Short Communications

Reinterpretation of the Effect of Haloperidol and Ethanol on Insulin Secretion

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Summary. We were unable to confirm the report of haloperidol induced dose-dependent inhibition of insulin and glucagon release from the isolated canine pancreas. The possibility that the inhibition was caused by ethanol, previously used as the solvent for haloperidol, was tested. Infusion of ethanol at increasing concentrations (15.8 to 252 mmol/l) caused a progressive inhibition of insulin (-17 ± 1 to $-69 \pm 2\%$) and glucagon (-13 ± 3 to $-67 \pm 3\%$) secretion, using a perfusate containing 200 mg/dl glucose and 2.65 mmol/l calcium. Haloperidol (5 to 20 μ mol/l) dissolved in ethanol (252 mmol/l) did not augment the inhibitory effects of ethanol. At a low calcium concentration (1.3 mmol/l) ethanol further inhibited insulin secretion ($-83 \pm 2\%$) with no additional inhibition by 20 μ mol/l haloperidol ($-80 \pm 3\%$). At a high calcium concentration (8.8 mmol/l) the inhibitory effect of ethanol on insulin or glucagon secretions was diminished and variable. This strongly suggests that the inhibition of insulin and glucagon secretion previously attributed to haloperidol was caused by the ethanol solvent.

Key words: Ethanol, haloperidol, insulin, glucagon, isolated canine pancreas.

Haloperidol, a dopaminergic antagonist, has been reported to cause a dose-dependent inhibition of insulin and glucagon release from the isolated perfused canine pancreas [1]. However, during studies on the presence of dopaminergic receptors in pancreatic islets, we were unable to demonstrate any effect of haloperidol on insulin or glucagon secretion.

In the former study [1] haloperidol had been dissolved in ethanol and infused at a maximum final ethanol concentration of 1.16% w/v (252 mmol/l). We therefore investigated whether the ethanol may have been the cause of the proposed "haloperidol effects".

Materials and Methods

Mixed breed male German shepherd dogs, weighing 20–25 kg were used as pancreas donors after an 18 h fast. Methods used for the pancreatectomy and perfusion are described in detail elsewhere [2]. Throughout the study, 200 mg/dl glucose was used to enhance the "basal" insulin response. Calcium levels in the perfusion medium were maintained at 2.65 mmol/l except where specifically noted. In these cases, calcium concentrations were adjusted to 1.3 mmol/l or 8.8 mmol/l. A flow rate of 20 ml/min was used and remained constant throughout the experiments.

Ethanol, or haloperidol dissolved in ethanol (K. Hermansen, personal communication 1979), was infused by means of a syringe pump at a constant flow rate (0.25 ml/min) into the medium perfusing the pancreas. The concentrations of ethanol were adjusted to yield a final concentration of 15.8, 31.5, 63, 126 and 252 mmol/l. Haloperidol (a gift from McNeil, Inc.) was dissolved in ethanol to yield final concentrations of 5, 10 and 20 μ mol/l haloperidol in 252 mmol/l ethanol delivered to the pancreas.

Samples were taken at one minute intervals from the efflux throughout the experiments. Timing of sample collection was corrected for a 60 s lag period. Results were calculated as % change from baseline values. The hormone concentrations for five min preceding an infusion were averaged and used as a baseline concentration. Chemicals were infused for 10 min. Calculations are based on minutes 1–10. There was a thirty min interval between infusions to allow for the reestablishment of baseline values.

Insulin was measured by radioimmunoassay using a charcoal separation technique [3]. 125 I-insulin was purchased from New England Nuclear (Boston, Mass). Glucagon was measured by radioimmunoassay using a charcoal separation method, according to the instructions given to purchasers of 30-K antiserum (R. Unger, Dallas, Texas). 125 I-glucagon was purchased from Nuclear Medical Labs., Inc. (Dallas, Texas). Results are expressed as mean $\pm$ SEM.

Table 1. Effects of ethanol, haloperidol and calcium on insulin and glucagon secretion

	2.65 mmol/l calcium ^a						8.8 mmol/l calcium ^b			
Ethanol mmol/l	15.75	31.5	63	126	252	252	252	252	252	252
Haloperidol μ mol/l	—	—	—	—	—	5	10	20	—	20
Insulin % change $\pm$ SEM	-17 ± 1^c	-26 ± 2^c	-35 ± 3^d	-61 ± 3^d	-69 ± 2^d	-68 ± 2^d	-69 ± 3^d	-73 ± 3^d	4 ± 70^c	-59 ± 3^c
Glucagon % change $\pm$ SEM	-13 ± 3	-16 ± 4	-42 ± 12	-53 ± 18	-67 ± 3	-62 ± 6	-30 ± 7	-63 ± 12	-2 ± 12	54 ± 25

^a Pre-infusion insulin 2795 μ U/ml

glucagon 95 pg/ml

^b Pre-infusion insulin 6675 μ U/ml

glucagon 419 pg/ml

^c n = 3^d n = 6 **Fig. 1.** Effect of ethanol (63, 126, 252 mmol/l) and (20 μ mol/l) haloperidol in ethanol (252 mmol/l) on the secretion of insulin in a representative experiment. The glucose concentration was 200 mg/dl with 2.65 mmol/l calcium. Samples were at 1 min intervals. Ethanol and ethanol/haloperidol (Hal) were infused for 10 min. Thirty minute periods between infusions were used to reestablish baseline values or as a recovery period

Results

1. Effect of Ethanol and of Addition of Haloperidol on Secretion of Insulin and Glucagon

The infusion of ethanol at concentrations ranging from 15.8–252 mmol/l caused a progressive inhibition of insulin secretion with increasing concentrations of ethanol (Table 1). The addition of haloperidol at concentrations ranging from 5–20 μ mol/l in ethanol (252 mmol/l) did not influence the degree of inhibition of insulin secretion. Ethanol also inhibited glucagon release. In terms of percentage change, the glucagon results were more variable than those for insulin. Nevertheless, the trend of dose dependent inhibition of glucagon secretion by

ethanol, and the failure of haloperidol to further inhibit glucagon secretion, are evident.

Figure 1, a representative experiment, shows that after the cessation of infusion, ethanol causes an off-response which is very similar to that previously described for haloperidol [1]. The off-response is shown as a rapid elevation of insulin secretion after its inhibition by ethanol.

2. Effect of Ethanol upon the Secretion of Insulin and Glucagon at Low and High Calcium Concentrations

At the low calcium concentration, ethanol strongly inhibited insulin secretion ($-83 \pm 2\%$) with no additional inhibition by 20 μ mol/l haloperidol ($-80 \pm 3\%$). At high calcium concentrations the effects of

ethanol were variable, with stimulation of insulin release in 1 of 6 ethanol infusions, and stimulation of glucagon release in 2 of 6 ethanol infusions.

Discussion

In this study we were unable to show any effect of haloperidol, dissolved in ethanol, on islet hormone secretion. Because ethanol was such an effective inhibitor of insulin secretion, it is difficult to avoid the conclusion that the various inhibitory effects, previously attributed to the infusion of haloperidol into the canine pancreas, were caused by ethanol.

Ethanol inhibited insulin secretion more consistently than glucagon secretion in this study. However, the conditions chosen favoured elevated baseline insulin values (a perfusate glucose concentration of 200 mg/dl) and made it difficult to demonstrate A-cell inhibition because pre-ethanol glucagon levels were low. The inhibitory effect of ethanol on insulin secretion was influenced by calcium the concentration in the perfusate, being enhanced by a very low calcium concentration and less evident at a very high calcium concentration. This phenomenon was formerly attributed to "haloperidol interfering with calcium handling by the pancreatic B cells" [1]. Perhaps the ethanolic inhibition of glucagon and insulin release is caused by changes in calcium-dependent events in cell membranes (structure) or microtubules (paralysis). The dose dependent nature of the inhibition of insulin secretion by ethanol confirms other *in vitro* studies [4, 5, 6].

High strength organic acids are an alternative solvent system for haloperidol. When acetic acid was used, haloperidol caused an increase in insulin release which was due to the acetate ion (unpublished observations). Other *in vitro* studies have failed to show any effect of haloperidol on insulin secretion [5]. However, haloperidol is notoriously

insoluble in aqueous solutions at neutral pH, and in studies which fail to mention solubilization methods, the absence of any effect is difficult to interpret. Studies utilizing ethanol or high concentrations of organic acids as solvents must be reexamined for the effect of the solvent on islet hormone secretions. We suggest that the inhibition of islet hormone secretion attributed to haloperidol [1] was caused by ethanol.

Acknowledgement. This study was supported by the Veterans Administration.

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Received: November 23, 1979,
and in revised form: February 5, 1980

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