Modification of Kinin Activity in Injured Tissue by Oral Kinases and Proteases. (27988)

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The anti-inflammatory action of orally given proteases has been documented, in the main, by uncontrolled clinical observations (1). In a recent animal study, however, bradykinin blocking effects followed orally given kinases and proteases(2). In view of the current interest in bradykinin as the biochemical mediator of the inflammatory reaction(3), the present study was undertaken to appraise any relationship between the anti-inflammatory activity of orally given enzymes(4,5) and kinin antagonism.

Materials and methods. This study comprised 48 male hamsters. A standard inflammatory stimulus was applied to the everted cheek pouch of the hamster. This stimulus consisted of a 2 minute immersion of the everted pouch in water pre-heated and maintained at 45°C. Animals were anesthetized with Nembutal (50-80 mg/kg) intraperitoneally. Twenty-four animals served as controls and received placebo tablets, orally, every 6 hours for 24 hours. The remaining 24 hamsters were divided into 3 groups of 8: one group received 30,000 units of Streptokinase* plus human plasma every 6 hours; the second group received 100 mg papain† every 6 hours; the third group received 400 casein units fungal protease‡ every 6 hours. All medications were suspended in saline and administered orally *via* stomach tube. The treatment period covered the 24 hours following injury. At the end of the treatment period a biopsy specimen was obtained for histologic examination and a strip of pouch tissue was excised. Tissue strips weighing 375 mg ± 5 mg were used for assay. The tissue was immediately homogenized in a Waring Blendor and each homogenate was eluted into a bath containing an intact rat uterus suspended in 17 ml Tyrode's solution at 37°C. 2 Bromolysergic acid diethylamide (BOL) 10-20 ng was added to the bath and allowed to stay in contact with the tissue 10-15 minutes before each sample was tested. In each experiment isotonic uterine contractions were recorded in the following order: (a) normal contractions, (b) following tissue homogenates from a control, (c) following tissue homogenates from the treated hamster, (d) fol-

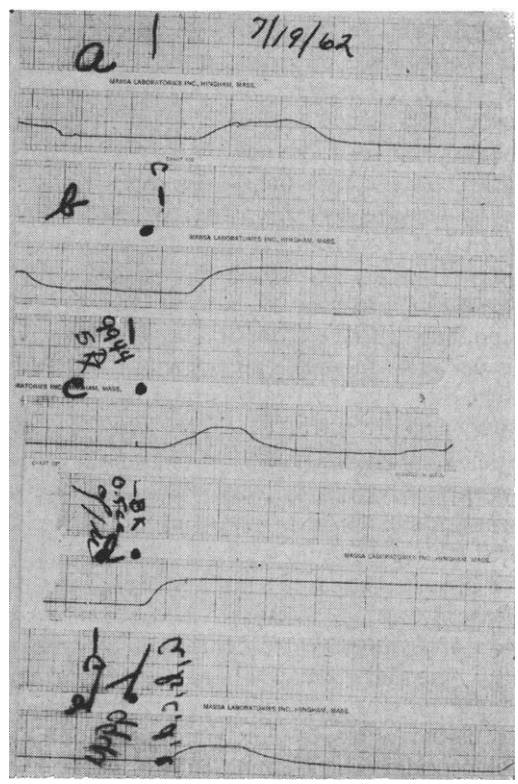

FIG. 1. Rat uterus contractions (a) normal (b) following control pouch homogenate, injured (c) following pouch homogenate, treated with fungal protease (SKF 9944) (d) following bradykinin 0.125 ng (e) following pouch homogenate from opposite, uninjured pouch.

* Varizyme, Lederle.

† Papase, Warner-Chilcott.

‡ Megazyme, Smith, Kline & French.

TABLE I. Characteristics of Uterine Contractions.

	BK	Injured control	SK	F.P.	Pap.	Uninjured control pouch
Observations	48	24	8	8	8	24
Elevated, sustained (Bradykinin-like)	48	21	1	2	1	0
	(100%)	(88%)	(12%)	(25%)	(12%)	(0%)
Recurrent, rhythmic (Normal)	0	3	7	6	7	24
	(0%)	(12%)	(88%)	(75%)	(88%)	(100%)

lowing 0.125 ng bradykinin,§ (e) following tissue homogenates from the opposite, uninjured, hamster pouch (Fig. 1).

Results and discussion. The results are summarized in Table I. Addition of bradykinin to the bath caused slow, sustained, uterine contractions. In 21 of the 24 control hamsters, pouch homogenates produced slow, sustained uterine contractions strongly resembling those following the addition of bradykinin. In these experiments the action of serotonin was excluded by the use of BOL. In 7 of 8 SK treated, 7 of 8 papain treated and 6 of 8 fungal protease treated hamsters, addition of tissue homogenates yielded normal uterine contractions. In each instance tissue homogenates from the opposite, uninjured pouch yielded normal uterine contractions. There was excellent correlation between the presence of necrosis and bradykinin-like uterine contractions. Similarly, tissue with minimal inflammatory involvement correlated well with normal uterine contractions (Fig. 2).

These studies suggest a relationship between bradykinin, or bradykinin-like polypeptides and extent of tissue injury, as measured by the rat uterus assay. Furthermore, the high incidence of normal uterine contractions in treated hamsters (83%) compared with 12% in untreated controls is consistent with the interpretation that orally given kinases or proteases may modify the extent of tissue injury by antagonizing kinin-like polypeptides which condition the inflammatory responses. Whether the modified polypeptide is actually bradykinin, or a peptide with pharmacologic properties resembling bradykinin cannot be determined from data obtained in this study.

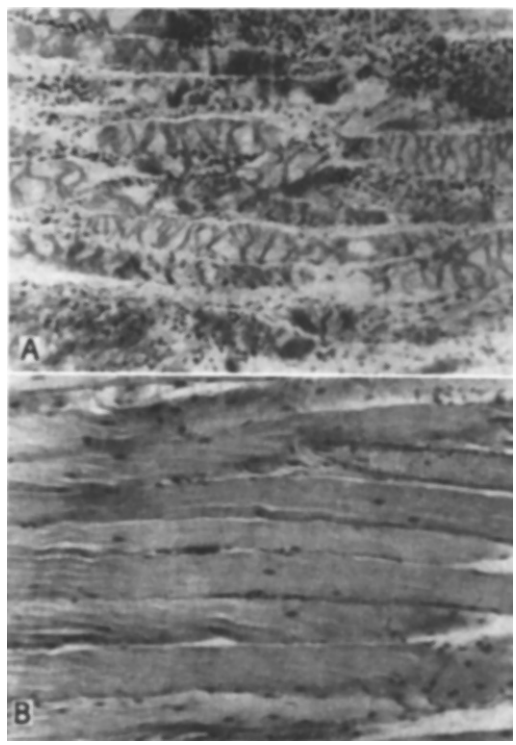

FIG. 2. Histologic appearance of pouch tissue. A. Untreated control 24 hr post injury. Note extensive necrosis and brisk leucocytic infiltration. B. Treated hamster 24 hr post injury. Necrosis is minimal with scanty leucocytes.

§ Synthetic bradykinin, Sandoz.

1. Sherry, S., Fletcher, A. P., Alkjaersig, N., *Physiol. Rev.*, 1959, v39, 343.

2. Innerfield, I., Hochberg, R., in press.

3. Lewis, G. P., *Nature*, 1961, v192, 596.

4. Innerfield, I., Wernick, T., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 506.

5. Innerfield, I., Hochberg, R., *Clin. Therap. Res.*, 1962, v4, 524.

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