Haloperidol, a Dopaminergic Antagonist: Somatostatin-Like Inhibition of Glucagon and Insulin Release from the Isolated, Perfused Canine Pancreas*

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Summary. The effect of haloperidol, a dopaminergic antagonist, on insulin and glucagon secretion was investigated using the isolated, perfused canine pancreas. Haloperidol at 4×10^{-7} to 10^{-5} mol/l caused a dose-dependent inhibition of glucagon release both at low (25 mg/100 ml) and high glucose concentrations (150 mg/100 ml). At the low glucose concentration insulin release was already maximally suppressed. At the high glucose concentration haloperidol (4×10^{-7} to 10^{-5} mol/l) also caused a dose-dependent inhibition of insulin release. Haloperidol (10^{-5} mol/l) inhibited dramatically pancreatic A and B cell responses to isoproterenol (2 ng/ml), acetylcholine ($1 \mu\text{mol/l}$) and arginine (5 mmol/l). The inhibitory effect of haloperidol on both glucagon and insulin release could be eliminated by increasing perfusate calcium concentration from 1.3 to 8.8 mmol/l. These findings suggested that haloperidol blocks glucagon and insulin release in a somatostatin-like manner by affecting a fundamental step of the stimulus-secretion coupling, probably by interfering with calcium handling of the pancreatic A and B cells.

Key words: Isolated, perfused canine pancreas, haloperidol, acetylcholine, isoproterenol, arginine, calcium, somatostatin-like inhibition, insulin secretion, glucagon secretion.

Falck and Hellman [1, 2] have demonstrated that in many species the endocrine pancreatic cells contain monoamines, particularly serotonin and dopamine [3, 4]. The physiological significance of these monoamines stored within the islet cells is unknown.

During investigations on monoaminergic mechanisms in pancreatic islets we employed the dopaminergic antagonist haloperidol, which is purported to be a specific dopamine receptor blocking agent in tissues such as brain [5], exocrine pancreas [6] and renal and mesenteric vessels [7]. Using the isolated, perfused canine pancreas it was noted that this dopaminergic antagonist inhibited release of both glucagon and insulin.

The purpose of the present study was to investigate further the effect of haloperidol on insulin and glucagon secretion from the isolated, perfused canine pancreas in order to elucidate the mechanisms by which this substance produces its bihormonal inhibitory actions.

Material and Methods

Mongrel dogs, fasted overnight, weighing 18–25 kg were used as pancreas donors. The technique for isolation of the canine pancreas and the perfusion system have been described in detail by Iversen [8, 9]. In brief, the preparation consisted of the pancreas and the proximal 10 cm of the attached duodenum. A nonrecirculating medium consisting of a Krebs Ringer bicarbonate buffer containing dextran 40 g/l (MW 75,000), 2 g/l bovine albumin, glutamate, fumarate and pyruvate, each at a concentration of 5 mmol/l was introduced through the splenic and the coeliac arteries, and the total portal effluent was collected every 1 min. Oxygenation of the Krebs-Ringer bicarbonate buffer was achieved by means of a rotating roller screen in an atmosphere of 94.4 per cent O_2 and 5.6 per cent CO_2 . Oxygen consumption has been measured in some experiments and was found to be constant during the perfusions around 0.005 ml/min/g tissue. PH of the perfusion fluid was constant at 7.4 throughout the experiments.

The perfusion pressure was 30–40 mmHg and the perfusion flow was 18–20 ml/min. Both parameters were constantly monitored and remained unchanged. The basic perfusion medium was composed with a calcium concentration of 1.3 mmol/l. CaCl_2 was added to result in final concentrations of 6.3 and 8.8 mmol/l. The chemicals employed and their sources were as follows: Haloperidol (4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4-fluorobutyrophenone) (a gift from Janssen Pharma, Copenhagen,

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Fig. 1. Effect of Haloperidol at concentrations of 4×10^{-7} mol/l (H I), 2×10^{-6} mol/l (H II) and 10^{-5} mol/l (H III) upon the secretion of glucagon and insulin during perfusion with low (25 mg/100 ml) and high (150 mg/100 ml) glucose concentrations in a representative experiment. The calcium concentration was 1.3 mmol/l

Denmark); 1-arginine hydrochloride (Sigma Chemical Comp., St. Louis, U.S.A.); 1-isoproterenol (Sigma Chemical Comp., St. Louis, U.S.A.); and acetylcholine (Sigma Chemical Comp., St. Louis, U.S.A.).

Glucagon and insulin were measured by a single antibody radioimmunoassay (10). In the glucagon assay an antiserum highly specific to pancreatic glucagon (K 964, a gift from Lise Heding, NOVO Research Lab., Copenhagen) was used. The sensitivity of the glucagon assay allowed differences in concentration of 10 pg/ml to be distinguished with 95 per cent confidence. The separation

of free from bound hormone was performed by wick-chromatography [10]. At the concentrations tested Haloperidol did not interfere in the radioimmunoassay.

Statistical analysis were made by Student's *t* test for paired comparisons.

Results

1. Effect of Haloperidol upon the Secretion of Insulin and Glucagon at Low (25 mg/100 ml) and at High (150 mg/100 ml) Concentrations of Glucose

In order to determine the effects of Haloperidol insulin and glucagon release in response to low (25 mg/100 ml) and high (150 mg/100 ml) glucose concentrations was studied during sequential 10 min infusions of Haloperidol at various concentrations. A representative experiment is shown in Figure 1. In the basal state with a calcium concentration of 1.3 mmol/l and a glucose concentration of 25 mg/100 ml insulin secretion was unmeasurable, while glucagon release was high. Within 1–2 min of introduction of Haloperidol at 3 different concentrations (4×10^{-7} , 2×10^{-6} and 10^{-5} mol/l), glucagon release was inhibited in a dose-dependent manner. At this low glucose concentration no further inhibition of insulin release could be detected. When the glucose concentration in the perfusing medium was increased from 25 to 150 mg/100 ml insulin release followed the typical biphasic response pattern and glucagon release was suppressed as expected. Infusions of Haloperidol for 10 min at the same concentrations as used before now caused a dose-dependent inhibition of both insulin and glucagon release. Upon removal of Haloperidol from the perfusate the hormone secretion after a short off-response attained preinhibition values. Data from 3 experiments demonstrating the clearcut dose-dependent inhibition of Haloperidol on insulin and glucagon secretion are shown in Figure 2. The relative inhibitory effect of Haloperidol on glucagon secretion is the same at low and high glucose concentrations. Identical results were obtained when Haloperidol was infused in decreasing concentrations.

2. Effect of Haloperidol upon Insulin and Glucagon Responses to Arginine, Isoproterenol, and Acetylcholine

The purpose of the following experiments was to determine whether Haloperidol inhibited pancreatic A and B cell responses to stimuli such as arginine (5 mmol/l), isoproterenol (2 ng/ml), and acetylcholine (1 µmol/l). These experiments were carried out with a glucose concentration of 75 mg/100 ml

Fig. 2. Dose-dependent inhibition of insulin and glucagon release with Haloperidol in three perfusion experiments indicated by three different symbols. The mean $\pm$ SD of the preinfusion levels from each experiment is shown above the 'zero point' on the logarithmic abscissa. The symbols on the curves represent mean values of the 5 last minutes hormone release during addition of various concentrations of Haloperidol

and at a calcium concentration of 1.3 mmol/l. The stimuli mentioned were infused for periods of 30 minutes. After 15 minutes of stimulation Haloperidol at a concentration of 10^{-5} mol/l was added for 8 minutes.

As can be seen in Figure 3 (left part) illustrating a representative experiment, Haloperidol powerfully suppressed the stimulated insulin and glucagon release. Upon termination of the Haloperidol infusion the concentration of hormones returned to control values after a short overshoot. Hormone release fell to prestimulatory levels when the infusions of secretagogues were stopped. Figure 4 shows the mean results $\pm$ SEM from 5 experiments. The percentage hormone release during Haloperidol infusion is calculated on the basis of total glucagon and insulin release in the presence of the stimulus.

3. Effect of Haloperidol upon the Secretion of Insulin and Glucagon at High (6.3 mmol/l and 8.8 mmol/l) Calcium Concentrations

To investigate a possible Haloperidol/calcium interrelationship for insulin and glucagon release a similar protocol was used. At a glucose concentration of 75 mg/100 ml both hormones were stimulated when calcium concentration was increased from 1.3 to 8.8 mmol/l (Fig. 3, right part). At this high calcium concentration no significant inhibition of either insulin or glucagon were obtained during Haloperidol infusion. A short-lived off-response appeared after withdrawal of Haloperidol possibly due to oscillations in calcium influx. Similarly, in the presence of a calcium concentration of 6.3 mmol/l (Fig. 4) Haloperidol was unable to inhibit glucagon secretion sig-

Fig. 3. Left part: Effect of Haloperidol (H) (10^{-5} mol/l) on arginine (5 mmol/l) stimulated insulin and glucagon secretion in the presence of a glucose concentration of 75 mg/100 ml. A similar pattern was seen when isoproterenol and acetylcholine were given. Right part: Effect of Haloperidol (H) (10^{-5} mol/l) on insulin and glucagon secretion stimulated by high calcium (8.8 mmol/l). The glucose concentration was 75 mg/100 ml. A representative experiment out of five

Fig. 4. A comparison of the inhibitory effect of Haloperidol (10^{-5} mol/l) on secretion of insulin and glucagon when stimulated by 1 μ mol/l acetylcholine (ACH: 23% and 45%), 2 ng/ml isoproterenol (ISO: 22% and 27%), 5 mmol/l arginine (ARG: 18% and 17%), 6.3 mmol/l calcium (CA I: 47% and 90%) and 8.8 mmol/l calcium (CA II: 93% and 94%). The glucose concentration was 75 mg/100 ml. Means of hormone release values during the last 5 min of the 8 min Haloperidol infusion are expressed in per cent of means of the levels during the 5 minutes preceding addition of the inhibitor. Insulin is depicted as open and glucagon as hatched columns. SEM of 5 experiments are shown as bars. a and b represent statistical significant inhibition of the stimulated hormone release. a. $2 p < 0.05$ and b. $2 p < 0.001$

nificantly. However, at 6.3 mmol/l calcium concentration insulin secretion was decreased somewhat by addition of Haloperidol ($2 p < 0.05$).

Discussion

In this study it has been shown that: 1) Haloperidol inhibits the release of insulin and glucagon dose-dependently, and this is an immediate and reversible phenomenon; 2) Haloperidol inhibits release of both hormones when stimulated by isoproterenol, acetylcholine and arginine; and 3) the inhibitory action of haloperidol is counteracted by an increase in calcium concentration.

Previous studies have shown that dopamine inhibits insulin release [11] and as we ourselves had found that Haloperidol exaggerates the dopamine induced inhibition of insulin secretion (unpublished results) it seemed unlikely that Haloperidol should act as a dopaminergic antagonist in the endocrine pancreas.

However, Feldman et al. [12] have reported results which are at variance with our data with respect to the effect of Haloperidol on insulin release. They did not find any effect on glucose stimulated insulin release at a Haloperidol concentration of 10^{-4} mol/l. The reason for the divergence in results may be due to lesser responsiveness of their preparation consisting of slices of rabbit pancreas.

However, a species difference or a difference in calcium levels in the media might be an alternative explanation. There are no further reports on the influence of Haloperidol on hormone release from the pancreas in vitro.

The present data indicate that calcium antagonises the inhibitory effect of Haloperidol on insulin and glucagon secretion. These findings are consistent with the observations of Seeman et al. [13] who found that Haloperidol inhibits neurosecretion and that the inhibition of the neurosecretion could be elevated by high calcium concentrations. Like other potent neuroleptics which are very lipophilic, Haloperidol binds reversibly with various membrane structures, expands membranes and competes with calcium for binding sites on the cell membrane in muscles and nerves [14]. It is tempting to postulate that Haloperidol acts directly on the endocrine pancreatic islet cells by displacing membrane-bound calcium in a manner analogous to its effect in muscle and nerve. This could explain in part the inhibitory effect of Haloperidol on hormone secretion from the endocrine pancreas since calcium is known to play a key-role in the secretion of insulin [15, 16] and glucagon [17, 18]. The dissociation between the inhibitory effect of Haloperidol on calcium-stimulated insulin and glucagon secretion observed in this report possibly represents a quantitative difference in the sensitivity of the pancreatic A and B cells to Haloperidol.

Using the isolated rat soleus muscle [19] Clausen (personal communication) found that Haloperidol is also a potent inhibitor of sodium flux across the cell membrane resulting in a decreased intracellular sodium concentration. Thus the decreased pancreatic hormone release observed during Haloperidol infusion may be ascribed partly to a decreased intracellular sodium concentration which secondarily leads to a fall in cytosolic calcium [20].

That the effect of calcium on Haloperidol action is not merely a function of generally increased secretion of insulin and glucagon is supported by the findings that other secretagogues for insulin and glucagon such as isoproterenol, arginine, and acetylcholine fail to overcome the inhibitory effect of Haloperidol.

It is of interest to note that Haloperidol influences the pancreatic hormone secretion in a somatostatin-like manner. The inhibitory action of somatostatin is also not restricted to basal conditions but includes pancreatic hormone secretion evoked by arginine [21, 22], isoproterenol [21], and acetylcholine [23]. Further, it has been observed that increasing calcium concentration counteracts the inhibitory effect of somatostatin [22, 24, 25]. Whether somatostatin and Haloperidol exert their

inhibitory action at the same step in the secretion process remains, however, unclear.

In conclusion, it is suggested that Haloperidol inhibits insulin and glucagon release by blocking a step in the stimulus-secretion coupling probably by interfering directly and/or indirectly with calcium handling by pancreatic A and B cells.

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