Effect of Ethanol, Acetaldehyde, and Acetate on Insulin and Glucagon Secretion in the Perfused Rat Pancreas

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SUMMARY

The effects of varying concentrations of ethanol (1, 10, and 30 mM) and its metabolites (1 mM acetate and 1 and 10 mM acetaldehyde) on insulin and glucagon secretion induced by glucose (11.1 mM) and arginine (20 mM) were studied in isolated perfused pancreas of Sprague-Dawley rats.

Ethanol and its metabolites did not significantly modify basal secretion of the two hormones. Ethanol reduced glucose-induced insulin secretion by means of a dose-related effect. Arginine-induced insulin output did not seem to be influenced to any significant degree. Acetate and acetaldehyde significantly inhibited glucose and arginine-induced insulin secretion. While ethanol (10 and 30 mM) did not modify glucagon output during arginine perfusion, acetate and acetaldehyde markedly enhanced it.

The block of insulin secretion and the increased secretion of glucagon could explain the diabetogenic effect of ethanol demonstrated in vivo. The mechanism by which ethanol acts on the pancreatic β- and α-cells is discussed. DIABETES *30*:705–709, September 1981.

While in man, ethanol in the fed state exerts a priming effect on the β-cell, thus potentiating insulin secretion induced by glucose and other secretogogues[1–5], in the rat it seems to have an inhibitory effect on insulin secretion.[6,7]

Few observations concerning the effect of ethanol on glucagon secretion can be found in the literature. Hyperglucagonemia concomitant with ethanol-induced hypoglycemia has been observed both in the animal[8–10] and in man.[11,12] Ethanol was found to have no effect on glucose-induced glucagon suppression[13] while glucagon's response to arginine was found to be potentiated by ethanol in man.[14,15]

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In vitro, ethanol does not modify spontaneous insulin secretion in the rabbit pancreas system, but reduces the glucose-induced output.[16,17] Moreover, while acetaldehyde has been found to have an inhibiting effect on insulin secretion, no influence or stimulating effect was attributed to acetate.[16,17] The latter, on the contrary, seems to enhance glucose-induced insulin secretion in vivo and also improves glucose tolerance.[6] Nothing is known about the possible influence in vitro of ethanol and its metabolites on glucagon secretion.

In this study, the effects of ethanol and its metabolites, acetaldehyde and acetate, on glucose and arginine-induced insulin and glucagon secretion in isolated and perfused rat pancreas are evaluated.

More specifically, it will be seen if ethanol has a direct or indirect effect on β-cells, and if hyperglucagonemia, demonstrated in vivo after ethanol administration, is due to the direct effect of ethanol or its metabolites on the α-cell.

MATERIALS AND METHODS

Sprague-Dawley rats weighing 250–300 g were employed in this study. The animals had free access to a commercially available chow and were kept in temperature-controlled quarters.

Following a 24-h fast, the animals were anesthetized by i.p. injection of sodium pentobarbital (50 mg/kg body weight, Farmotal, Carlo Erba, Milan). The pancreas was isolated according to the Sussman technique,[18] in which the duodenalpancreatic block is extracted without the stomach and spleen, and only a small part of the duodenum is included. Polyethylene cannuale were inserted into the abdominal aorta and portal vein. After its removal the preparation was immediately transferred to a perfusion chamber connected with a thermostatic unit (Juccheim Laborte-Chimik, Germany) and immersed in a 38°C water bath. The pancreas was perfused by means of a cannula placed in the aorta. The perfusate was oxygenated by means of an oxygenator made up of silastic tubes, and the flow rate was kept at 3 ml/min at 30–40 mm Hg by means of a peristaltic pump (Holter model 912, U.S.A.). Lateral infusion of the

stimulating substances at a flow rate of 0.1 ml/min was effected by a connected perfusor (Harvard model 975, U.S.A.). The effluent was collected at preestablished intervals from the portal catheter into plastic test tubes. Duodenal and exocrine pancreatic secretion was drained into the duodenum. The perfusate consisted of a Krebs-Henseleit solution supplemented with 0.50 g/L bovine albumin (V fraction), 400 U/ml trasylol (Bayer), and 4.4 mM glucose.

After the pancreas was connected to the perfusate system, an equilibrating period of 20 min, during which the organ was perfused with the basic buffer solution, preceded each trial.

At the end of each trial and following a 10-min period of stabilization by perfusing with a standard medium, the functional integrity of the pancreas was evaluated by means of a rapid infusion of glucose (11.1 mM). The perfusate was collected from the portal effluent, and stored immediately at −20°C until insulin and glucagon were assayed.

Insulin was determined by the radioimmunoassay method of Herbert et al.[19] using rat insulin as the standard (Novo Company, Copenhagen). Glucagon was assayed according to Faloona and Unger[20] using K-30 antibody kindly furnished by Dr. Unger. Preliminary tests were carried out which ruled out a possible interference by ethanol or its metabolites on the radioimmunoassay of insulin and glucagon.

The following experiments were carried out in groups of six rats:

(1) Perfusion of ethanol followed by glucose. Different concentrations of ethanol (1, 10, and 30 mM) were added to the standard medium throughout the study. After 40 min of perfusion, 11.1 mM glucose was added to the medium and perfused for 40 min.

(2) Perfusion of ethanol followed by arginine. Arginine (20 mM) was added to standard medium containing ethanol (10 and 30 mM) after 20 min of perfusion and perfused 20 min further.

(3) Perfusion of acetate or acetaldehyde followed by glucose. After acetate (1 mM) and acetaldehyde (10 mM) had been perfused for 20 min, glucose (11.1 mM) was added for a further 30 min of perfusion.

(4) Perfusion of acetate or acetaldehyde followed by arginine. Arginine (20 mM) was added to the perfusion medium containing acetate (1 mM) or acetaldehyde (1 and 10 mM) after 20 min of perfusion and perfused 20 min further.

The medium used for the control perfusions did not contain ethanol, acetate, or acetaldehyde.

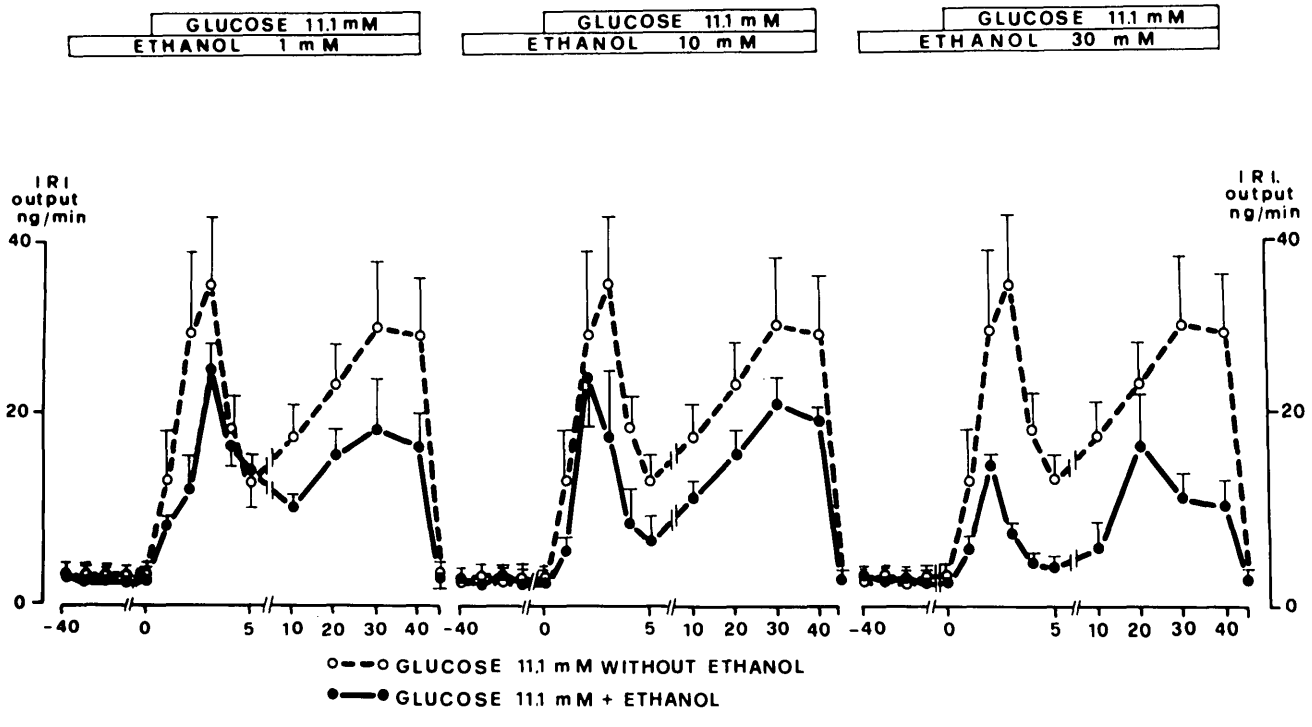
The secretion rate of insulin and glucagon was calculated by multiplying the concentration in the respective perfusate samples by the flow rate and was expressed as ng/min. The cumulative hormone secretory responses were expressed as the area under the response curve from 0 to 5 min and from 5 to 30 min for glucose perfusion, and from 0 to 5 min and from 5 to 20 min for arginine perfusion.

All the results were expressed as mean standard error (SEM). Statistical evaluation was performed employing the analysis of variance in comparing the various groups, and Dunnets test in comparing the differences between the treated and control groups.

RESULTS

Ethanol, acetate, and acetaldehyde effect on basal secretion of insulin and glucagon. Under glucose steady-state conditions (4.4 mM), perfusion with varying concentrations of ethanol, acetate, or acetaldehyde induced no significant variation in insulin and glucagon basal output.

FIGURE 1. Effect of ethanol at different concentrations (1, 10, and 30 mM) on insulin (IRI) output induced by glucose (11.1 mM) in isolated perfused pancreas of groups of six Sprague-Dawley rats (mean ± SEM). The continuous line shows the perfusions with ethanol, the dotted line without ethanol.



Ethanol effect on glucose-induced insulin secretion. Ethanol perfusion induced a reduction in glucose-induced insulin output both in the early and late secretion phases. The inhibitory effect appeared to be dose-related and was higher with 30 mM ethanol (Figure 1). Calculation of the areas of the first and second secretion phases confirmed this finding. Insulin output during the first 5 min of 11.1 mM glucose perfusion was 66 ± 2 ng/min in the presence of 1 mM ethanol, 52 ± 9 with 10 mM ethanol, and 27 ± 7 with 30 mM ethanol in contrast to 101 ± 22 ng/min in the absence of ethanol. During the second secretion phase (5–30 min), insulin output fell from 532 ± 118 in the presence of 11.1 mM glucose alone to 342 ± 70 with 1 mM ethanol, 343 ± 71 with 10 mM ethanol, and 261 ± 95 ng/min with 30 mM ethanol.

Ethanol effect on arginine-induced insulin and glucagon secretion. Ethanol (10 mM) reduced, though not significantly, the initial phase of arginine-induced insulin secretion. Moreover, this reduction was absent during perfusion with 30 mM ethanol. The second phase of insulin secretion did not seem to be affected by the presence of ethanol. No significant variation in the early and late glucagon output in the presence of 10 or 30 mM ethanol was observed (data not shown).

Acetate and acetaldehyde effect on glucose-induced insulin secretion. Acetate (1 mM) as well as acetaldehyde (10 mM) brought about a significant inhibition in both phases of glucose-induced (11.1 mM) insulin release (Figure 2). Evaluation of the integrated insulin output disclosed a significant inhibition in the perfusion with 1 mM acetate (23 ± 7 ng/min versus 101 ± 22 without, $P < 0.05$) during the first 5 min, and within 5–30 min (202 ± 66 ng/min versus 532 ± 118 without). Acetaldehyde findings were 37 ± 11 ng/min versus 101 ± 22 during the first 5 min and 178 ± 36 ng/min versus 532 ± 118 within 5–30 min; $P < 0.05$.

Acetate and acetaldehyde effect on arginine-induced insulin and glucagon secretion. Acetate (1 mM) and acetaldehyde (1 and 10 mM) induced a significant inhibition in arginine-induced insulin secretion, particularly in the first phase of secretion (Figures 3 and 4). The integrated values of hormone secretion emphasize this trend: arginine-induced insulin output was blunted during the first 5 min by acetaldehyde, 1 mM (35 ± 6 ng/min versus 166 ± 21, $P < 0.05$) and 10 mM (76 ± 32 ng/min versus 166 ± 21, $P < 0.05$), and 1 mM acetate (76 ± 25 ng/min versus 166 ± 21, $P < 0.05$).

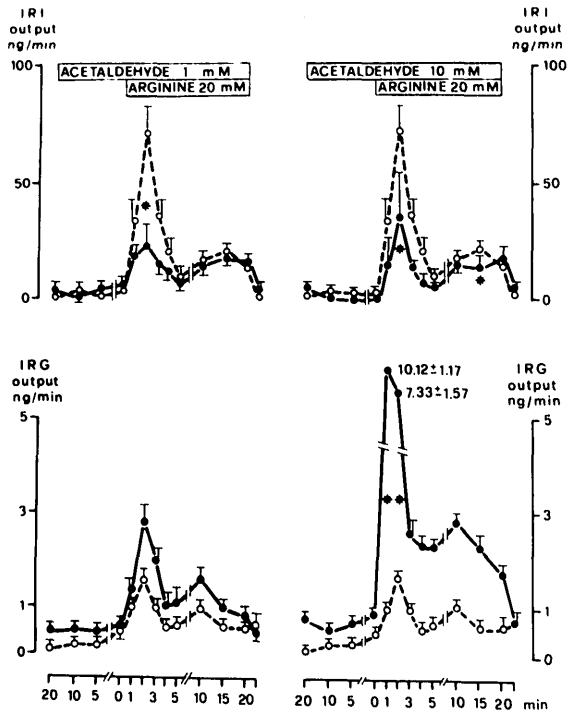


FIGURE 3. Effect of acetaldehyde (1 and 10 mM) on insulin (IRI) and glucagon (IRG) output induced by arginine (20 mM) in isolated perfused pancreas of groups of six Sprague-Dawley rats (mean ± SEM). The continuous line shows the perfusions with acetaldehyde, the dotted line without acetaldehyde. (*P < 0.05; **P < 0.01)

On the other hand, acetate and acetaldehyde at different concentrations had a stimulatory effect on glucagon secretion which was significantly increased in both the first and second secretion phase (Figures 3 and 4). The glucagon area in the first 5 min of perfusion was enhanced by 1 mM acetate (18 ± 2.7 ng/min versus 5 ± 0.6, $P < 0.05$) and by acetaldehyde, 1 mM (8 ± 0.4 ng/min) and 10 mM (24 ± 8 ng/min versus 5 ± 0.6, $P < 0.05$). The integrated glucagon secretion in the following 15 min, from 5 to 20 min of perfusion, was also higher during the 1-mM acetate infusion

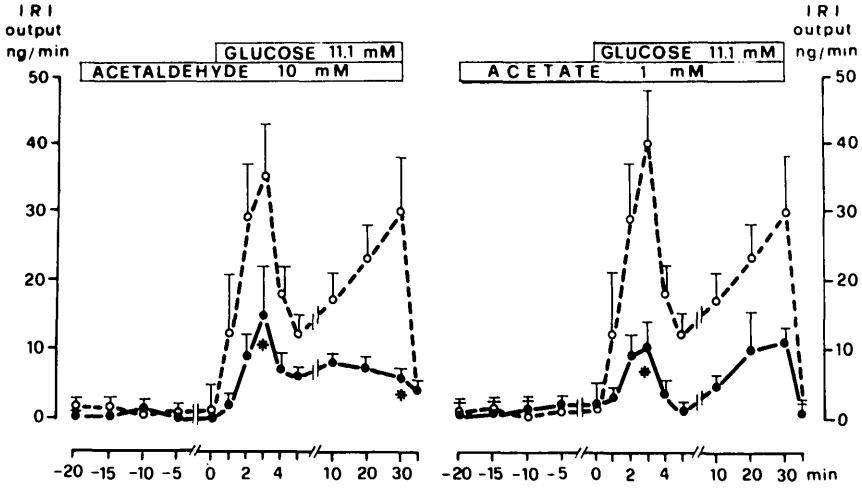


FIGURE 2. Effect of acetaldehyde (10 mM) and acetate (1 mM) on insulin (IRI) output induced by glucose (11.1 mM) in isolated perfused pancreas of groups of six Sprague-Dawley rats (mean ± SEM). The continuous line shows the perfusions with acetaldehyde and acetate, the dotted line without ethanol's metabolites. (*P < 0.05)

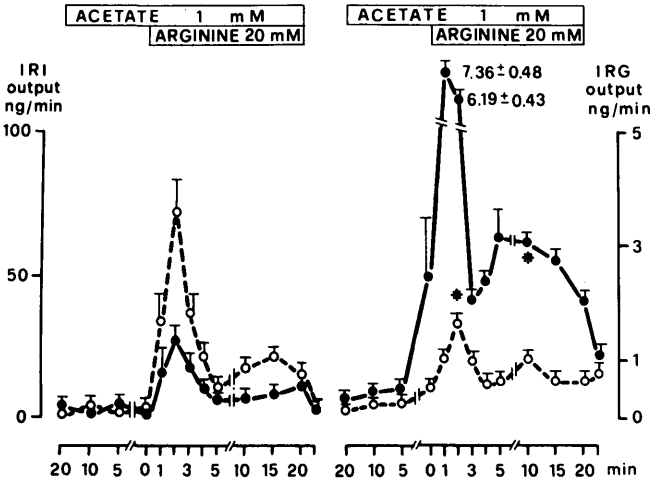


FIGURE 4. Effect of acetate (1 mM) on insulin (IRI) and glucagon (IRG) output induced by arginine (20 mM) in isolated perfused pancreas of groups of six Sprague-Dawley rats (mean ± SEM). The continuous line shows the perfusions with acetate, the dotted line without acetate. (*P < 0.05; **P < 0.01)

(37 ± 10 ng/min versus 11 ± 1.9) and acetaldehyde, 1 mM (19 ± 6 ng/min) and 10 mM (36 ± 9 ng/min versus 11 ± 1.9).

DISCUSSION

The results obtained show that, even though they do not exert a primary effect on pancreatic β- and α-cell spontaneous secretion, ethanol and its metabolities are capable of modifying their reactivity to secretogogue stimuli.

It has been suggested that inhibition of insulin secretion might be mediated in vivo by an excess of circulating catecholamines or by an ethanol-induced hypoglycemia. This inhibitory effect, in fact, was less pronounced in adrenalectomized rats[9] and after normalization of glucose levels.[10] The hyperglucagonemia observed in vivo following acute ethanol administration[8,9,11,12] has been attributed to concomitant glucopenia or to a direct or mediated ethanol effect.

While ethanol does not influence basal insulin secretion, it is capable of reducing glucose-induced secretion in vitro. This inhibitory effect seems to be dose-related, as shown in rabbit pancreas slices,[16] isolated rat islets,[17] and isolated canine pancreas;[21] this effect has been demonstrated in vivo in the pig.[22] Ethanol action on the β-cell might be mediated through its metabolites, acetaldehyde and acetate, which have shown a marked blocking effect on insulin secretion both by glucose and arginine. It is possible that the pancreatic islet cells contain alcohol-dehydrogenase, the enzyme which controls ethanol metabolism.[16] On the other hand, when alcohol-dehydrogenase is blocked by pyrazole, ethanol's inhibitory effect on insulin secretion persists. This inhibitory effect on glucose-induced insulin secretion by ethanol's metabolites has already been demonstrated for acetaldehyde.[16,17] However, acetate-induced suppression of insulin secretion in isolated rat pancreas is at variance with the findings of Patel and Singh[17] in isolated rat islets, Bivens and Feldman[16] in hamster pancreas (but not rabbit pancreas), and Shah et al. [6] in the intact rat. Employing a higher glucose concentration (16.7 mM) than was used in the present study (11.1 mM), these workers demonstrated that acetate exerted a stimulatory effect on glucose-induced insulin secretion. However, it should be noted that ethanol metabolism has not been delineated in rat islets, and different experimental conditions are found in the various protocols.

How ethanol and its metabolites block insulin secretion induced by secretogogue stimuli is not easily explained. The inhibitory effect may be exerted through a block in the active transport of Na^+ and K^+ [23] or a reduction in the plasma concentration of Ca^{2+} [24] involving the microtubular system[25] or the cAMP-dependent pathways of insulin secretion.[26] Ethanol and, above all, acetaldehyde could influence the β-cells by means an amino-like action by releasing pancreatic monoamines, which are potent inhibitors of insulin secretion.

The stimulating effect of acetaldehyde and acetate on α-cell reactivity to arginine stimulation emerges from our findings. This suggests that in vivo ethanol-related hyperglucagonemia might be directly dependent on the effect of its metabolites, and that glucopenia is not the only trigger during ethanol metabolism. There are no reports in this regard in the literature. Ethanol-induced hyperglucagonemia observed in the pig,[8] man,[11,12] and rat,[9] therefore, could be a primary effect related to the metabolism of ethanol itself, and not merely secondary to coexisting hypoglycemia. The stimulating effect in vitro of acetaldehyde and acetate on α-cell function as opposed to the inhibitory effect on the β-cell suggests a catecholamine effect through the stimulation of the β-adrenergic receptors of the α-cell. However, acetaldehyde could stimulate not only epinephrine release from the adrenal medulla but also norepinephrine from the sympathetic nerve endings.[27] The block in insulin secretion, as well as the reduction in the intra-islet concentration of insulin and the lessening in the normal relationship between α- and β-cells, could explain the hyperreactivity of the α-cells.[28] Ethanol's metabolites may influence arginine-induced glucagon secretion perturbing the intraislet redox state. As shown by Edwards and Taylor,[29] the reduction in substrate oxidation and energy production within the α-cell stimulates glucagon release.

The data obtained in vitro could account for the diabetogenic effect of ethanol seen in vivo. The reduced glucose tolerance observed following ethanol ingestion[30–33] could be due, at least in part, to a direct inhibition of pancreatic insulin secretion and direct stimulation of pancreatic glucagon by ethanol or its metabolites.

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