9.5±1.7	10.7±2.3	12.3±2.6	10.7±2.1
	2. Ethanol, 10 mM	(6)	5.2±1.1	12.3± 1.7	13.5± 1.8	16.8±2.9	19.8±4.5	20.5±3.6	19.0±2.7	16.0±3.7
Hepatic	3. Ethanol, 19 mM	(5)	6.2±0.7	9.2± 1.6	10.4± 1.2	9.6±1.4	10.0±2.0	10.2±1.6	10.4±1.2	9.0±1.2
Veins	p (2 vs. 1)		N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.
	p (3 vs. 1)		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	1. NaCl	(6)	4.7±0.9	7.5± 1.3	8.0± 1.4	10.5±1.2	9.0±1.8	10.5±1.6	9.0±1.6	8.8±2.1
	2. Ethanol, 10 mM	(6)	4.8±1.2	12.5± 1.3	13.2± 1.4	12.7±1.5	18.8±4.8	20.0±6.3	18.3±5.1	16.8±5.5
Jugular	3. Ethanol, 19 mM	(5)	6.2±0.7	9.2± 1.7	8.0± 1.4	10.5±1.2	7.2±1.4	7.0±1.3	10.4±3.0	8.6±2.1
Vein	p (2 vs. 1)		N.S.	<0.05	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
	p (3 vs. 1)		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Average of the −10 and −5 minute values.
Numbers of experiments are given in the brackets.

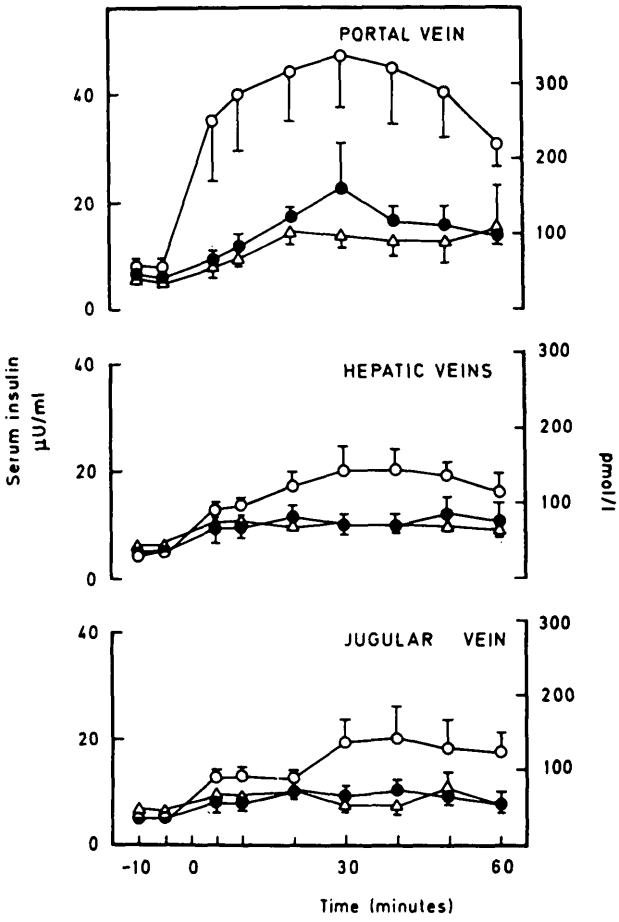

FIG. 2. Serum insulin concentrations (mean ± S.E.M.) in portal, hepatic, and peripheral venous blood during intravenous glucose infusion tests following preinfusion with ethanol or saline (●-●) for four hours. In a group of six pigs the mean serum ethanol concentration was 10 mM (o-o), and in a group of five pigs it was 19 mM (△-△).

generally lower at the portal sampling site; differences were, however, not significant. Even if serum insulin concentration increased significantly at all three sampling sites no real peaks were seen. Instead, plateaus were reached after approximately 20 mintues.

Figure 3 shows the mean ± S.E.M. incremental insulin areas calculated from serum insulin concentrations in portal and hepatic venous blood. In the 10-mM-ethanol group, the portal as well as the hepatic incremental insulin area was significantly increased. In contrast, the incremental insulin areas of the 19-mM-ethanol group did not differ significantly from those of the control group.

Figure 4 shows a plot of the individual portal incremental insulin areas versus the corresponding average values of serum ethanol in the experiments. An inverse relationship was found ($p < 0.02$), indicating that the potentiating effect of ethanol on glucose-induced insulin release is concentration-dependent and disappears at serum ethanol levels of about 20 mM.

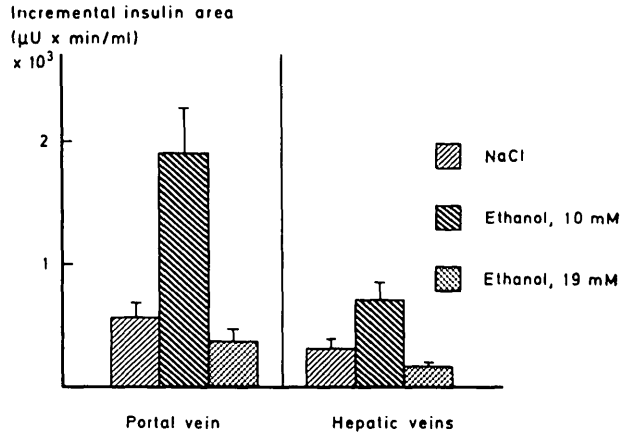

FIG. 3. Incremental insulin areas (mean ± S.E.M.) in portal and hepatic venous blood in experiments shown in figure 2.

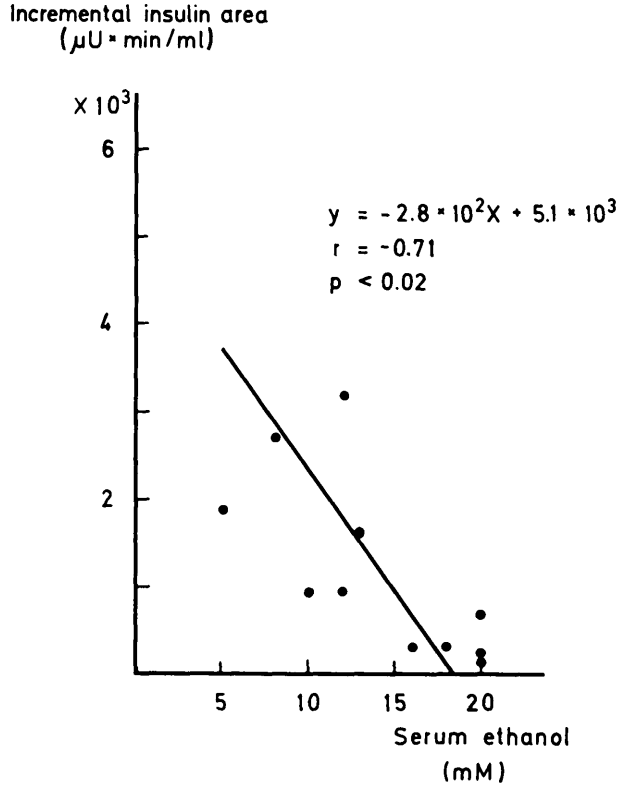

FIG. 4. Correlation between the average serum ethanol concentrations in the experiments (abscissa) and the corresponding portal incremental insulin areas (ordinate). r = coefficient of correlation.

Effect of Intravenous Ethanol on Hepatic Insulin Extraction Ratio (Figure 5)

Figure 5 shows mean values ± S.E.M. for the hepatic insulin extraction ratios for the two series of ethanol experiments and the control experiments. Differences are not significant, indicating that neither concentration of serum ethanol influenced hepatic insulin extraction.

DISCUSSION

The finding that ethanol can potentiate glucose-mediated insulin release in pigs is in agreement with findings previously reported in humans.[1,2,10,11] Furthermore, the data presented here give new information regarding the effect of ethanol on insulin secretion. First, it seems that ethanol influences the glucose-induced insulin release through an action directly on the pancreas, portal serum insulin concentrations being markedly enhanced when serum ethanol concentration was 10 mM (figure 2). Second, in the serum ethanol concentration range studied, a negative relationship existed between the amount of insulin released after glucose stimulation and the average serum ethanol concentration in the experiment (figure 4). Finally, ethanol, in the concentration range used, did not seem to influence the hepatic insulin extraction (figure 5). Hence, serum insulin levels in peripheral blood were not enhanced after ethanol simply as a result of diminished hepatic insulin degradation. The mode of action of ethanol on the pancreas is still unknown. Ethanol has been shown to inhibit the glucose-induced insulin secretion from small pieces of rat pancreas,[12] rabbit pancreas,[13] and hamster pancreas.[13] However, the ethanol concentrations used in these studies were uniformly above 20 mM, i.e., in the concentration range in which ethanol was not found to influence insulin secretion in the present study. On the other hand, the recent finding of a stimulating effect of ethanol (approximately 10 mM) on the adenyl cyclase in homogenates of rat islets[14] suggests that ethanol affects glucose-induced insulin secretion through an increased formation of cyclic adenosine monophosphate. In accordance with our finding of an inverse relationship between the average serum ethanol concentration and the potentiating effect on the glucose-induced insulin release, stimulation of the adenyl cyclase was maximal at low ethanol concentrations (8.6 mM), whereas the stimulation was reduced at higher ethanol concentrations.[14] The possibility that accumulation of ethanol metabolites might be operative in the potentiating effect on insulin secretion also exists. Our finding in humans that preinfusion with ethanol for one hour did not enhance glucose-induced insulin release while preinfusion for four hours did[2] might favor this view.

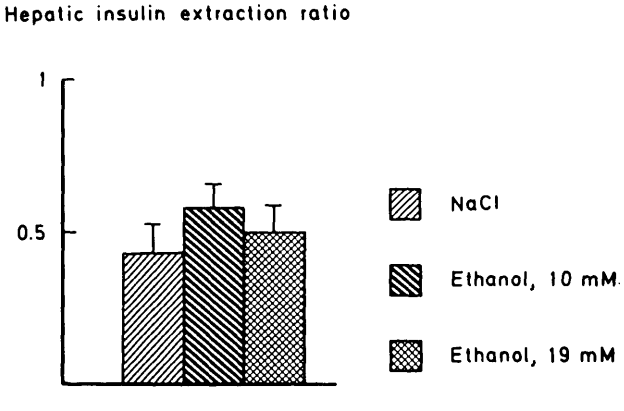

FIG. 5. Hepatic insulin extraction ratios of the ethanol and control experiments (mean ± S.E.M.) calculated from the data shown in figure 3.

It is of interest that the mean glucose concentration curves of the three series of experiments almost merged (figure 1), since it could be expected that the considerably higher serum insulin levels in the 10-mM-ethanol group would increase the glucose disposal rate. It may be suggested that the amounts of insulin released in the control experiments as well as in the 19-mM-ethanol experiments already were sufficient for maximal rate of glucose transport away from the vascular bed. Whether this suggestion is correct or not remains to be clarified, however.

The finding that the mean hepatic insulin extraction ratio was unaffected by constant serum levels of ethanol does not favor the hypothesis that ethanol enhances insulin levels in peripheral blood by diminishing the hepatic insulin degradation. However, our data must be interpreted with caution, since factors such as the portal and hepatic venous blood flow, the admixture of peripheral venous blood to the hepatic venous blood at the inferior vena cava sampling site, and, furthermore, the amount of insulin brought to the liver by the hepatic artery also have to be taken into account when hepatic insulin extraction ratios are calculated. Unpublished data from our laboratory indicate, however, that ethanol in the concentration range used in these experiments does not influence portal or hepatic blood flow. This finding is supported by previously reported findings in dogs.[15,16] Even though we have no data regarding the degree of admixture of peripheral venous blood and hepatic venous

blood, it appears that insulin levels in samples drawn through the inferior vena cava catheter were as a rule higher than those obtained simultaneously through the jugular vein catheter (table 1). Furthermore, it is well established that the hepatic insulin extraction ratio is relatively constant at about 0.5 at physiologic levels of portal insulin,[17-20] a figure that compares well with those found in the present investigation. Finally, both the hepatic insulin extraction ratios and the mean insulin concentration curves in portal and hepatic venous blood were similar in the 19-mM-ethanol group and the control group (figures 2 and 5).

Therefore, it must be concluded that ethanol affects the glucose-mediated insulin response by acting directly on the pancreas. The nature of this action is still unknown. The liver does not seem to be involved in the genesis of enhanced postglucose serum insulin levels after ethanol preinfusion.

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