Stimulation of histamine release by the peptide kinetensin

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Abstract

The peptide kinetensin isolated from pepsin-treated human plasma induced a dose-dependent release of histamine when exposed to rat peritoneal mast cells. The threshold concentration was around $10^{-6}$ M, the ED$_{50}$ was $10^{-5}$ M, and the optimal concentration of between $10^{-4}$ to $10^{-3}$ M released 80% of the total histamine. Kinetensin was 10 to 100 times less potent than neurotensin and equipotent with the opioid peptide dynorphin. The histamine release was clearly temperature-dependent, with no release occurring at 0°C or 45°C and with an optimum around 37°C. The histamine release was significantly reduced in the absence of extracellular calcium. Kinetensin also induced a dose-dependent increase in vascular permeability when injected intradermally into rats. The findings indicate that kinetensin is a potent histamine releaser in the rat and may serve as an inflammatory mediator.

Introduction

An increasing number of peptides have been reported to release histamine ever since the report from Johnson and Erdös in 1973 [1], describing the effect of some vasoactive peptides like bradykinin and substance P. The histamine releasing effect of peptides is believed to be coupled to the presence of basic amino acids especially arginine. During recent years, there have been several reports concerning structure activity relationships [2–4]. However, the exact receptor interaction with the basic peptides is still an unsolved question. One reason might be that it is a rather unspecific receptor [5] or even a mechanism independent of receptor activation [6]. Kinetensin (Ile-Ala-Arg-Arg-His-Pro-Tyr-Phe-Leu) was originally isolated from pepsin-treated human plasma [7]. This neurotensin-like peptide with two arginine amino acids is basic and has sequence homologies with certain regions of human serum albumin. As neurotensin is a very potent histamine releaser, the aim of the present study was to investigate the effect of kinetensin on mast cells.

Methods

Mast cells were obtained from peritoneal and pleural washings of male Sprague-Dawley rats. The cells were washed three times and used without purification. Mast cell concentrations were usually around 150,000/ml and the incubation volume was 0.2 ml. The incubation was stopped by the addition of 1.8 ml of ice-cold buffered saline and cells were separated from supernatant by centrifugation. The incubation medium was a buf-

Figure 1
(a) Log dose-response curve for the release of histamine induced by kinetensin. Rat peritoneal mast cells were incubated with kinetensin at 37°C for 10 min. Histamine release is expressed as a percentage of the total histamine in the mast cells and the spontaneous release has been deducted. Values are means ± SEM for 6-8 experiments except for 10⁻³ M kinetensin which was tested in 3 experiments. (b) Log dose-response curve for the release of histamine induced by different basic peptides. Mast cells were incubated with the different secretagogues at 37°C for 10 min. Histamine release is expressed as a percentage of the total histamine, when induced by neurotensin (o, NT), dynorphin (△, Dyn), kinetensin (○, KT), substance P (●, SP), β-endorphin (■, BE) and luteinizing hormone-releasing hormone (Δ, LH-RH). Values are means for 3-12 experiments.

Results
Kinetensin induced a dose-dependent release of histamine from rat peritoneal mast cells (Fig. 1a). The histamine release started at 10⁻⁶ M and the ED₅₀ was around 10⁻⁵ M. Kinetensin at 10⁻⁴ - 10⁻³ M induced an optimal release of around 80% of the total histamine. When comparing the effect of kinetensin to that of other basic peptides (Fig. 1 b), it was found to be 10 to 100 times less potent than neurotensin but having a higher intrinsic activity and inducing a greater maximum release of histamine. Kinetensin was about equipotent with dynorphin and about one order of magnitude more potent than substance P and β-endorphin.

Rat peritoneal mast cells were incubated with kinetensin (10⁻⁵ M) for 10 min at different temperatures (Fig. 2). The kinetensin-induced histamine release was temperature-dependent, with no release occurring at 0°C and with a decreased responsiveness at 45°C. There was an optimal release at around 30-37°C.

Vascular permeability
Anesthetized Sprague-Dawley rats were given ¹²⁵I-albumin i.v. Samples were then injected intradermally in 5 x 2 spots on the back and comprised saline as a control or kinetensin in different doses in 100 µl saline. After 20 min, skin biopsies of 7 mm diameter were cut out, weighed and transferred to a gamma-counter. Results are expressed as: (counts per min (cpm) in tissue per gram wet weight/cpm in plasma per ml plasma). Kinetensin was synthetized and kindly provided by Drs. C. and N. Yanaihara, Shizuoka, Japan.

All values are given as means ± SEM for the number (n) of experiments noted.