DIAZEPAM: IN VITRO EFFECTS ON GLUCAGON AND INSULIN RELEASE

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SUMMARY: Diazepam suppressed arginine-induced glucagon release from the isolated perfused rat pancreas in a dose-dependent manner, with an IC_{50} of approximately 65 μM. In contrast, insulin release was enhanced by 10-50 μM diazepam, but inhibited by higher concentrations of drug. Thus, 50 μM diazepam simultaneously suppressed glucagon and increased insulin release in this model. The potentiation of insulin release may result from phosphodiesterase inhibition. The inhibitory effects on hormone release are discussed in terms of diazepam's molecular conformation, which is similar to that of diphenylhydantoin, an inhibitor of both glucagon and insulin release in the isolated perfused rat pancreas. The possibility is also considered that the conformation of both compounds might be similar to the apparent active site of the hormone release inhibitor somatostatin.

The hyperglycemia associated with the diabetic state may be due to hyperglucagonemia as well as to insulin deficiency (1). Therefore, agents capable of suppressing glucagon secretion may have potential therapeutic value in the treatment of diabetes. Somatostatin (somatotropin release inhibiting factor, SRIF) has generated considerable interest as such an agent (2). However, its use, and even its pharmacological and clinical evaluation, are limited by its short biological half-life, lack of selectivity for glucagon suppression, and the necessity for parenteral administration. Many analogs of SRIF have been synthesized (3), but none thus far are devoid of these problems. It therefore seems that effort should also be directed toward nonpeptide molecules as potential orally effective, long-acting, selective glucagon suppressants. That nonpeptides can mimic SRIF, at least functionally, seems to have been demonstrated by recent reports that diphenylhydantoin (DPH) blocked both arginine-induced insulin and glucagon secretion from the isolated perfused pancreas (4,5).

Diazepam, like DPH, is a potent anticonvulsant agent. Although the chemical structures of these two drugs appear unrelated, Camerman and Camerman reported that their 3-dimensional conformations, as seen in CPK models constructed on the basis of x-ray crystallographic data, are closely related and may explain their similar anticonvulsant properties (6,7).

Consequently, we tested diazepam in the isolated perfused rat pancreas and the results are reported in this communication.

MATERIALS AND METHODS

Our isolated perfused rat pancreas model combined a surgical procedure modified from that described by Weir et al. (8) and a perfusion apparatus similar to that of Grodsky and Fanska (9). The pancreas was totally isolated with only a 5-cm section of duodenum remaining attached. The perfusion medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4% dextran T-70 and 0.2% bovine serum albumin, fraction V; it was constantly stirred under an atmosphere of 95% O_2 - 5% CO_2. Glucose, arginine, and other test substances were added to this buffer as required; the perfusion medium was changed rapidly by use of a 5-way stopcock. The flow rate was maintained at 4.5-5 ml/min using a Buchler Polystaltic Pump; the temperature was 38°C; the perfusion pressure was 30-40 mm Hg. Perfusate was collected at 60 or 30-sec intervals in tubes containing 25 µl of 15% disodium EDTA, which were held in a rack partially immersed in a slurry of ice.

Basal glucose (80 mg %) was perfused for 20 min preceding the start of each experiment and then 60-sec fractions were collected for 5 min to monitor basal levels of glucagon and insulin secretion. Arginine (20 mM) in buffer containing glucose (80 mg %) served as secretagogue. Diazepam, when present, was dissolved in the same buffer containing arginine and glucose. The intervals of perfusion with each buffer and the concentration of diazepam used are shown in Fig. 1.

Insulin and glucagon were determined in each fraction by radioimmunoassay. After completion of each perfusion, aliquots for the glucagon assays were pipetted into tubes containing 100 µl of Trasylol. These tubes, together with the remaining fractions of perfusate, were stored at -20°C until assayed.

Glucagon was measured according to the procedure from Unger's laboratory (10) using their 30K antibody and [^{125}I]glucagon from Cambridge Nuclear Corp. Porcine glucagon from Eli Lilly was used as standard. Antibody-bound glucagon was separated from free glucagon by precipitation with polyethylene glycol, using 100 µl of lamb serum as carrier protein, according to the procedure of Desbuquois and Aurbach (11); the pellets were counted in a gamma counter.

Insulin was measured by a modification of the procedure of Wright et al. (12) using antibody to bovine insulin from Miles Laboratories, Inc., and [^{125}I]insulin from Cambridge Nuclear Corp. Rat insulin, purchased from Novo Research Institute, Copenhagen, Denmark, was used as standard.

Trasylol was obtained from FBA Pharmaceuticals, dextran T-70 from Pharmacia Fine Chemicals, Inc., polyethylene glycol (Carbowax 6000) from Fisher Chemical Company; normal lamb and guinea pig serums from Grand Island Biological Co., and Albumisol (5%) from Merck, Sharp & Dohme. Bovine serum albumin, fraction V, was purchased from Armour Pharmaceutical Co.; it was purified by passage of a 30% solution through a column of Amberlite MB-3, an ion exchange resin obtained from Mallinckrodt. Portions of purified albumin were stored frozen until used in the preparation of buffer for each experiment.

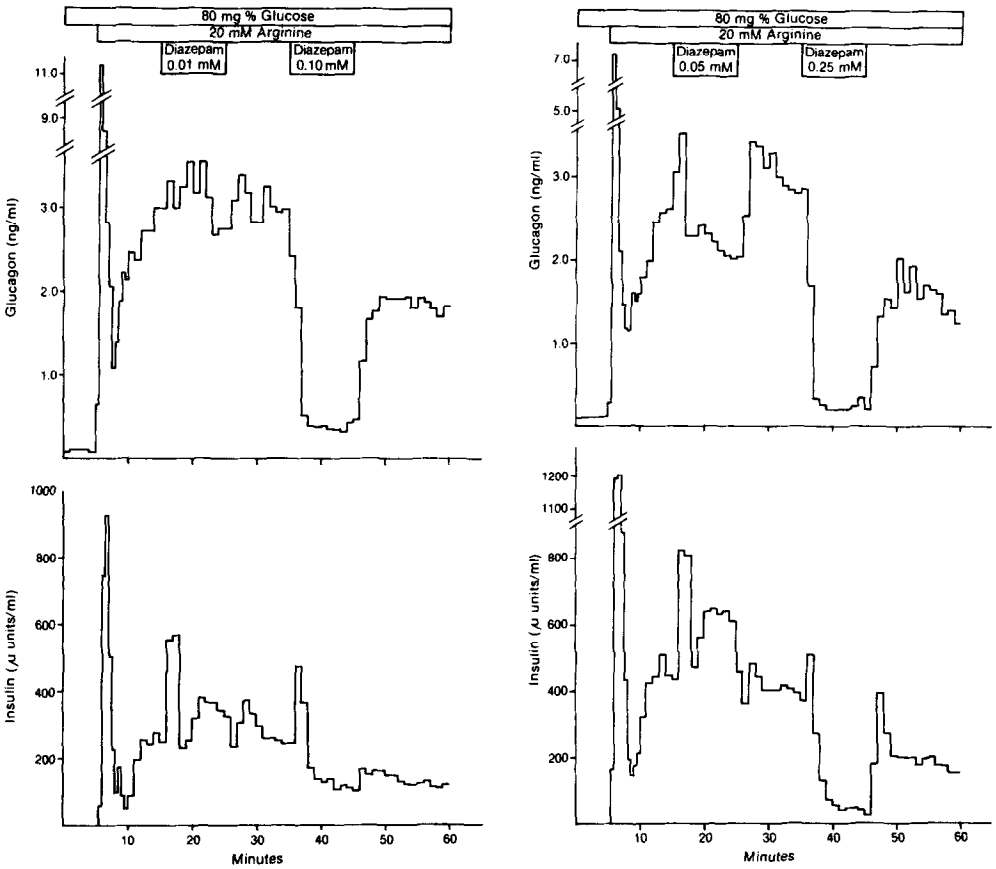

Fig. 1. Effect of varying concentrations of diazepam on the release of glucagon and insulin from the isolated perfused rat pancreas. The procedures used are described under "Materials and Methods". One preparation was used to test two concentrations of diazepam. The data shown are the average of three experiments for each pair of concentrations; the secretory patterns appeared very similar in each experiment.

RESULTS AND DISCUSSION

Diazepam modified both arginine-induced glucagon and insulin secretion from the isolated perfused rat pancreas. Fig. 1 illustrates the biphasic release of glucagon and insulin produced by arginine and the effects of diazepam on the second phase of secretion. These effects were quantified further, as shown in Table I.

It is seen that diazepam suppressed arginine-induced glucagon release in a dose-dependent manner; the IC_{50} was approximately 65 μM. In

Table I. Effect of Diazepam on the Release of Glucagon and Insulin from the Isolated Perfused Rat Pancreas

Diazepam Concentration	Hormone Release	
	Glucagon	Insulin
μM	Percent of control	
10	99 ± 4	139 ± 14
50	65 ± 2	130 ± 11
100	22 ± 2	94 ± 13
250	18 ± 4	33 ± 3

Data are derived from the experiments shown in Fig. 1, using the area under the curve as a measure of hormone secretion. Control release was obtained via interpolation of hormone secretion before and after perfusion with diazepam.

contrast, arginine-induced insulin release was enhanced at 10 and 50 μM diazepam, but inhibited at higher concentrations, although even at the higher concentrations a transient burst of insulin release was always observed prior to inhibition (see Fig. 1). At 50 μM diazepam, glucagon release was markedly suppressed, whereas insulin release was appreciably increased. Responses were seen immediately with diazepam; upon discontinuation of its perfusion, the secretion rate rapidly returned to that expected without drug.

Although glucagon suppression combined with insulin elevation was observed at only one concentration of diazepam, these studies demonstrate that a drug can elicit opposing effects on pancreatic hormone secretion in the desired direction for a potential antidiabetic agent. Further, a non-peptide molecule is more likely to be orally effective and of longer duration of action than SRIF. However, the clinical use of diazepam itself is unlikely to affect pancreatic hormone release, since the concentrations required for such effects are more than 10-fold greater than the blood levels reached after chronic administration of massive doses of drug (13).

The suppression of both glucagon and insulin release by diazepam, observed at concentrations greater than 0.1 mM, is similar to the inhibitory patterns seen with DPH and SRIF (5,14). However, the latter two agents did not enhance insulin release under any conditions tested. The stimulation of insulin secretion by diazepam may result from its ability

to inhibit phosphodiesterase (15). At 0.05 mM, diazepam inhibited the phosphodiesterase of rat pancreas, whereas SRIF (1 μg/ml) and DPH (5 mM) had no effects (16). Inhibition of beta cell phosphodiesterase could increase the intracellular concentration of cyclic AMP, and thereby potentiate insulin release. This mechanism has been proposed to explain similar effects seen with theophylline and other phosphodiesterase inhibitors, including tolbutamide (17).

The similarity between diazepam and DPH in their inhibitory effects on hormone secretion may be a consequence of their similar molecular conformations, as proposed by Camerman and Camerman to explain their anticonvulsant properties (6,7). Furthermore, a similar mechanism might underlie both the anticonvulsant and the antisecretory effects of these drugs, perhaps via interaction with a membrane receptor which controls ion transport. Therefore, it is of interest that SRIF also was reported to exhibit anticonvulsant activity (18).

A tentative molecular conformation for SRIF has recently been proposed by Holladay and Puett, based on physico-chemical studies (19). They proposed that SRIF exists in solution as a hairpin loop around the Trp^8 residue, and is held in this position by the disulfide bond between Cys^3 and Cys^{14}; the ellipsoid shape is further stabilized by three intramolecular hydrogen bonds. The aromatic ring moieties of Trp^8 and of the Phe residues in positions 6, 7, and 11 create a hydrophobic domain at one end of the molecule, which appears to include the active site for possible receptor interaction. Molecular models suggest the possibility that the two aromatic rings of diazepam and DPH might be coincident with residues in the hydrophobic region at the hairpin of the proposed SRIF conformation. Thus, diazepam and DPH might interact with a membrane receptor for SRIF on the membranes of α and β cells. Alternatively, these drugs might elicit their effects on hormone secretion by stimulating the release of SRIF from the delta cells of islets, where its presence has been demonstrated (20).

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