

## Effects of a Gastrin-Like Peptide on Uterine Smooth Muscle (34583)

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The documented actions of gastrin are restricted to the gastrointestinal tract. The hormone stimulates secretion in the stomach, pancreas, and liver, and initiates smooth-muscle activity in the stomach, gut, and gallbladder (1). Gastrin also increases blood flow to the stomach (2, 3); however, the vascular response appears to be secondary to the metabolic changes associated with augmented secretion (4). Despite early evidence for a weak depressor action with large doses of gastrin (1), this has not been confirmed in our experience with conscious dogs.

It has been reported (5) that extracts of gastrin were without effect on smooth muscle outside the gastrointestinal tract. Recently, a synthetic pentapeptide possessing the physiological actions of gastrin has come into wide use. The pentapeptide contains the C-terminal tetrapeptide sequence of the natural molecule plus beta alanine. The present investigation was directed toward assessing the effect of this pentapeptide on extragastrointestinal smooth muscles, particularly the uterus.

*Methods. In vitro smooth-muscle preparation.* White virgin female rats weighing between 200 and 250 g were ovariectomized 2 weeks prior to use. Each animal received an injection of 100  $\mu$ g of estradiol 48 hr before being sacrificed. The uterine horns were removed and suspended in a 10-ml tissue bath containing oxygenated Krebs-Henseleit solution at a constant temperature of 37°. Muscle contractions were recorded with a force transducer connected to a recorder (6).

As a control for gastrin activity, strips of guinea pig ileum were suspended and tested in a 10-ml oxygenated Tyrode bath at 37°. In a similar manner, we tested the following

tissues: strips of rabbit aorta and veins (oxygenated Krebs bicarbonate solution at 37°), guinea pig seminal vesicles and vas deferens (oxygenated Tyrode's solution at 37°), and tracheal chain (carbogenated Krebs-Henseleit solution at 37°).

The pentagastrin (Paptavlon, Ayerst Laboratories) was dissolved in a small volume of 0.1 *N* ammonium hydroxide, the pH was adjusted to 7.0 with 0.1 *N* HCl, and the final volume was made up with Krebs-Henseleit solution. The muscle strip was allowed to stabilize for 30 min before beginning any experiment. Responses to acetylcholine and gastrin-like peptide were obtained at varying dose levels. The bath was flushed with fresh solution after a given contact time, and a blank (pentagastrin solvent) preceded and followed each test dose. There was a 5-min waiting period between each dose, and this period was standard for all substances tested.

*In vivo smooth-muscle preparation. Rat.* Fifteen white virgin female rats were anesthetized with sodium pentobarbital intraperitoneally, 3–5 days after being primed with 100  $\mu$ g of estradiol. The abdomen was opened with a midline incision, and a single uterine horn was isolated and ligated at the junction of the horn and body of the uterus. A 22-gauge needle connected to a polyethylene tube was inserted into the uterine cavity and secured with a second ligature. The uterine cavity was then distended with a small volume of normal saline to facilitate recording of pressure changes. The polyethylene tubing was connected to a pressure transducer and recorder. After allowing the preparation to stabilize for 30 min, responses to topically applied acetylcholine and gastrin-like peptide were recorded in the exteriorized uterine

horn. The uterine horn was thoroughly washed with normal saline after each test dose, and there was a 5-min waiting period between test doses.

**Dog.** Four pregnant dogs near term were anesthetized with sodium pentobarbital, 30 mg/kg, and their uteri exteriorized through a midline incision. A 22-gauge needle attached to a pressure transducer with polyethylene tubing was inserted directly into the amniotic sac of the uterus. Gastrin-like peptide was injected intravenously and pressures were measured in centimeters of water. In nine other dogs, a polyethylene catheter was inserted into the femoral artery under anesthesia, and the animals were allowed to recover. Several days later the heparin-filled catheter was connected to a strain-gauge transducer for recording of pressures. Pentagastrin in doses of 5–10  $\mu$ g was injected into a foreleg vein, and the effect on arterial pressure was noted.

**Baboon.** A female baboon, 110 days pregnant, was lightly anesthetized and placed in a restraining chair. A polyethylene catheter was threaded through an amniocentesis needle into the uterine cavity and connected to a pressure transducer and recorder. The animal was permitted to regain consciousness for 1 hr before the experiment was started. Pentagastrin was injected intravenously into the baboon.

**Results.** Our data, summarized in Table I, demonstrate that pentagastrin stimulated uterine contractions in all four preparations. As shown in Fig. 1, no clear-cut dose-response relationship could be demonstrated,

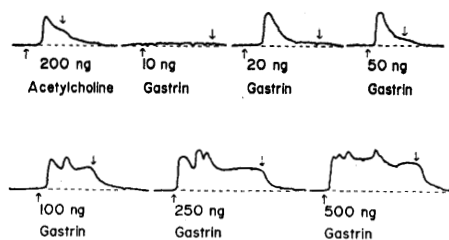


Fig. 1. Comparison of uterine muscle strip responses to various doses of gastrin-like peptide and acetylcholine. Upright arrows indicate time of drug injection and inverse arrows time of washing the bath to remove the drug.

TABLE I. Effect of Pentagastrin on Various Smooth Muscle Preparations.

Preparation	Number of experiments	Experiments with positive responses	Threshold dose for pentapeptide	Tachyphylaxis	Potency of pentapeptide compared with acetylcholine	Dose-response relationship
Rat, uterine strip <i>in vitro</i>	100	39	$2 \times 10^{-8}$ g/ml bath	+	Greater	—
Rat, uterus <i>in vivo</i>	15	11	$8 \times 10^{-7}$ g/kg body wt	+	Smaller per gram Greater per mole	—
Dog, anesthetized and pregnant	4	4	$2.5 \times 10^{-6}$ g/kg body wt			
Baboon, conscious and pregnant	1	1	$1 \times 10^{-5}$ g/kg body wt			
Guinea pig, ileal strip <i>in vitro</i>	50	50	$5 \times 10^{-10}$ g/ml bath	+	Greater	+
Guinea pig, gallbladder strip <i>in vitro</i>	10	7	$1 \times 10^{-8}$ g/ml bath	—	Greater	+

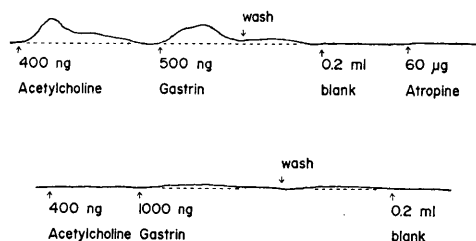


FIG. 2. Comparison of *in vitro* uterine responses to gastrin-like peptide and acetylcholine before and after adding atropine to the bath.

although higher doses of the pentapeptide produced somewhat more intense and prolonged responses. Comparing responses of pentagastrin and acetylcholine (Fig. 1), the pentapeptide seemed to be a more potent stimulator of uterine contractility. The responses to acetylcholine and pentagastrin were blocked by atropine in doses of  $10 \mu\text{g}$  (sulfate)/ $10 \text{ ml}$  bath (Fig. 2). Tachyphylaxis, which was frequently seen in the uterine strips (Fig. 3), could be prevented by interspersing gastrin doses with occasional doses of acetylcholine.

*In vivo* studies in the rat, employing the exteriorized uterus and topical application of agents, demonstrated a higher frequency of positive responses to pentagastrin (Table I). The threshold dose of the pentapeptide on rat uterus was greater with the *in vivo* method, but atropine still blocked the response to both acetylcholine and gastrin-like peptide (Fig. 4).

When the potencies of pentagastrin and acetylcholine were compared on a molar basis in either the *in vitro* or *in vivo* rat preparations, pentagastrin was clearly the more potent agent.

Other *in vivo* studies employing the preg-

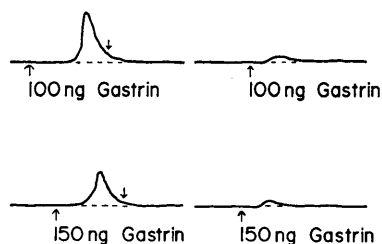


FIG. 3. Diminishing *in vitro* uterine responses to successive administrations of the gastrin-like peptide.

nant baboon and dog demonstrated that gastrin-like peptide stimulated uterine activity in these species as well, but at a much higher dose (Fig. 5 and Table I).

For comparison, the guinea pig ileum and gallbladder were tested *in vitro* (Table I). With these tissues, the frequency of positive contractile responses to pentagastrin was greater than with rat uterine strips. The threshold dose of pentagastrin was less with the ileum and greater with the gallbladder than was observed with the uterus. In both the ileum and gallbladder, atropine blocked the action of pentagastrin, as well as acetylcholine. Tachyphylaxis to the pentapeptide was observed in the ileum but not in the gallbladder.

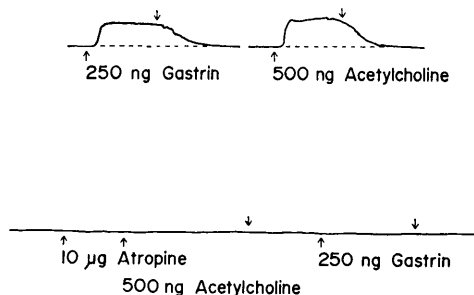


FIG. 4. Comparison of *in situ* rat uterine responses to various doses of gastrin-like peptide and acetylcholine before and after adding atropine to the bath. Inverse arrows indicate washing of uterine surface to remove the drug.

Strips of rabbit aorta and veins, guinea pig seminal vesicles and vas deferens, and isolated tracheal chain and tracheal segment of the guinea pig did not respond to the pentapeptide over the dose range of  $10^{-9}$  to  $10^{-4}$  g/ml of the tissue bath.

Systemic arterial pressure responses to intravenously injected pentagastrin in conscious dogs were inconstant, rising more than  $10 \text{ mm Hg}$  in three animals and declining by  $10 \text{ mm Hg}$  or more in three other dogs.

*Discussion.* Our findings indicate that the gastrin-like peptide induces uterine contractions. Contractile responses were observed in: (1) rat uterine muscle strips; (2) *in situ* uteri of anesthetized rats and gravid dogs; and (3) the gravid uterus of a conscious

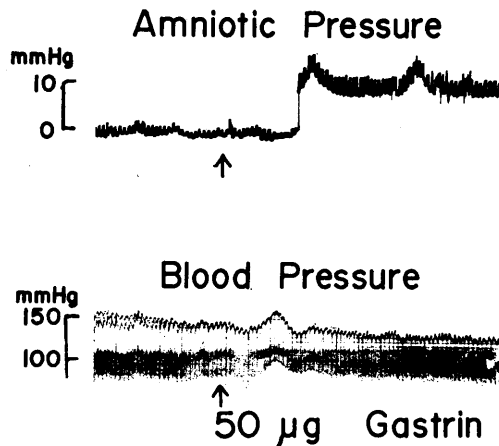


FIG. 5. Amniotic pressure response to intravenously injected gastrin-like peptide in a conscious gravid (110 days) baboon. Amniotic pressure values had been stable, except for minor respiratory variations, for over 30 min prior to injecting the pentapeptide. Pressure remained elevated for 30 min subsequently. The time interval from injection to uterine response was approximately 1 min.

baboon. The *in vitro* uterine strip was less sensitive to gastrin than was the guinea pig ileum.

A large proportion of uterine strips failed to respond positively to pentagastrin. In a number of experiments not listed, we varied bath conditions and the composition of the bath solution to ascertain the cause of negative uterine responses to pentagastrin. We were unable to identify the factors responsible for uterine insensitivity to the peptide *in vitro*.

The block of pentagastrin activity by atropine suggests that a cholinergic mechanism mediates the effect of the polypeptide in the uterus. Further indirect support for this explanation may be derived from the finding that uterine and ileal strips developed tachyphylaxis to gastrin which could be prevented

by alternating doses of acetylcholine and gastrin. This conforms to Bennett's view of the action of gastrin on gastrointestinal muscle (6).

Our findings raise the possibility of a physiological role for gastrin at parturition in some species. This speculation is based upon the following considerations. At term, the uterus nearly fills the abdominal cavity and compresses the viscera. The stomach would then be less able to relax and accommodate a bolus of food, and its walls would be stretched more than is usual. The major normal stimulus for release of gastrin into the circulation is stretching of the pyloric gland area of the stomach. Circulating gastrin may either play a permissive role in priming the uterus for other neurohumoral stimuli or may be synergistic with other agents.

**Summary.** Pentagastrin stimulated uterine contractions in several animal species *in vitro* and *in situ*. The response could be blocked by atropine. The uterus was not as responsive to the pentapeptide as was the ileum, but the peptide was approximately as potent a uterine stimulus as acetylcholine.

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