--Original Article--

IN VITRO AND IN VIVO EFFECT OF PROGLUMIDE ON CHOLECYSTOKININ-STIMULATED AMYLASE RELEASE IN MOUSE PANCREATIC ACINI

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Summary

The effect of proglumide on cholecystokinin (CCK)-stimulated amylase release was studied in vitro and in vivo in dispersed acini from mouse pancreas. In an in vitro study, proglumide at concentrations between 0.3-10 mM inhibited CCK-stimulated amylase release dose-dependently, while proglumide did not influence the basal amylase release at concentrations between 0-3 mM. Dose-response curves to CCK for amylase release shifted to the right with increase in proglumide concentration. This inhibition by proglumide was reversible. In addition, the effect of proglumide was selective for CCK and its related peptide, and this drug did not inhibit other secretagogues such as carbachol or gastrin releasing peptide in mouse acini.

In contrast to its inhibitory effect in vitro, in vivo administration of proglumide (500 mg/kg/day, i.p., for 5 days) to mice did not cause the rightward shift of the dose-response curve to CCK for amylase release from dispersed acini.

Key Words: cholecystokinin, amylase release, proglumide, mouse pancreatic acini.

Introduction

Proglumide, an anti-gastrin drug\(^1,2\), has been widely used in both Europe and Japan for treatment of gastric ulcer\(^3\). Cholecystokinin (CCK) has a similar amino acid sequence in the c-terminal portion to gastrin, and the effects of proglumide on the actions of CCK and its analogues have been reported in several animals and in various tissues\(^4-6\).

Mantovani et al.\(^4\) reported that proglumide inhibits caerulein-stimulated pancreatic secretion and gallbladder contraction in a guinea pig. Hahne et al.\(^5\) reported that proglumide inhibits CCK-stimulated amylase secretion in direct proportion to the inhibition of \(^12\)I-CCK binding in dispersed guinea pig acini. Recently, we reported that proglumide competitively inhibits CCK-stimulated 2-deoxyglucose uptake in mouse pancreatic acini\(^6\).

Cifarelli et al.\(^7\) previously reported that \(^12\)I-gastrin binding was decreased in membranes obtained from proglumide-administered rats.
It is not known, however, whether proglumide administration alters the sensitivity of acini to CCK or not. In the present study, therefore, we examined the effect of proglumide on CCK-stimulated amylase release in mouse pancreatic acini in vitro and in vivo.

Materials and Methods

Materials

Proglumide sodium salt was a gift from Kaken Pharmaceutical Co., Tokyo, Japan; synthetic CCK octapeptide (CCK8) was a gift from Dr. M.A. Ondetti, Squibb Institute for Medical Research, Princeton, NJ, U.S.A.; synthetic CCK tetrapeptide (CCK4) and synthetic porcine gastrin releasing peptide (pGRP) were generous gifts from Prof. N. Yanaihara, Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy, Shizuoka, Japan. The following reagents were purchased: hyaluronidase type-1, soybean trypsin inhibitor, carbamylcholine chloride from Sigma Chemical, St. Louis, MO; chromatographically purified collagenase and chymotrypsin from Worthington Biochemicals, Freehold, NJ. All other reagents and chemicals were of analytical grade. Krebs-Henseleit bicarbonate (KHB) buffer and N-2-hydroxyethyl piperazine-N'-2 ethane sulfonic acid (HEPES)-Ringer (HR) buffer, containing an essential amino acid supplement and 0.01% soybean trypsin inhibitor were prepared as previously described by Williams et al.8).

Preparation of dispersed acini

Pancreatic acini were prepared from overnight fasted male ICR mice by the method of Williams et al.8). Briefly, pancreatic tissue (0.9–1.1 g) were obtained from 5–6 mice, then the pancreas was injected with 5 ml KHB buffer with 0.1 mM Ca²⁺ containing purified collagenase (60–75 U/ml), chymotrypsin (15–30 µg/ml) and hyaluronidase (1.8 mg/ml), following by incubation of the tissue at 37°C with shaking for 50 min. Acini were dissociated by pipetting through plastic pipettes with narrow orifices, filtered through nytex mesh, and centrifuged through KHB buffer containing 4% bovine serum albumin. The dispersed acini were washed twice in the same buffer and then once in HR buffer containing 0.5% bovine serum albumin. Prior to the experimental procedures, the acini were preincubated for 60 min in HR buffer at 37°C.

In the in vivo study, 500 mg/kg of proglumide sodium salt in saline was injected into male ICR mice intraperitoneally twice a day for 5 days. The pancreas was then obtained from proglumide-administered mice after overnight fasting, and acini were prepared as described above.

Measurement of amylase release

Amylase release from mouse acini was studied as described previously by Williams et al.8). Briefly, the acini at 0.5–1.0 mg protein/ml were incubated in plastic flasks containing 2 ml of fresh HR buffer. After a 10-min incubation period, proglumide (sodium salt) was added, and then hormones or secretagogues were added. The incubation continued for 30 min at 37°C, shaking at 60 cycles/min, and the incubation was terminated by taking 1 ml aliquots, followed by immediate centrifugation.

The supernatant was reserved on ice for subsequent amylase assay, which was carried out by the method of Bernfeld9) as modified by Kanno10). One unit of amylase activity in the medium was defined as the amount of enzyme which produced 1 mg of maltose during a 5 min incubation with the diluted medium at 37°C. The initial time 0 value of amylase release was measured by taking the acinar suspension at the start of the incubation, and was subtracted from values obtained after incubation to obtain amylase release during the incubation. The pellet obtained at the start of