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DIFFERENCE IN EFFECTS OF PIRENZEPINE AND ATROPINE ON CARBACHOL-INDUCED PEPSINOGEN SECRETION FROM ISOLATED GASTRIC GLANDS

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Summary: The effect of pirenzepine on carbamylcholine (carbachol)-stimulated pepsinogen secretion was compared with that of atropine in the isolated guinea pig gastric glands. Pirenzepine and atropine caused a dose dependent inhibition of carbachol-stimulated pepsinogen secretion. Moreover, pirenzepine as well as atropine produced a rightward shift in the dose response curve of carbachol-stimulated pepsinogen secretion but did not alter the maximum increase in pepsinogen secretion. Results therefore demonstrate that pirenzepine acts as a specific receptor antagonist in the interaction of carbachol with its receptor on gastric chief cells. However, pirenzepine was 50 times less potent than atropine in inhibiting pepsinogen secretion. Half maximal inhibitory concentration of pirenzepine was 2×10^{-5}M when a maximally effective concentration of carbachol was used, while that of atropine was 4×10^{-7}M. Results, therefore, suggest that muscarinic receptor on gastric chief cells to which pirenzepine binds may be an intermediate affinity type.

Pirenzepine, a tricyclic pyridobenzodiazepine derivative, has been shown to reduce gastric acid secretion by an antimuscarinic mechanism (1)(2). Hammer et al. (3)(4)(5) have demonstrated that muscarinic receptors can be classified into three classes based upon binding studies of and pharmacologic responses to pirenzepine in both isolated tissues and intact systems. Its inhibitory effect on gastric acid secretion seems similar to those of classical anticholinergics and its potency of inhibiting carbachol-induced gastric acid secretion is nearly the same as that of atropine (1)(2)(6)(7).

Abbreviations used are: carbachol, carbamylcholine chloride; and BSA, bovine serum albumin.

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By contrast to gastric acid secretion, relatively little is known about the action of secretagogues with respect to pepsinogen secretion. Exceptionally, carbachol has been shown to stimulate pepsinogen secretion and atropine is a well known antagonist against carbachol-induced pepsinogen secretion (8)(9). Such studies have suggested that cholinergic muscarinic receptors also play an important role on pepsinogen secretion. However, the effectiveness of pirenzepine on muscarinic receptors in gastric chief cells is still unknown. There is no direct evidence as to whether pirenzepine reduces gastric pepsinogen secretion with the same potency as that observed with respect to inhibition of gastric acid secretion.

Therefore, the present study was undertaken to evaluate the antimuscarinic properties of pirenzepine on carbachol-stimulated gastric chief cell function and to characterize muscarinic receptors subtypes in gastric chief cells using isolated guinea pig gastric glands.

MATERIALS AND METHODS

Gastric Glands Preparation

The isolated guinea pig gastric glands were prepared as described previously (10). Stomach was removed by blunt dissection immediately after decaptitation. After stripping the smooth muscle coat, the fundus and corpus were minced and digested for 50 min at 37°C in a incubation solution containing 0.1% collagenase. At the end of incubation, the glands were filtered through nylon mesh to remove coarse fragments and rinsed. Finally, the glands were suspended in the medium consisting of 132 mM NaCl, 4.7 mM KCl, 1.1 mM $MgCl_2$, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.28 mM $CaCl_2$ and 0.5% BSA at pH 7.4.

Pepsinogen Determination

Glands suspension was pre-incubated at 37°C for 30 min. Agents were then added and the suspension was incubated for an additional 30 min. An aliquot of the suspension was taken and centrifuged, and the supernatant separated for pepsinogen determination. Pepsinogen was measured by the method of Anson and Mirsky (11) using acid-denaturated hemoglobin as the substrate. The reaction was stopped by adding trichloroacetic acid solution. Supernatant samples, read at 280 nm, were caliculated using a standard curve for peptic activity of crystallized pepsinogen. Pepsinogen released into the medium was then corrected as the percentage of the total peptic activity present in the glands before incubation.

Chemicals

Pirenzepine was kindly donated by Nippon Boehringer Ingelheim Co., (Kawanishi, Japan). The following was purchased: carbamylcholine chloride (carbachol), atropine, soybean trypsin inhibitor (type 1s) and type I collagenase from Sigma Chemical Co., (St. Louis, MO), crystallized pepsinogen from Worthington Biochemicals (Freehold, NJ).

RESULTS

Pepsinogen secretory responses to carbachol are shown in Fig. 1. Carbachol caused a dose-dependent increase in pepsinogen secretion from isolated guinea pig gastric glands. Maximal stimulation of pepsinogen secretion occurred with 10^{-3}M carbachol. Atropine, as expected, antagonized the action of carbachol on pepsinogen secretion in the present study. $3x10^{-8}$M atropine caused a rightward shift of the dose response curve for carbachol-induced pepsinogen secretion and 10^{-6}M atropine inhibited carbachol-induced pepsinogen secretion more profoundly.

Pirenzepine considered to be an antimuscarinic agent also antagonized the action of carbachol on pepsinogen secretion (Fig. 2). 10^{-6}M pirenzepine reduced pepsinogen secretion induced by graded concentrations of carbachol, but did not alter the maximum increase in pepsinogen secretion. With higher concentration of

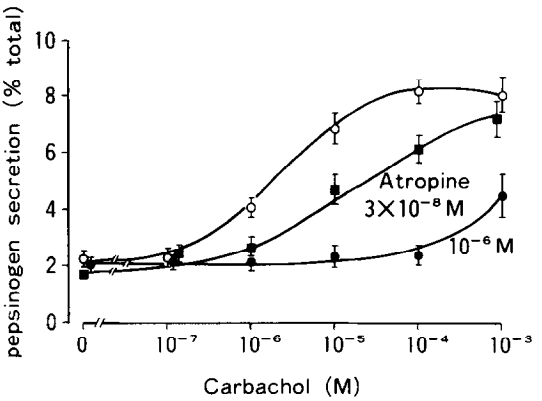
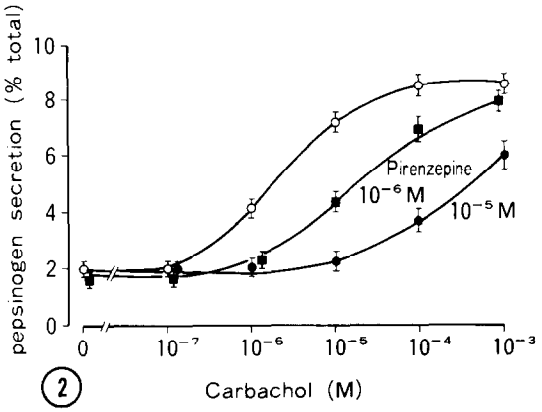


Fig. 1 Effect of atropine on carbachol-induced pepsinogen secretion from isolated guinea pig gastric glands. Gastric glands prepared as described under "Methods" were incubated at 37°C for 30 min with increasing concentrations of carbachol alone (o——o) or in the presence of either $3x10^{-8}$M atropine (■——■) or 10^{-6}M atropine (●——●). Each value shown is the mean ± SE of triplicate determinations from a representative of three separate experiments.



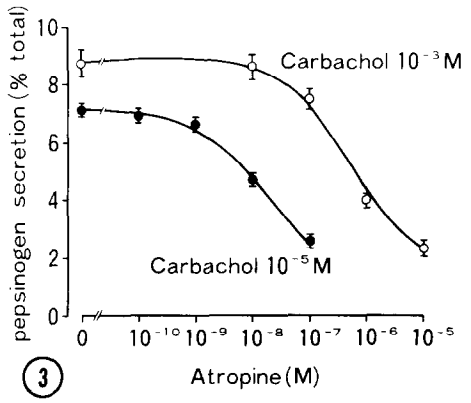
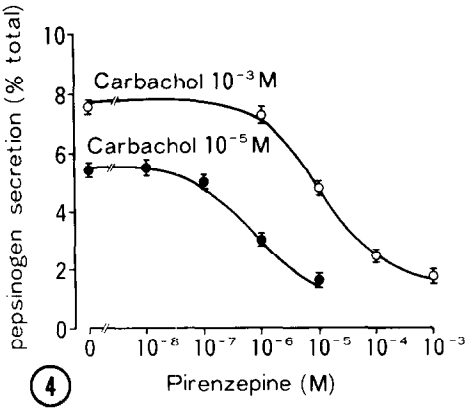


Fig. 2 Effect of pirenzepine on carbachol-induced pepsinogen secretion from isolated guinea pig gastric glands. Gastric glands were incubated with various concentrations of carbachol alone (o——o) or in the presence of either 10^{-6}M pirenzepine (■——■) or 10^{-5}M perenzepine (●——●). Values shown are the mean ± SE of triplicate determinations from a representative of three separate experiments.

Fig. 3 Effect of graded concentrations of atropine on carbachol-induced pepsinogen secretion. Gastric glands were incubated with either 10^{-3}M carbachol (o——o) or 10^{-5}M carbachol (●——●) in the presence of atropine at indicated concentrations. Values are the mean ± SE of triplicate determinations and this experiment is a representative of three separate experiments.

pirenzepine, more profound inhibition of pepsinogen secretion was also observed, although pepsinogen secretory responses to supramaximal concentration of carbachol could not be observed.

The inhibitory action of atropine and pirenzepine was further characterized. The effect of increasing concentrations of atropine and pirenzepine was examined on a maximally or submaximally stimulated pepsinogen secretion. Atropine and pirenzepine caused a dose-dependent inhibition of pepsinogen secretion induced by 10^{-3}M or 10^{-5}M carbachol as shown in Fig. 3 and Fig. 4. This antagonism was competitive judging from that, with higher concentration of carbachol, higher concentrations of atropine or pirenzepine were required to produce detectable inhibition of pepsinogen secretion. This action of atropine and pirenzepine was specific for carbachol, because atropine or pirenzepine inhibited the increase in pepsinogen secretion caused



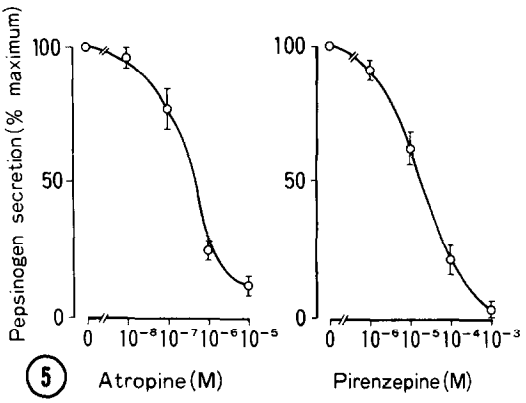


Fig. 4 Effect of graded concentrations of pirenzepine on carbachol-induced pepsinogen secretion. Gastric glands were incubated with either 10^{-3}M carbachol (o——o) or 10^{-5}M carbachol (●——●) in the presence of pirenzepine at indicated concentrations. Values are the mean ± SE of triplicate determinations from a representative of three separate experiments.

Fig. 5 Effect of atropine or pirenzepine on pepsinogen secretion induced by a maximally effective dose of carbachol. Gastric glands were incubated with 10^{-3}M carbachol in the presence of either atropine or pirenzepine at indicated concentrations. Each value is expressed as the percent of a maximally stimulated value and the mean ± SE from three separate experiments.

by carbachol but not that caused by cholecystokinin (data not shown). However, the potency of pirenzepine to antagonize carbochol-induced pepsinogen secretion was weak when compared with that of atropine as shown in Fig. 5. Half maximal inhibitory concentration of pirenzepine was 2×10^{-5}M when caliculated from Fig. 5, while that of atropine was 4×10^{-7}M. Pirenzepine was, therefore, about 50 times less potent than atropine on a molar basis in inhibiting carbochol-stimulated pepsinogen secretion.

DISCUSSION

Present study demonstrates that pirenzepine inhibits carbachol-induced pepsinogen secretion from isolated gastric glands in vitro. As in the case of atropine, pirenzepine act as a competitive antagonist for inhibiting carbachol-stimulated pepsinogen secretion. Pirenzepine as well as atropine caused a

rightward shift in the dose response curve of carbachol-stimulated pepsinogen secretion, but did not alter the maximum increase in pepsinogen secretion. However, pirenzepine was found to be about 50 times less potent than atropine on a molar basis in inhibiting the stimulated pepsinogen secretion. Since pirenzepine has been shown to be only 2-10 times less potent than atropine in inhibiting gastric acid secretion (1)(2)(6)(7), it seems that the potency of pirenzepine to antagonize the action of carbachol on gastric chief cell is lower than that of pirenzepine on gastric parietal cell. Results, therefore, suggest that affinity of pirenzepine for muscarinic receptor on gastric chief cell may be different from that of pirenzepine on parietal cell.

Hammer et al. (5) have demonstrated that pirenzepine can distinguish subclasses of muscarinic receptors to which atropine is equipoment and thereby binds to receptors of various tissues with a wide heterogeneity of affinities. In tissues with high affinity muscarinic receptors, pirenzepine and atropine exhibit similar potency (2-10 times less potent than atropine), whereas in tissues with intermediate and low affinity receptors, pirenzepine is up to 200 times less potent than atropine. Competitive inhibition study of ^{3}H-N-methylscopolamine binding to gastric mucosal homogenate by pirenzepine reveals that more than one site of muscarinic receptors exist with high and intermediate affinity for pirenzepine (12). Thus Hammer by himself proposes that binding study of and pharmacologic responses to pirenzepine are necessary in each gastric cell to further characterize gastric muscarinic receptors. In the present study, the concentration of pirenzepine required to inhibit the secretory response of pepsinogen to carbachol was 50 times higher than that of atropine. Taking into considerations of our results, it may be

suggested that muscarinic receptor on gastric chief cell is a intermediate affinity type as compared with high affinity type of muscarinic receptor on gastric parietal cell.

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