Effect of cisapride on gastrin releasing peptide(14-27)-stimulated gastrin and somatostatin release from the isolated perfused rat stomach

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ABSTRACT

The purpose of this study was to examine the effect of cisapride on gastrin releasing peptide(14-27)-stimulated release of somatostatin and gastrin from the isolated perfused rat stomach. The results show that cisapride can inhibit somatostatin release, but does not affect gastrin release.

Cisapride is a pharmacologic agent that affects gastrointestinal motility in both experimental animals and humans (1, 3, 7, 8). Cisapride can enhance gastric emptying and antagonize dopamine-induced slowing of gastric emptying. Cisapride is also thought to stimulate the release of acetylcholine (3). Acute treatment with cisapride causes release of PP and CCK in humans (4, 5). The effects of cisapride on release of somatostatin from the stomach are not known. The objective of this study therefore is to examine the effect of cisapride on basal and gastrin releasing peptide (GRP)-induced gastric somatostatin release from the isolated perfused rat stomach.

Peptides and drug pGRP(14-27), the active fragment of porcine GRP (pGRP), was prepared by a conventional method of peptide synthesis (6) and its purity was assessed extensively by routine analytical criteria. Cisapride was supplied by Jansen Pharmaceuticals (Belgium). In control experiments, the cisapride solvent (CC15FIC), consisting of distilled water (1.0 ml) with 25 mg of mannitol and 8.0 mg of glacial acetic acid, was used.

pGRP(14-27) was dissolved in saline containing 0.2% bovine serum albumin. Both

agents were infused by a compact syringe pump at a rate of 0.1 ml/min.

Perfusion of rat stomach The stomach was isolated from the 24-h fasted male Wistar rats weighing 200-250 g under pentobarbital anesthesia (50 mg/kg, i.p.) by the method of Chiba *et al.* (2). Polyethylene cannulae were inserted into the left gastric artery and vein. All other vessels were ligated and the entire pancreas was excluded by ligation. After the cardia was ligated, a catheter was inserted into the stomach through the pyloric ring to drain gastric juice. Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% dextran (Meito Sangyo, Nagoya), 5.5 mM glucose, and 0.2% bovine serum albumin (perfusate) was infused into the left gastric artery at a rate of 2.0 ml/min without recirculation. The perfusate was equilibrated immediately before use with 95% O₂/5% CO₂. Following a pre-perfusion period (20 min) where the perfusate alone was infused, pGRP(14-27) was infused at a rate of 0.1 ml/min with or without cisapride into the gastric artery for 20 min using a Harvard compact syringe pump (Harvard Apparatus, Model 975, U.S.A.). Perfusates were collected and frozen for subsequent measurement of

Fig. 1 Effect of vehicle CC15FIC (0.1%) on GRP(14-27)-stimulated changes in IR-gastrin and IR-somatostatin release in isolated perfused rat stomach

gastrin and somatostatin levels by radioimmunoassay. The effluent was collected from the portal vein cannula at 2-min periods into chilled tubes containing aprotinin (1,000 KIE), frozen immediately, and stored at -20°C until assayed.

Radioimmunoassays Immunoreactive gastrin and somatostatin (SRIF) levels were measured using specific and sensitive radioimmunoassays. Radioimmunoassay for gastrin was carried out in a manner described previously (9). Radioimmunoassay for SRIF was done as described earlier, but with modifications (10). Briefly, the tracer was ^{125}I - α -N-Tyr-SRIF, and the SRIF antiserum (OAL-272, Otsuka Assay Laboratory, Tokushima) was used at a final dilution of 1:60,000. Synthetic SRIF (prepared in this laboratory) was used as standard. The standard buffer consisted of 0.1 M histidine (pH 6.5) containing 70 mM EDTA, 0.2% NaN_3 , and 0.1% gelatin. Bound and free radiolabeled ligands were separated by the dextran-coated charcoal method. Radioactivity contained in the supernatant (bound form) was counted in a gamma counter.

Fig. 2 Effect of cisapride (10^{-9} M) on GRP(14-27)-stimulated changes in IR-gastrin and IR-somatostatin release in isolated perfused rat stomach

Fig. 3 Effect of cisapride (10^{-7} M) on GRP(14-27)-stimulated changes in IR-gastrin and IR-somatostatin release in isolated perfused rat stomach

All results are expressed as mean $\pm$ SE, and differences were analyzed by Student's *t*-test.

Experiment 1 In the control study, basal gastrin (50–100 pg/min) and SRIF (300–400 pg/ml) levels did not change significantly ($P > 0.05$) during the infusion with the perfusate alone. Infusion of pGRP(14-27) at 10^{-7} M, caused a biphasic release of gastrin, with an initial peak for 10 min (maximum, 800% of the basal secretion) followed by a second phase of sustained gastrin secretion. pGRP(14-27) stimulated a prompt release of SRIF (about 300% of the basal), followed by a gradual decrease.

Experiment 2 Perfusion of the cisapride solvent alone at a final concentration of 0.1%, did not affect basal gastrin and SRIF secretion while it enhanced pGRP(14-27)-stimulated SRIF release significantly (Fig. 1). The solvent did not affect pGRP(14-27)-induced gastrin release. Cisapride (10^{-9} M– 10^{-5} M) did not

Fig. 4 Effect of cisapride (10^{-5} M) on GRP(14-27)-stimulated changes in IR-gastrin and IR-somatostatin release in isolated perfused rat stomach

affect basal and pGRP(14-27)-stimulated gastrin release (data not shown). The total amounts of gastrin release during the administration of various doses of cisapride were $96.6 \pm 18.8\%$ (10^{-9} M), $106.5 \pm 20.4\%$ (10^{-7} M), and $114.7 \pm 21.4\%$ (10^{-5} M) of the control. Cisapride at 10^{-9} M did not affect SRIF release ($104.4 \pm 14.2\%$ of control group) (Fig. 2). In contrast, 10^{-7} M cisapride suppressed pGRP(14-27)-stimulated SRIF release ($69.3 \pm 10.3\%$), although it did not affect basal SRIF release (Fig. 3). Cisapride at 10^{-5} M suppressed pGRP(14-27)-stimulated SRIF release ($64.8 \pm 12.7\%$) significantly (Fig. 4).

The present study indicates that cisapride does not affect basal release of gastrin or SRIF from the isolated-perfused rat stomach, whereas it can inhibit GRP-stimulated release of SRIF significantly. Interestingly, the cisapride solvent, CC15FIC, enhanced GRP-stimulated release of SRIF significantly. The present observations agree with previous reports regarding the effects of cisapride on gastrin release (4, 5). These reports have indicated that cisapride does not affect gastrin, but the effects of cisapride on SRIF release have not been known.

Since the cisapride solvent can augment GRP-stimulated release of SRIF, the inhibitory effect of cisapride may be more dramatic.

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