Glucose- and Tolbutamide-Mediated Insulin Response after Preinfusion with Ethanol

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SUMMARY

Intravenous glucose tolerance tests (IVGTT) were carried out in five normal subjects after ethanol preinfusion for one, two or four hours. Constant serum level of ethanol was maintained throughout the experiments. Control experiments were performed with saline. Basal levels of serum glucose and insulin were unaffected by ethanol. As regards glucose tolerance a nonsignificant rise in the glucose assimilation constant was seen. During IVGTT peak insulin concentration was unaffected by preinfusion with ethanol for one or two hours, but significantly elevated after four hours. The declining part of the insulin concentration curve was significantly elevated after one or four hours of preinfusion. The insulin increment per glucose increment (the insulinogenic index) rose with the duration of the preinfusion period and, compared to the control experiment, a twofold increment was seen after four hours. When tolbutamide (1 gram) was given intravenously to two of the subjects after preinfusion with ethanol for four hours, the subsequent insulin response followed the same pattern as seen in the four-hour-IVGTT experiment. As the potentiation increases with the duration of the preinfusion period, it might be explained by cummulative changes in the beta cell. However, a diminished hepatic degradation rate of insulin cannot be ruled out. DIABETES *23*:821-26, October, 1974.

The effect of ethanol on glucose tolerance and serum insulin response to glucose has been investigated in recent years. While studies on patients suffering from 'alcohol diabetes' agree on the existence of a state of glucose intolerance with inappropriate high levels of serum insulin after a challenge with ethanol,[1,2] reports on normal human beings are conflicting. Thus, ethanol has been reported both to improve[3,4] and impair[1,2,5] glucose tolerance in the normal subject. It is well documented that ethanol per se does not stimulate insulin secretion,[6,7] but it is still uncertain if ethanol potentiates the action of well known insulinogogues on the beta cell. A potentiating effect on the glucose mediated insulin secretory response has been reported[3] as well as an inhibitory effect on the glucagon mediated response.[4] Both of these experiments included a twelve hour 'priming' period during which about 120 grams of ethanol was given by mouth or intravenously before administration of the insulinogogue. It is well known that ethanol inhibits gluconeogenesis in the liver and decreases peripheral glucose utilization.[8,9,24] The metabolic state in the persons under study[3,4] consequently was far from the ideal fasting state and normal glucose metabolism at the beginning of the experiment, thereby impeding the interpretation of the results.

The present investigation was designed to test the possibility that a relationship exists in normal subjects between the duration of the ethanol preinfusion and the potentiation of the subsequent insulin response to glucose.

The results of a supplementary study concerning the action of ethanol on tolbutamide-induced insulin release is also reported.

MATERIALS AND METHODS

Subjects

The subjects investigated were five male students, aged between twenty-four and twenty-nine years, without recognized diabetes mellitus, liver disease or abuse of alcohol. The subjects weighed approximately between 128 and 160 pounds and all were within 10 per cent of their desirable weight.[10] None had close relatives suffering from diabetes. Only persons with a

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normal K-value (i.e.> 1.05) from the intravenous glucose tolerance test[11] and with normal serum levels of aspartate aminotranspherase, alkaline phosphatase and creatinine were included in the study.

Experimental procedure

All subjects were on a carbohydrate-rich diet before and between investigations. Each subject was tested four times at one-week intervals. Two of the subjects participated in two supplementary tests carried out at similar intervals. The tests were performed in the morning after ten to twelve hours of fasting and at a minimum of twenty-four hours of abstinence from alcohol. An indwelling plastic cannula was inserted in an antecubital vein on each side. Blood was collected in clean tubes on ice and the serum stored in plastic tubes at -21°C until analyzed. Between drawings of samples the cannula was kept patent by a slow saline drip.

Ethanol was administered intravenously as a 5 per cent solution in 0.9 per cent sodium chloride. This solution was given simultaneously in one arm by means of a pump and in the other by means of a drip. The drip was finished after fifty minutes at which time the serum level of ethanol was approximately 50 mg. per 100 ml. The pump infused 140 ml. per hour throughout the experiment in order to compensate for the amount of ethanol metabolized during the procedure.

1. Ethanol-glucose-insulin study

Administration of ethanol started one, two or four hours before an intravenous glucose tolerance test (IVGTT) was initiated by injecting 50 ml. of 50 per cent glucose over four minutes, time zero being the middle of the injection. The tests will be referred to as the one-hour test, the two-hours test and the four-hours test, respectively. In the two-hours test and the four-hours test, samples were drawn with intervals of sixty minutes in the pre-IVGTT period, and in all tests blood samples were drawn ten and five minutes before and 5, 10, 20, 30, 40, 50 and 60 minutes after injection of glucose. A control experiment with administration of identical volumes of 0.9 per cent sodium chloride without ethanol was performed following the schedule of the two-hours test.

2. Ethanol-tolbutamide-insulin study

Ethanol was administered as described above for the four-hours test. Blood samples were drawn with intervals of sixty minutes in the preinfusion period, ten and five minutes before and 2, 5, 10, 20, 30, 40, 50 and 60 minutes after the injection of 20 ml. of 5 per cent tolbutamide (Rastinon, Hoechst). In the control experiment 0.9 per cent sodium chloride without ethanol was given.

Laboratory investigations and calculations

Serum glucose was determined on a Technicon AutoAnalyzer by a hexokinase method.[12] Serum insulin was estimated using a sensitive radioimmunoassay utilizing wick separation.[13] In our laboratory the assay has a sensitivity of $<1\ \mu$U./ml. and a between-assay reproducibility of 3 μU./ml. ($\pm$ 1 S.D.) at a level of 20 μU./ml. The presence of ethanol in the serum does not affect the assay. Serum ethanol was measured by a gas chromatographic method.[14] The glucose concentrations were plotted versus time on semilogarithmic paper, and the glucose assimilation constant, K, calculated according to Lundbaek.[11]

The insulinogenic index[15] was calculated by dividing the integrated area under the insulin curve with the integrated area under the glucose curve using fasting levels as a base line.

Statistical significance was calculated by means of the Mann-Whitney nonparametric rank sum test.[16]

RESULTS

The mean concentration of serum ethanol in all tests was 66 $\pm$ 11 (S.D.) mg. per 100 ml. (range: 50-110 mg. per 100 ml.). None of the subjects appeared intoxicated during the tests.

Effect of intravenous ethanol alone on serum glucose and insulin concentration

The levels of serum glucose and insulin during the pre-IVGTT and pre-tolbutamide period were constant and unaffected both by saline and ethanol infusion (data not shown).

Effect of intravenous ethanol on glucose tolerance

Figure 1 gives the mean glucose concentration in the control experiment compared to the three ethanol-IVGTT experiments. The average K-value in the control experiment was 1.24 $\pm$ 0.06 (mean $\pm$ S.E.M.), in the one-hour test 1.64 $\pm$ 0.19, in the two-hours test 1.36 $\pm$ 0.13 and in the four-hours test 1.60 $\pm$ 0.18. The differences are not significant.

Effect of intravenous ethanol on serum insulin concentration during the intravenous glucose tolerance test

Mean values $\pm$ S.E.M. for serum insulin concentrations during the control experiment and the three

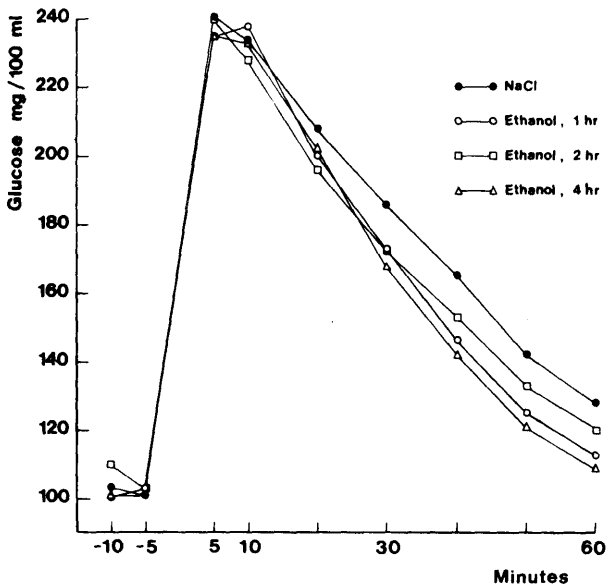

FIG. 1. Mean serum glucose concentration in five normal subjects during an intravenous glucose tolerance test following preinfusion with ethanol for one, two or four hours or saline for two hours.

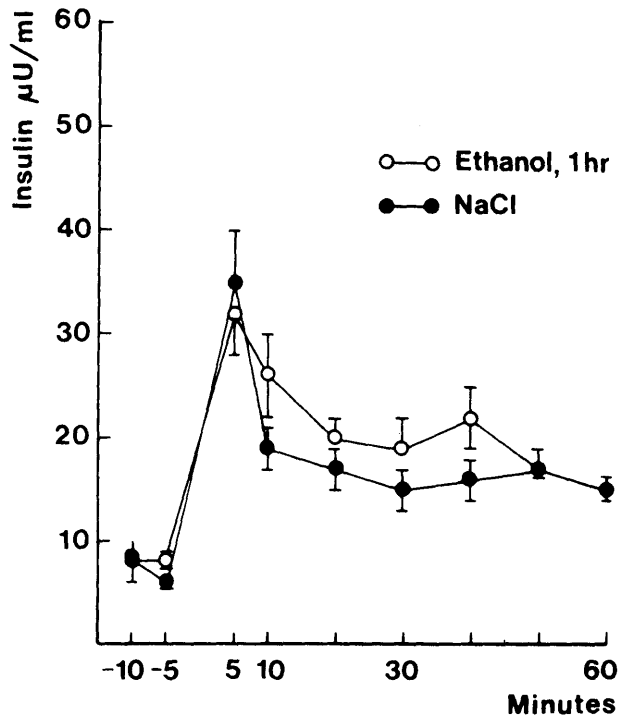

FIG. 2 A. Serum insulin concentration (mean ± S.E.M.) in five normal subjects during intravenous glucose tolerance tests following preinfusion with ethanol for one hour as compared to saline for two hours.

ethanol experiments are shown in figures 2 A-C. One or two hours of ethanol preinfusion did not influence either the peak insulin concentration or the time at which it occurred compared to the control experiment. A significant increase in the peak insulin concentration ($p < 0.05$) was, however, found after four hours preinfusion.

The insulin concentration curve after the peak value (i.e. from the tenth-minute value to the sixty-minute value) was found to be elevated after ethanol preinfusion for one and four hours compared to the control experiment ($p<0.01$). After two hours preinfusion the tenth-minute value was the only point significantly elevated, ($p<0.05$).

To see if observed differences in insulin response might be due to differences in levels of serum glucose only, the insulinogenic index for the total test was calculated (figure 3). Generally, the insulin increment per glucose increment was found to increase with the duration of the preceding ethanol infusion. However, the increment after four hours of infusion was the only one to reach the 5 per cent level of significance when compared to the control experiment. The increment after four hours of infusion was also significantly greater than the increment after one hour ($p<0.05$).

Effect of intravenous ethanol on serum insulin concentration during the intravenous tolbutamide test

This test was performed as a supplementary study in two of the subjects in order to see if ethanol could modify the insulin response after stimulation with another insulinogogue, tolbutamide. Four hours of ethanol preinfusion was chosen for the study because this period had been able to provoke the quantitatively largest alterations in the insulin response in the ethanol/IVGTT part of the study. The results from the two subjects were quite similar and figure 4 gives the results obtained in one of them. The post-ethanol insulin concentration curve displayed almost the same pattern as observed in the IVGTT study. Peak insulin concentration was 63 μU. per ml. compared to 25 μU. per ml. in the control experiment. The integrated areas under the curves were 1010 and 320 μU. per ml. per minute, respectively. In the experiment, not shown, peak values were 76 and 36 μU. per ml. and the areas were 1798 and 787 μU. per ml. per minute, in the ethanol and control experiment, respectively.

DISCUSSION

Our results confirm the finding that ethanol potentiates the glucose mediated insulin response.[3] The

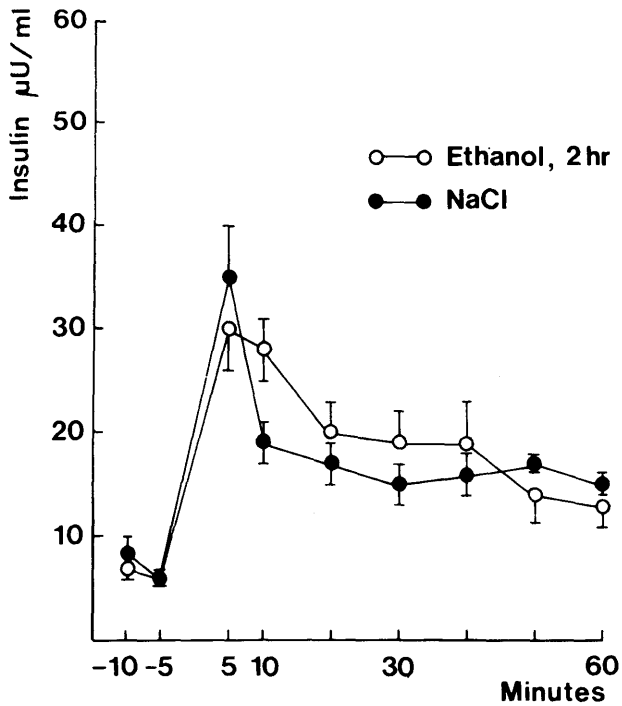

FIG. 2 B. Serum insulin concentration (mean ± S.E.M.) in five normal subjects during intravenous glucose tolerance tests following preinfusion with ethanol for two hours as compared to saline for two hours.

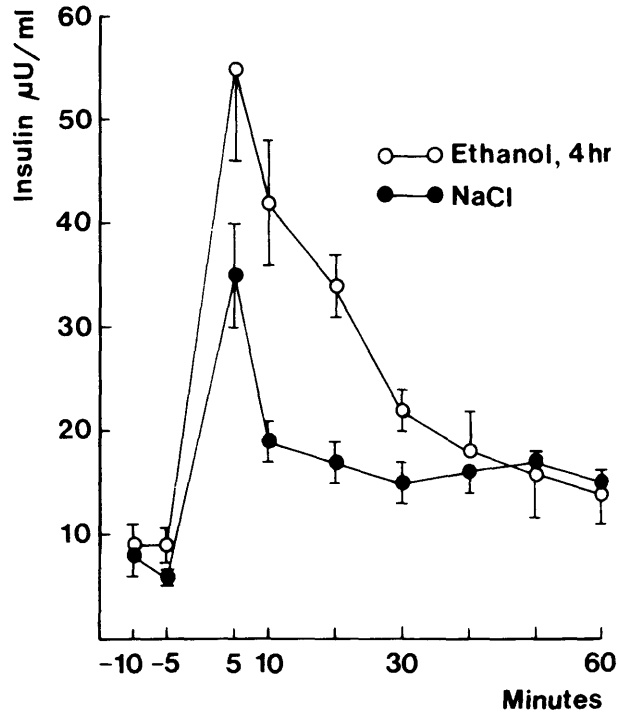

FIG. 2 C. Serum insulin concentration (mean ± S.E.M.) in five normal subjects during intravenous glucose tolerance tests following preinfusion with ethanol for four hours as compared to saline for two hours.

possibility that ethanol in the concentration range used in this investigation might act on the membranes of the beta cells in a nonspecific manner is not supported. If so, maximal potentiating effect of ethanol would be expected to occur as soon as steady-state concentration of ethanol was achieved. In well perfused organs, as the endocrine pancreas, steady-state concentration of ethanol must be achieved at least within one hour. However, ethanol preinfusion for four hours induced a significantly greater potentiation of the insulin response, than did infusion for only one hour employing identical ethanol concentrations (figure 2A vs. C). Ethanol preinfusion for two hours resulted in a greater insulin response than after one hour (not significant) (figure 2B vs. A), but a significantly lesser response than after four hours (figure 2B vs. C). This time-response dependency may explain some of the discrepancy reported in the literature concerning whether ethanol potentiates glucose mediated insulin response[3-5] or not.[17,18] Large differences in the glycemic stimulus cannot account for the differences in the insulin response since the glucose concentrations during the different tests were almost identical (figure 1). The declining part of the glucose concentration curve tended to be steeper in the ethanol experiments than in the control experiment. Consequently, the insulin secreted seemed to be biologically active.

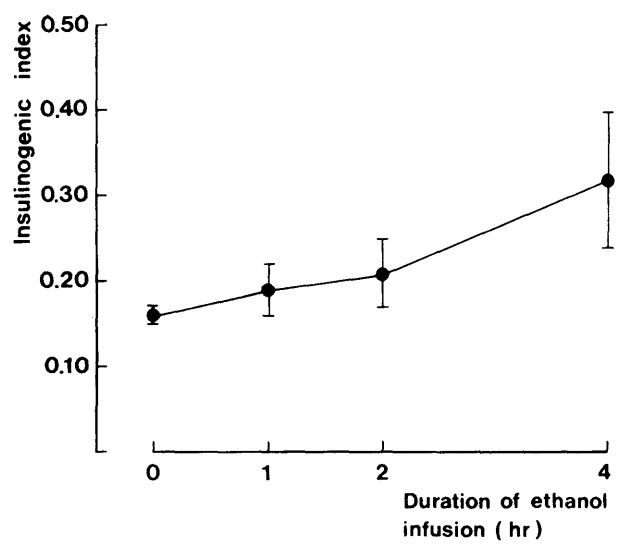

FIG. 3. Insulinogenic index (mean ± S.E.M.) in the experiments shown in figures 1 and 2.

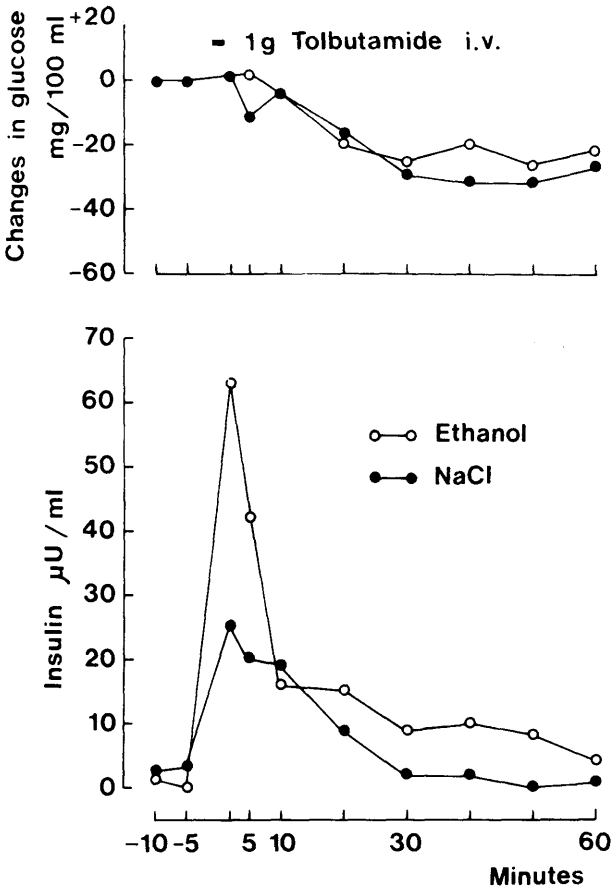

FIG. 4. **Serum insulin concentration and changes in serum glucose concentration in one subject during an intravenous tolbutamide test preceded by infusion of ethanol or saline for four hours.**

The results raise the possibility that ethanol acts through accumulation of some product in the beta cell or in the liver.

In vitro experiments with small peices of rat pancreas have shown inhibition of insulin secretion in the presence of ethanol. The results were interpreted as an interference of ethanol with the microtubular system.[19,20] The ethanol concentration employed was, however, 1 gm. per 100 ml. which is around twenty times larger than the concentration used in our investigation. In another study ethanol concentration close to ours (i.e. 50 mg. per 100 ml.) stimulated the adenyl cyclase in homogenates of rat islets, whereas the stimulation was abolished when the concentration was elevated to 500 mg. per 100 ml.[21] As cyclic adenosine monophosphate (cAMP) is thought to be involved in the process of insulin secretion[22] this finding may explain our in vivo results: Ethanol might in the lower concentration range stimulate the islet adenyl cyclase system and thereby enhance the subsequent insulin response to glucose.

Recently it has been suggested that sulphonylureas act on insulin secretion by inhibiting the phosphodiesterase system responsible for the breakdown of cAMP.[23] The rise in the intracellular cAMP concentration may then trigger insulin release and the insulin response to tolbutamide will thus be enhanced by the raised level of cAMP induced by ethanol. As pancreas glucagon is thought to mediate insulin secretion by stimulating the membrane bound adenyl cyclase,[20] the findings of Friedenberg and coworkers[4]—that ethanol inhibits glucagon stimulated insulin response—on the other hand, are unexplained by this theory. Colwell et al.[18] recently found that the insulin secretion after close arterial injection of free cAMP in dogs was inhibited by simultaneous ethanol injection. The ethanol concentration employed was, however, approximately 1 gm. per 100 ml., which is in the range previously found to inhibit insulin secretion.[19,20]

Glucose metabolism in liver is profoundly influenced by ethanol. Thus ethanol inhibits hepatic gluconeogenesis[24] and blocks the Cori cycle.[9] Furthermore, ethanol has been reported to inhibit degradation of some substances (i.e. galactose) in the liver.[25] As the samples analyzed in this study were achieved from peripheral veins, it is possible that the elevated glucose-induced insulin level observed after ethanol preinfusion merely reflects a diminished hepatic insulin degradation rate. The striking similarity between our post-ethanol insulin curves and those reported from IVGTT studies on patients suffering from various disseminated liver diseases[26-29] might support this view. However, during a single trans-hepatic circulation the liver normally retains about 50 per cent of the insulin presented[30,31] and even a total inhibition of liver insulin degradation caused by ethanol could not account for the net increase in the areas under the insulin curves observed in the tolbutamide part of the study. This suggests that ethanol, at least in part, acts directly on the beta cell.

Therefore, in order to clarify the mechanism by which ethanol influences glucose and tolbutamide mediated insulin response, further studies are needed including the simultaneous measurement of the insulin concentration in the portal and hepatic veins.

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