Short communications

A kallikrein-like substance in cat nasal secretion

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Incubation of cat nasal secretion with cat plasma releases a substance which will contract the cat isolated jejunum, a tissue specifically sensitive to bradykinin and similar peptides. The smooth muscle stimulating activity of the incubate is potentiated and prolonged by the addition of the kininase inhibitor, EDTA. From the results it is concluded that the incubation of cat nasal secretion and plasma releases a kinin-like substance, and that this kinin may have a role in the secretory and vasomotor activity of the nasal mucosa.

The presence of kallikrein was first demonstrated in dog urine by Frey (1926) and in the human pancreas by Kraut, Frey & Werle (1930).

More recently, Hilton & Lewis (1957), Lewis (1959) and Hilton (1970), have shown that many glandular secretions, such as saliva, contain a plasma kinin-forming enzyme or kallikrein, and that incubation of the secretion with whole plasma or plasma pseudoglobulin releases a substance with vasodilator and smooth muscle stimulating properties. The activity of the incubate declines rapidly owing to the presence of kininases in the plasma and glandular secretion, but it is possible to inhibit these enzymes by the addition of ethylenediamine-tetraacetate (EDTA) to the incubate mixtures (Erdös & Yang, 1970).

The present study was undertaken to determine whether cat nasal secretion, when incubated with cat plasma, releases a kinin like substance.

Methods.—Cats were anaesthetized with pentobarbitone sodium, 40 mg/kg intraperitoneally. Nasal secretion was induced either by the intravenous administration of carbaminoyl choline, 1–3 μg/kg, or by electrical stimulation of the Vidian nerve (Eccles & Wilson, 1973). The secretion was stored for up to 48 h in a polythene container at 4°C and diluted with distilled water (up to 1 in 50 dilution) before use.

Blood was collected in a glass centrifuge tube from the anaesthetized, heparinized cat (250 units mucous heparin/kg) through a polythene cannula inserted into the femoral artery. The plasma was separated by centrifuging at 5,000 *g* for 20 min and stored for up to 48 h in a polythene container at 4°C.

The jejunum was taken from the anaesthetized cat and cut longitudinally into several strips approximately 8 mm wide and 2–3 cm long. The strips were washed and then stored in Krebs solution at 4°C for up to 48 h before use. The cat jejunum has previously been reported to be specifically sensitive to bradykinin and similar peptides (Ferreira & Vane, 1967).

Detection of kinin-like substances

To test whether nasal secretion acted on plasma to produce a substance with smooth muscle stimulating activity, samples were incubated with fresh whole cat plasma both with and without EDTA, at room temperature (21°C), for up to 30 min (the concentration of trisodium EDTA in the incubates was 0·013 g/ml). The incubates were then tested on the isolated cat jejunum suspended in a 10 ml organ bath containing atropinized (150 μg/l atropine) Krebs solution (NaCl 6·9, KCl 0·35, $CaCl_2.6H_2O$ 0·5, KH_2PO_4 0·16, $MgSO_4$ 0·29, glucose 1, $NaHCO_3$ 2·1, g/l in distilled water) at 37° C. The bath was bubbled with a mixture of 5% CO_2 and 95% O_2. The preparation was allowed to equilibrate with a resting tension of 2 g for a period of 2 hours. In all experiments the viability of the tissue was tested by the addition of synthetic bradykinin (Sandoz) to the tissue bath, and any tissue failing to respond to the peptide was discarded. The incubate mixtures remained in contact with the isolated tissue for 1 min and, after two washes, 4 min was allowed for the tissue to relax. Contractions of the preparations were recorded with an isotonic transducer (Devices type 2LD01) and pen recorder (Devices M2).

Results.—In thirteen experiments the addition of either cat nasal secretion or plasma to the tissue bath failed to give a response. Similarly, no response could be obtained when nasal secretion and EDTA, or plasma and EDTA were incubated in a polythene container for periods of 10 min

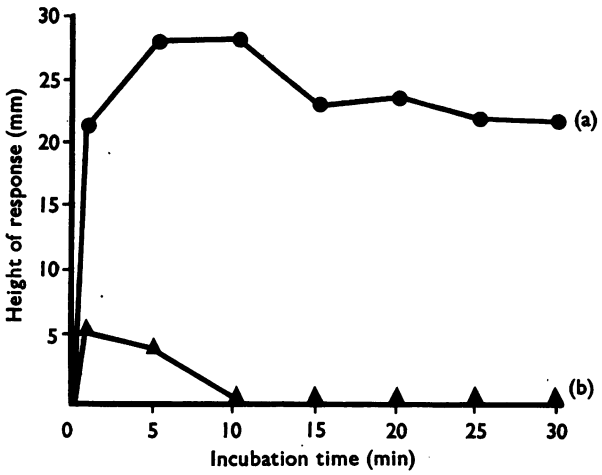

FIG. 1. Contractions of the cat jejunum induced by 0·3 ml aliquots of the incubates. (a) 0·7 ml nasal secretion, 0·7 ml plasma, 0·7 ml EDTA solution (final EDTA conc. 0·013 g/ml). (b) 0·7 ml plasma, 0·7 ml nasal secretion, 0·7 ml distilled water.

at room temperature and then added to the tissue bath. In these experiments, however, slow graded contractions of the tissue were caused by the addition of synthetic bradykinin, 0·05–5·40 ng/ml. When nasal secretion and plasma were incubated together in equal volumes (0·5–2·0 ml) in a polythene container at room temperature with EDTA solution or an equal volume of distilled water, the incubates caused a slow contraction of the jejunum. Aliquots (0·3 ml) of the incubates were added to the tissue bath at different periods of time to determine the activity during a 30 min period of incubation. The incubates containing EDTA developed maximal activity after 10 min incubation and still caused a considerable contraction of the jejunum after 30 min incubation. In comparison, similar incubates without EDTA developed maximal activity after 0·5–5 min incubation, which declined or disappeared during the 30 min incubation period. The results of one experiment are shown in Fig. 1 which demonstrates that the activity of the incubated secretion and plasma was much greater in the presence of EDTA.

Discussion.—These results show that a substance with smooth muscle-stimulating activity is released on incubation of cat nasal secretion and plasma. The substance is probably a kinin since it contracts the isolated cat jejunum, a tissue specifically sensitive to kinins. The maximum activity of the incubate occurs within 0·5–5·0 min in the absence of EDTA, and then declines rapidly and has no smooth muscle-stimulating activity after 20–30 minutes. These results are similar to those of Lewis (1959), who incubated human saliva and dog pseudoglobulin and found maximal activity after 90 s–5 min but no smooth muscle-stimulating activity after 20 min incubation. The inactivation of the kinin is probably due to kininase enzymes which are known to be present in glandular secretions and plasma (Erdös & Yang, 1970). The kininase enzymes are inhibited by EDTA and this could explain the potentiation and prolongation of the smooth muscle-stimulating activity of the incubate when nasal secretion and plasma are incubated in the presence of EDTA.

The release of kinin on incubation of nasal secretion and plasma could be due to the presence of a kallikrein in nasal secretion similar to that demonstrated in cat saliva (Hilton, 1970). It is also possible that nasal secretion contains a kallikrein activator which acts on a plasma pre-kallikrein to initiate the release of plasma kallikrein (Eisen & Vogt, 1970). Although this possibility cannot be excluded it is unlikely that a plasma pre-kallikrein is involved in the release of kinin by nasal secretion because any plasma pre-kallikrein would probably be exhausted by contact with glassware during the collection and spinning of the whole blood.

The results demonstrate that cat nasal secretion releases a kinin from cat plasma. The kinin may play a part in the normal secretory and vasomotor processes of the nasal mucosa and could also have a role in inflammatory nasal disorders.

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