Pharmacological characterization of kinin-forming activity in rat intestinal tissue

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Amundsen & Nustad (1965) demonstrated the presence of a small but distinct kinin-forming capacity in isotinic saline extracts of rat intestinal mucosal cells. The presence of kinin-forming activity in hypotonic and hypertonic aqueous extracts of rat intestinal tissue has also been reported by Zeitlin (1970). This communication describes some studies on the nature of the kinin-forming enzyme (KFE) in rat intestinal tissues.

Fasting Wistar rats were killed and the aorta was cannulated just above the diaphragm and ligatured below the renal arteries. The portal vein was cut to allow drainage of the perfusate. Heparinized Krebs solution (10 units/ml) followed by plain Krebs solution was passed through the cannula to flush blood from the intestine. The ileum was removed, washed with Krebs solution and homogenized in distilled water (10 ml/g wet weight). The homogenate was centrifuged at 2,000 *g* for 1 hour. To determine the KFE content of the supernatant, the aliquots were incubated for 15 min with excess of the stable substrate of Amundsen, Nustad & Waaler (1963) in the presence of *o*-phenanthroline (0·001 M) in Krebs solution at 37°C. The enzymic action was stopped by boiling and the incubates were frozen until used for assay of the released smooth muscle stimulant on the isolated oestrous rat uterus preparation. KFE activity was measured in terms of bradykinin equivalent, one unit of KFE being defined as that amount of enzyme which formed 1 μg bradykinin in 15 minutes. The substance formed also inhibited the isolated rat duodenum and contracted the guinea-pig ileum confirming that the released activity was a kinin.

The KFE activity of the tissue was about 1 unit/g wet weight and increased by 5–6 times when the tissue homogenate was incubated for 18 h at 20°C before removal of the supernatant. A similar increase occurred when the homogenate was incubated at 55°C for 10 minutes. Acidification of the homogenate with acetic acid to 0·05 M followed by incubation at 4°C for 18 h as described by Oates, Melmon, Sjoerdsma, Gillespie & Mason (1964) for the extraction of carcinoid kallikrein, produced only a 2-fold increase in KFE. These findings indicate that, as in the pancreas, KFE is present in intestinal tissue as an inactive precursor.

Standard KFE prepared from incubated rat intestine was used for further experiments. The optimum pH of this enzyme was 8·5. The presence of the protease inhibitor aprotinin almost completely inhibited the enzyme (Table 1). The activity was thus distinguished from rat pancreatic kallikrein which is not inhibited by aprotinin (Vogel & Werle, 1970). The enzyme was unaffected by the presence of N-α-*p*-tosyl-L-lysine chloromethyl ketone HCI (TLCK), a specific trypsin inhibitor, and only slightly inhibited by soybean trypsin inhibitor (SBTI) which distinguishes it from plasmin and plasma kallikrein.

TABLE 1. *Effect of inhibitors on rat intestinal kallikrein*

Inhibitor present	Inhibitor concentration	Kinin released as % of control ($n=4$) mean	S.D.
None		100·0	10·5
Aprotinin	100 units/ml	2·6	1·3
TLCK	10^{-3} M	105·3	12·0
SBTI	100 μg/ml	92·5	21·1

Rat intestinal kinin-forming enzyme thus appears to be distinct from trypsin, plasmin, plasma kallikrein and rat pancreatic kallikrein.

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Inhibition by spinal transection of renin release from ischaemic rat kidneys

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The re-establishment of renal blood flow after ischaemia of 4 h duration causes a pressor response in rats anaesthetized with Nembutal (50 mg/kg i.p.). This response is related to the release of renin from the ischaemic kidney and is totally abolished by transection of the spinal cord at any level rostral to thoracic 1 (Hayden & Targett, 1971). These qualitative studies suggested that less renin was released by the ischaemic kidney after spinal transection than by the kidney of an intact preparation.

We have compared the plasma renin activities of arterial blood samples taken before, and 3 min after, re-establishment of the circulation from spinal and intact rats in which the left kidney pedicle had been totally occluded for 4 h (Hayden & Targett, 1971). In the spinal rat preparations the cord was cut between cervical 2 and cervical 3. The mean B.P. of the anaesthetized rats was 80–120 mmHg and 40–60 mmHg in the spinal rats. Plasma renin activity was estimated by the method of McKenzie, Ryan & Lee (1967).

The results of these experiments are given in Table 1.

TABLE 1. *Plasma renin activity of rat arterial blood samples taken before and after re-establishment of blood flow through an ischaemic kidney*

Sample	Plasma renin activity ± S.E.M.†
Before re-establishment of renal blood flow in intact rat	13·4 ± 2·9 (n=11)
3 min after re-establishment of blood flow in intact rat	35·7 ± 4·5 (n=7)
3 min after re-establishment of blood flow in spinal rat	18·6 ± 5·5 (n=8)

†Expressed as ng angiotensin II formed by 0·025 ml plasma incubated at 42° C for 6 h in the presence of an excess of renin substrate.

These results show that a central mechanism is concerned in the release of renin from an ischaemic kidney under these conditions and that the failure of the spinal