DEMONSTRATIONS

The assay of tissue kallikrein in rat intestine

N.H. FRANKISH & I.J. ZEITLIN

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW

Werle (1960) reported the presence of trypsin-activated hypotensive activity, thought to be kallikrein, in the gut wall of rats. He found that a change from normal diet to diets consisting solely of meat, eggs or fat caused increases in this activity in the small intestinal wall. Kallikreins form kinins which have powerful actions on blood flow, vascular permeability and gut motility. A method is described for measuring localized changes in kinin-forming activity in intestinal tissue.

A kinin-forming substrate (KFS) was prepared from human plasma by a modification (Zeitlin, Singh, Lembeck & Theiler, 1976) of the method of Amundsen, Nustad & Waaler (1963). Fasted male Wistar rats were killed, exsanguinated and the gut freed of blood as described by Zeitlin *et al.* (1976).

The stomach, duodenum, jejunum, terminal ileum, caecum, proximal colon and distal colon were removed, opened, washed in Krebs solution and homogenized separately in 0.1 N-HCl (10 ml per g wet weight). Homogenates were allowed to stand for 15 min to destroy kininase, neutralized using 2 N-NaOH and activated autolytically (Zeitlin, 1971). Homogenates were centrifuged and aliquots of supernatant were incubated with excess KFS in 0.1 M-glycine buffer (pH 8.5, 37°C). Enzymic action was stopped by boiling and bradykinin-like activity was assayed using contractions of isolated superfused oestrous rat uterus. The gut extracts contained negligible kininase. Formation of bradykinin-like activity was linear with time and enzyme concentration until 80% substrate consumption. Bradykinin-like activity was characterized by parallel bio-assay.

In normally fed rats, all gut tissues contained kallikrein (Figure 1) with least in the stomach (0.02 μg bradykinin equiv. min^{-1} g^{-1} tissue) and a single large peak in the caecum (0.5 μg min^{-1} g^{-1}). In 24 h fasted rats allowed water *ad libitum*, the level in the duodenum more than doubled to 0.14 μg min^{-1} g^{-1} ($P < 0.05$) while that in the caecum fell to less than half of the fed value ($P < 0.05$).

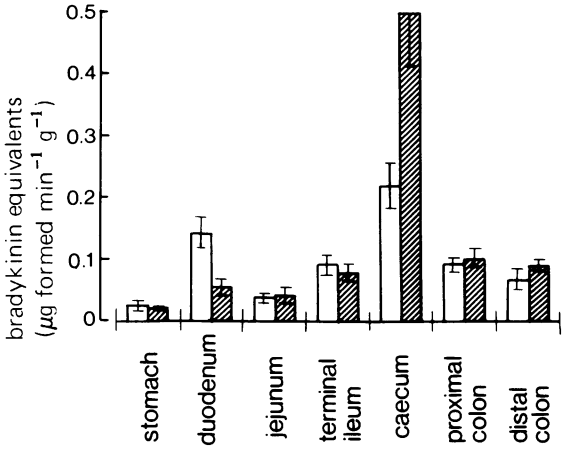

Figure 1 Concentrations of kallikrein in rat intestine, expressed as μg bradykinin equivalent formed min^{-1} $gram^{-1}$ wt of wet tissue. Open columns refer to starved animals while cross-hatching denotes normally fed rats. (Means of 6 rats ± s.e.)

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