Parathyroid Hormone and Parathyroid Hormone-Related Protein Inhibit Phasic Contraction of Pig Duodenal Smooth Muscle

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Abstract. **Parathyroid hormone (PTH) and a newly discovered PTH-related protein (PTHrP), which has amino-terminal homology with PTH, are potent relaxants of rat gastrointestinal tissues. Since their gastrointestinal relaxant effects have been described only in the rat, we examined their actions in another mammalian species in order to evaluate whether the relaxant property was more generally applicable. Longitudinal smooth muscle strips were obtained from the pig duodenum. The mucosa was removed, the strips were mounted in a tissue chamber, and changes in phasic contraction were detected with a force-displacement transducer and recorded using a polygraph. Acetylcholine-induced phasic contraction was inhibited rapidly in a dose-related manner by [Nle^{8,18},Tyr^{34}]-bPTH-(1–34)-amide, or hPTHrP-(1–34). The IC_{50} values for these peptides were 2.6 n*M* and 6.1 n*M*, respectively. The maximal effect of both peptides was observed at 60 n*M* with an 84% decrease of the acetylcholine-induced contraction. At 400 n*M*, the PTH antagonist, [Nle^{8,18},Tyr^{34}]-bPTH-(3–34)-amide, had no effect by itself. However, the same 400 n*M* concentration of this peptide totally blocked the decrease in phasic contraction induced by 10 n*M* of the bPTH-(1–34) analogue or hPTHrP-(1–34). Our results show that receptors for PTH or PTHrP are present in the muscular layer of the pig duodenum and that activation of these receptors inhibits the phasic contraction of the tissue. Furthermore, the ability of PTH-related peptides to relax gastrointestinal smooth muscle is not restricted to the rat.** [P.S.E.B.M. 1989, Vol 191]

Although parathyroid hormone (PTH) is well known as a major calcium regulating hormone, it also has been found to relax smooth muscle in tissues obtained from blood vessels, trachea, uterus, vas deferens, and gastrointestinal tract (1, 2). Recently we reported that *N*-terminal peptides representing the active regions of both rat (r) and bovine (b) PTH were potent, highly effective relaxants of the rat gastric fundus, duodenum, ileum, and colon (3, 4). Furthermore, a synthetic analogue of the newly discovered, tumor-related human PTH-related protein (hPTHrP), which has amino-terminal homology with PTH (5), also had dose-dependent effects similar to rPTH in relaxing the rat fundus (6). These findings indicated that receptors for PTH or PTHrP are present in smooth muscle throughout the rat gastrointestinal tract.

To date, the gastrointestinal relaxant effects of PTH and PTHrP have been described only for the rat. Therefore, it is not known whether such effects are unique for the rat or whether PTH-like peptides also can relax gastrointestinal tissues from other mammalian species as well. In this study, we tested the effects of the active, synthetic bPTH analogue, [Nle^{8,18},Tyr^{34}]-bPTH-(1–34)-amide, and of synthetic hPTHrP-(1–34) on the pig duodenal smooth muscle. The results show that both peptides inhibit the phasic contraction of the duodenum in a similar dose-dependent manner.

Materials and Methods

Sections of the proximal duodenum were removed from adult Large White pigs of either sex weighing ~150 kg and anesthetized with pentobarbital. Each duodenal section was dissected free of mucosa, and the remaining muscular tissue, which included both the longitudinal and circular muscle layers, was cut into 1-

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cm × 0.2-cm longitudinal strips. One end of the strip was mounted in a tissue bath, and the other end was tied to a Grass FT.03 force-displacement transducer. Changes in phasic contraction were recorded using a Grass model 5E polygraph. Earle's balanced salt solution (m*M*: NaCl, 116; NaH_2PO_4, 1; $NaHCO_3$, 26; $CaCl_2$, 1.8; KCl, 5.4; $MgSO_4$, 0.8; D-glucose, 5.5; pH-7.4) was used as incubation medium. It was kept at 37°C and aerated with 95% O_2-5% CO_2. A length-tension study of both passive and active (acetylcholine stimulated, 0.5 μM) tension was conducted. Passive tension increased nonlinearly as tissue was progressively stretched. Acetylcholine (Ach)-induced tension increased linearly to a plateau. A passive tension of 0.25 g was observed to be the load at which tissues could be studied without excessively stretching them and the one at which tissue would still generate maximal tension with Ach. Therefore, a basal tension of approximately 0.25 g was applied to each duodenal strip and the tension was allowed to stabilize for at least 30 min. Thereafter, phasic contraction was induced by introducing medium containing 0.5 μM ACh into the bath.

In cumulative dose-response experiments, the lowest dose of peptide was first added to the tissue bath; then it was followed by the next higher dose at 5-min intervals. In order to quantify the responses, the amplitude of each phasic contraction was summated for the 2-min period immediately before the initial dose of peptide, and this was considered as a basal value. The sum of phasic tension for the 3–5-min period after each peptide treatment was calculated and expressed as the percentage of change from the basal value. Evaluation of responses using this procedure did not measure exclusively amplitude or frequency of contraction, but rather components of both parameters. All data are shown as mean ± SE. The peptide concentration which inhibited Ach-induced phasic contraction by 50% (IC_{50} value) was established by plotting each individual log dose-response curve, and the geometric mean IC_{50} was calculated using these individual values (7). In control experiments, vehicle was used instead of the peptides. Only one peptide, i.e., either PTH 1–34 or PTHrP 1–34, was tested with each duodenal strip.

The specificity of the relaxant effects of PTH and PTHrP was tested by evaluating the antagonistic action of [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide. In this experiment, the action of 10 n*M* [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide was first examined. This was followed by several washes over the next 20–30-min period using medium without any added peptide. Then the tissues were incubated with 400 n*M* [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide alone for 5 min before the effect of the same dose of the bPTH-(1–34) analogue used initially, i.e., 10 n*M*, was tested again for 5 min. The data were evaluated using analysis of variance and a Scheffe test (8). The combined effect of the bPTH-(3–34) and bPTH-(1–34) analogues was compared with the initial bPTH-(1–34) treatment alone. A probability value of less than 0.05 was considered to be significant. The same protocol also was used in similar experiments in two pigs in which the antagonistic action of [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide on the effect of hPTHrP-(1–34) was studied.

[$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide and [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide were purchased from Peninsula Laboratories, Inc. (San Carlos, CA). Synthetic hPTHrP-(1–34) was purchased from Bachem Inc. (Torrance, CA). The peptides were dissolved in 10 m*M* acetic acid as stock solutions. They were kept at −70°C in 50-μl aliquots until used. Sigmacote (Sigma, St. Louis, MO), a silicone solution, was used to coat the tissue bath before each experiment in order to prevent possible binding of peptides to the glass surface.

Results

Figure 1 shows representative tracings of duodenal tension for two pigs in which [$Nle^{8,8}$,Tyr^{34}]-bPTH-(1–34)-amide and hPTHrP-(1–34) were tested at doses of 1–10 n*M*. As shown in these representative tracings, both peptides decreased the frequency and amplitude of duodenal contractions. However, because there was variation in both the rate and frequency of contraction from animal to animal, we chose to use a measure involving both components as described in Materials and Methods rather than rely on only one component. The cumulative dose-response curves for these peptides in inhibiting ACh-induced phasic contraction in all pigs tested are presented in Figure 2. The IC_{50} for [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide was 2.6 n*M* (range, 1–6 n*M*), and the IC_{50} for hPTHrP-(1–34) was 6.1 n*M* (range, 2–16 n*M*). Both peptides were equally effective in reducing phasic contraction to −84% of the basal value at a dose of 60 n*M*. Addition of vehicle alone to duodenal strips (control) did not inhibit phasic contraction.

The specificity of the PTH-induced inhibition of pig duodenal contraction is shown in Figure 3. In this

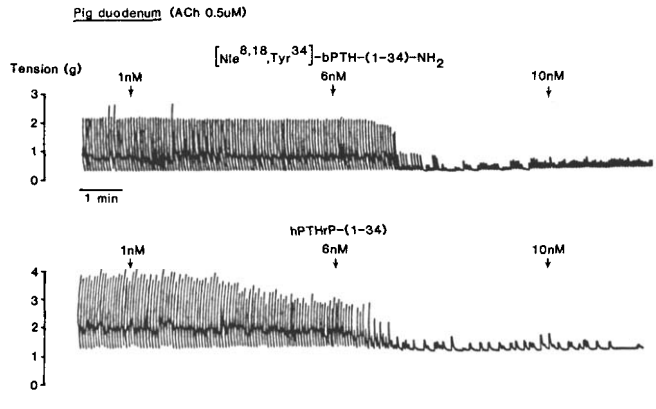

Figure 1. Representative tracings showing the inhibitory effects of different doses of [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide and hPTHrP-(1–34) on acetylcholine-stimulated (0.5 μM) phasic contraction in duodenal strips from two pigs.

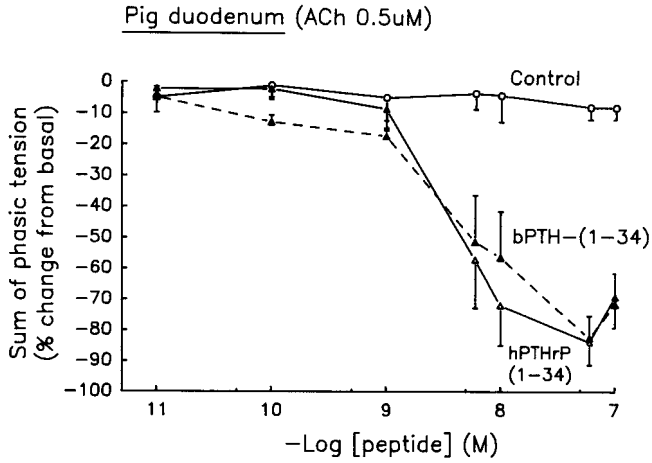

Figure 2. Dose-response curves for bPTH-(1–34) and hPTHrP-(1–34) in inhibiting acetylcholine-stimulated phasic tension. The IC_{50} values for bPTH-(1–34) and hPTHrP-(1–34) were 2.6 and 6.1 n*M*, respectively. Control represents vehicle alone. bPTH-(1–34) = [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide. Mean ± SE, $n = 5$.

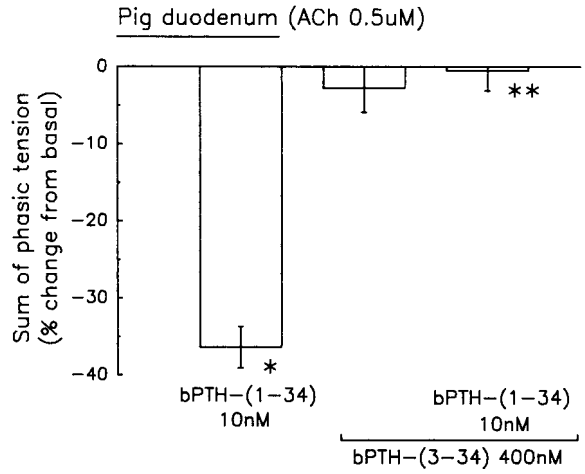

Figure 3. Ability of 400 n*M* bPTH-(3–34) to block the inhibitory effect of 10 n*M* bPTH-(1–34). bPTH-(1–34) significantly inhibited the phasic contraction produced by acetylcholine, whereas a 40-fold excess of bPTH-(3–34) alone had no effect. The same dose of bPTH-(3–34) blocked the inhibitory effect of bPTH-(1–34) when both peptides were tested together. bPTH-(1–34) = [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide, bPTH-(3,34) = [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide. * $P < 0.05$ compared with the phasic tension before bPTH-(1–34) treatment, ** $P < 0.05$ compared with the effect of bPTH-(1–34) alone. Mean ± SE, $n = 3$.

experiment as in Figure 2, 10 n*M* [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide significantly inhibited the acetylcholine-induced duodenal phasic contraction. The addition of 400 n*M* [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide alone, a peptide identical to the bPTH-(1–34) analogue, except for the absence of the first two amino acid residues, failed to inhibit the phasic contraction. However, when both of these peptides were tested together, the relaxant effect of the bPTH-(1–34) analogue was totally eliminated, showing that a 40-fold molar excess of [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide could antagonize the effect of PTH on pig duodenal contraction. In similar experiments in two pigs not shown, the inhibitory effect of 10 n*M* of the hPTHrP-(1–34) alone on duodenal phasic contraction also was antagonized by a 40-fold excess dose of the bPTH-(3–34) analogue. In these experiments, following a washout period of 20–30 min, a full response to bPTH-(1–34) or hPTHrP-(1–34) again could be observed (not shown).

Discussion

Several studies have shown that PTH is a potent vasodilatory peptide in dogs and rabbits, and, in rats, PTH is known to relax the blood vessel, uterus, vas deferens, and gastrointestinal tissues. PTH also has been found to relax tracheal smooth muscles in the guinea pigs. These and other studies reviewed by Pang *et al.* (1) and by Nickols (2) have shown that PTH is an effective smooth muscle relaxant in several mammalian species. One group has reported that PTH causes relaxation of porcine vascular smooth muscle (9). The present study is the first to demonstrate that PTH-like peptides can inhibit intestinal smooth muscle contraction in the pig. The effect was shown using porcine duodenum and was evident in the absence of the mucosal layer.

Until this study, our knowledge of the relaxant effect of PTH on gastrointestinal smooth muscle has been limited to the rat. Our own previous studies have shown that rPTH-(1–34) and [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide are potent relaxants of the tonic tension in the rat fundus, duodenum, ileum, and colon (3, 4). The ED_{50} for rPTH-(1–34) was 5–6 n*M* in the fundus and 2 n*M* in the colon. The maximal relaxation produced in the fundus was 90% at 40 n*M* (3). Although there also was a decrease in the phasic components of contraction, i.e., the frequency and amplitude, these effects were not measured because they varied considerably from rat to rat. Furthermore, dose-response experiments were not carried out using rat duodenal tissues, so that the potency of PTH in this gastrointestinal region was not known. However, these earlier studies did show that the classical PTH antagonist, [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide, was inactive by itself and that excess doses of this peptide could block the relaxant action of rPTH-(1–34) or [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide. The present study using porcine duodenal smooth muscle shows clearly that [$Nle^{8,18}$,Tyr^{34}]-bPTH-(1–34)-amide had a dose-dependent, inhibitory effect on ACh-stimulated phasic contraction. The potency and efficacy of the bPTH-(1–34) analogue were similar to those found using the rat fundus. Likewise, a high dose of the PTH antagonist, [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide, alone had no effect on the porcine duodenum. However, pretreatment with a 40-fold molar excess of this peptide totally eliminated the inhibitory effect of the bPTH-(1–34) agonist on phasic contraction. The results of this study are totally consistent with the actions of the PTH-(1–34) and (3–

34) analogues using the rat gastrointestinal tissues (3, 4).

Recently, the complete amino acid sequence of hPTHrP was reported by Suva *et al.* (10). This peptide has 60–70% homology with bovine, rat, or human PTH within the first 13 amino-terminal residues, and, therefore, it also has PTH-like biologic activities. A synthetic analogue of this peptide, hPTHrP-(1–34), is known to relax the rabbit renal artery (11) and the rat fundic strip (6). The IC_{50} for hPTHrP-(1–34) using the two tissues was 1.3 and 10 n*M*, respectively. In rat fundic strips, the relaxant effect of hPTHrP-(1–34) could be blocked by the PTH antagonist, [$Nle^{8,18}$,Tyr^{34}]-bPTH-(3–34)-amide (6). Furthermore, rPTH-(1–34) and hPTHrP-(1–34), showed cross-desensitization for one another (12). Therefore, receptors for PTH or PTHrP are present in the rat gastrointestinal tract. In the present study, hPTHrP-(1–34) inhibited the phasic contraction of porcine duodenum. Furthermore, the IC_{50} of this peptide was 6.1 n*M*, a value in good agreement with those in the rabbit renal artery and the rat fundic strip. The efficacy of this peptide was also similar to that reported in the rat (6). In the pig, like the rat, the bPTH-(3–34) analogue was an antagonist of hPTHrP-(1–34). Whether PTH or PTHrP serves as the endogenous ligand for the receptors in the porcine duodenal smooth muscle which mediate relaxation remains to be determined. If PTH were the normal ligand, it presumably would be derived from circulating PTH. If PTHrP were the endogenous ligand, it might be derived locally and act as a paracrine factor, since mRNA for PTHrP has been detected in the rat gastrointestinal tract (13).

rPTH-(1–34) and hPTHrP-(1–34) produce the same smooth muscle relaxant effect in the rat fundus and in the pig duodenum. Although the present study was done in a nonhomologous system using synthetic peptide analogues and tissues from different species, it can be argued that the relaxant effects of PTH-(1–34) and PTHrP-(1–34) in pigs and rats are remarkably similar with respect to potency and efficacy. These peptides could help regulate gastrointestinal motility, but the mechanisms of action and the physiologic function of PTH and PTHrP in the gastrointestinal tract remain to be established.

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