Bradykinin Antagonism Following Oral Protease Therapy. (28020)

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Bradykinin is considered the mediator of several classical responses comprising the inflammatory reaction(1). These responses include vasodilatation, enhanced capillary permeability, accumulation and migration of leucocytes, pain and erythema. In a recent study involving analgesic-antipyretic compounds, anti-inflammatory effects were found only in substances previously characterized as bradykinin antagonists: aspirin, salicylates, amidopyrine and phenylbutazone. Such analgesics as salicylamide, acetanilide, phenacetin and N-acetyl p-aminophenol which do not antagonize bradykinin were devoid of anti-inflammatory properties(2).

Anti - inflammatory effects have been claimed for orally given proteolytic enzymes (3). Supporting experimental evidence has been meager. In view of the possibility that anti-inflammatory action may relate to bradykinin antagonism, it seemed pertinent to ascertain whether orally given proteases induce bradykinin blocking effects.

Materials and methods. 1. *Antagonism to bradykinin hypotensive effect.* This study comprised 15 dogs. Three dogs served as controls and received placebo tablets plus bradykinin* injections. Three dogs received oral doses of papain;† 3 dogs received oral doses of aspergillus oryzae;‡ 3 dogs received oral streptokinase;§ 3 dogs received oral doses of trypsin.‖

Under Nembutol anaesthesia, the carotid artery was cannulated and attached in the usual fashion to a mercury manometer. Bradykinin (0.5 μg per kg) was injected intravenously and a control blood pressure tracing was obtained. Placebo and enzyme tablets were introduced *via* stomach tube. The following single doses were given: Papain, 100 mg/kg; SKF9944, 1600 casein units/kg; Streptokinase plus human plasma, 20,000 units/kg; Trypsin, 10 mg/kg. Following treatment, bradykinin was given intravenously every 15 minutes for a 2 hour period and blood pressure tracings were recorded.

2. *Antagonism to bradykinin smooth muscle stimulation.* This study comprised 28 female Sprague-Dawley rats. Treated rats were divided into groups of 3, each group receiving 3 hourly doses of one of the following oral enzyme preparations: Papain, 100 mg; A. oryzae, 800 casein units; SK plus human plasma, 200,000 units.

Isotonic rat uterus contractions were recorded from the intact rat uterus suspended in Tyrode solution in a 17 ml bath at 37°. One ml whole blood was obtained from control and treated rats *via* heart puncture and immediately introduced into the bath. Bradykinin (0.125 ng) was then added to the bath.

3. *Antagonism to bradykinin enhancement of permeability.* In this study 12 rabbits were used and served as their own controls. The rabbits were shaved over a ventral area extending between the pectoral and pelvic girdles and extending 6 cm on either side of the mid-line. Bradykinin in doses of 0.1 ng was administered intradermally at 2 sites 10 minutes after injecting intravenously 1 ml of 5% trypan blue. Thirty minutes after these control injections, enzyme preparations were given orally. Half an hour later, an additional 1 ml injection of trypan blue was given, and 10 minutes later 0.1 ng bradykinin was injected intracutaneously at other sites. Responses were measured at areas by tracing the zone of bluing 30 minutes after intradermal injection onto mm squared paper.

The 12 rabbits were divided into groups of 4. Each group received one dose of one of the following enzyme preparations orally: Streptokinase plus human plasma (Varizyme) 100,000 units; Papain, 500 mg; A. oryzae, 8,000 casein units.

Results and discussion. On the basis of

* Bradykinin, Synthetic, Sandoz.
† Papase, Warner-Chilcott.
‡ 9944, Smith, Kline & French.
§ Varizyme, Lederle.
‖ Inflamase, Smith, Miller & Patch.

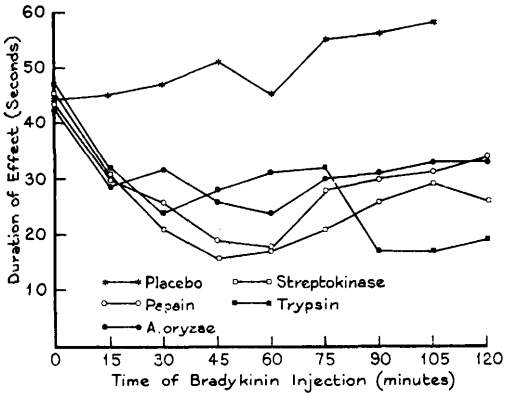

FIG. 1. Duration of hypotensive effect in dogs.

these experiments, orally given proteases appear to modify 3 different biological responses to bradykinin: hypotension, smooth muscle contraction and permeability. The antagonism toward bradykinin hypotension appears partial. Control and treated dogs showed similar decreases in blood pressure. During a 2-hour observation period the mean fall in blood pressure was 25 mm Hg in placebo treated dogs and 23 mm Hg in enzyme treated dogs. Duration of the hypotensive effect was significantly shorter in enzyme treated animals, varying with the enzyme preparation used (Fig. 1).

Analysis of variance computations indicated a highly significant difference between the placebo and treated groups(4). The F ratio was 18.67 between groups; 69.50 in placebo *vs.* treatments; 1.73 in the enzyme treated groups. There was little variation in average treatment effect among the preparations tested.

By far the greatest sensitivity to bradykinin is displayed by the isolated rat uterus, which responds to as little as 0.003 ng/ml (5). A significantly decreased rat uterus response to bradykinin followed orally given proteases in this study (Table I).

TABLE I. Rat Uterus Contraction.

No. of rats	Treatment	Mean contraction time (sec)	Standard deviation
7	Control	190	15.9
7	Papain + bradykinin	121	13.6
7	SK + bradykinin	116	14.6
7	A. oryzae + bradykinin	128	19.7

Bradykinin has been characterized 10 to 15 times more active than histamine in enhancing capillary permeability(6). In the present study, a marked decrease in size and intensity of the bluing response to bradykinin followed oral enzyme administration (Table II).

It therefore appears that orally given proteases induce bradykinin blocking action in the 3 areas considered in this study. The 2 remaining actions of bradykinin, accumulation of leucocytes and pain stimulation, were not evaluated.

In a prior study involving rabbit ear cellulitis and venous thrombosis, one of us (I.I.) reported marked regression of inflammatory infiltration following proteolytic enzyme therapy(7).

TABLE II. Antagonism to Bradykinin Enchancement of Permeability.

Bradykinin Dose: 0.1 ng

No. of rabbits	Treatment	Mean area of bluing (Mm^2)	Standard deviation
4	Control	41	8.8
	Streptokinase	21	7.5
4	Control	38	5.6
	Papain	17	3.8
4	Control	39	8.7
	A. oryzae	23	4.5

In clinical studies the analgesic effects of enzyme therapy in acute inflammation are well documented(8,9).

Summary. Orally given proteolytic enzymes appear to antagonize 3 bradykinin physiologic responses: hypotension, smooth muscle contraction and enhanced capillary permeability. Whether these observations relate to anti-inflammatory activity of orally given proteolytic enzymes requires further study.

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Influence of the Parathyroids on Composition of Rat Milk.* (28021)

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The demonstration of an effect of parathyroid hormone on lactation has been, in the past, based on differences produced in the rate of weight gain of the litters(1-7). The first report concerning the effect of this hormone on the composition of milk was that of Manunta and Mureddu(8) who reported an increase in calcium concentration of milk following parathyroid extract administration. Mosimann(9) ascribed the increased calcium in the milk of ewes in which lactation was induced by estrogens to an increase in parathyroid activity. However, recently, Toverud and Munson(10,11) noted a paradoxical increase in concentration of milk calcium of lactating parathyroidectomized rats in spite of a depressed serum calcium level. They ascribed most, but not all, of this increase to an increase in concentration of total milk solids. Administration of parathyroid extract immediately after parathyroidectomy prevented the decreased water content of the milk as well as the reduction in level of serum calcium, but the increase in calcium concentration of the milk was not so clearly prevented. Munson suggested that parathyroid hormone influenced the calcium concentrating mechanism of the mammary gland directly.

Studies were undertaken in this laboratory to determine whether the changes in composition of milk which are known to occur following parathyroidectomy in the rat could be attributed directly to a loss of the influence of parathyroid hormone on the mammary epithelium or are the result of changes in the serum calcium which occur following parathyroidectomy.

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Methods. Holtzman albino rats 2 to 3 months old were parathyroidectomized or sham-operated under light ether anesthesia on the 4th, 11th, 12th, or 13th day of lactation. In the days immediately preceding their milking, groups of rats were given diets which varied in calcium and protein content. Some groups were isolated from their litters immediately following parathyroidectomy, some were suckled for 24 hours, and some were returned to their litters for 9 to 10 days before isolation and milking. After 16 hours isolation from the litter, the unanesthetized rat was milked with a specially designed glass milking tube connected to a motor-driven suction apparatus. In order to insure milk letdown, the rat was injected with 1 USP unit of oxytocin or syntocinon prior to milking. Blood was taken by heart puncture at the conclusion of milking.

Calcium determinations were carried out on milk and serum by a method modified from that of Collip and Clark(12). This procedure involves precipitating the calcium with ammonium oxalate, redissolving in perchloric acid and reading on a Beckman Flame Spectrophotometer. Milk samples from groups 3 and 5 were ashed, dissolved in .5N HCl, and filtered before precipitation with ammonium oxalate. Milk samples from groups 1, 2, 4, 6, and 7 were analyzed for calcium directly by precipitating samples of whole milk with ammonium oxalate.

The percentage of total milk solids was determined by drying the samples to a constant weight in a 37°C oven. All calcium determinations on the milk were corrected for total solids.

The specific treatments of different groups