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Effects of ranatensin, a polypeptide from frog skin, on isolated smooth muscle

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Summary

1. The actions of bretylium tosylate on neuromuscular transmission in the rat phrenic nerve diaphragm preparation have been investigated by electro-

2. On the guinea-pig ileum, threshold doses elicit repeated maximal spike contractions which are blocked by atropine. In the presence of atropine, higher concentrations of ranatensin elicit small contraction spikes superimposed on a relatively weak sustained contraction. These latter two actions are not blocked by increasing the concentration of atropine.

3. Other smooth muscle preparations respond as follows: rat uterus, rhythmic contractions; rat duodenum, relaxation; rabbit aortic strip, contraction. Atropine has no effect on the above responses. The rat aortic strip does not respond to ranatensin. Ranatensin is four times as active as bradykinin on the rat uterus.

4. Ranatensin can be readily distinguished from other known peptides such as angiotensin, bradykinin and the eledoisin-like peptides, by bioassay.

Introduction

Ranatensin is a potent, biologically active peptide (Pyr-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met·NH_2) which occurs free in the skin of *Rana pipiens* (Nakajima, Tanimura & Pisano, 1970). The structure was established by classical degradation techniques and confirmed by synthesis. It bears a distant resemblance to eledoisin-like peptides in that it contains eleven residues and has pyroglutamic acid at the first, and methionine amide at the last, position. The remaining structure and the spectrum of pharmacological actions are, however, quite different.

This report presents the findings on several isolated smooth muscle preparations. Ranatensin has a wide spectrum of smooth muscle effects including some actions that are similar to angiotensin, bradykinin and eledoisin-like peptides. The peptide has, in addition, some unique actions. Using parallel bioassays, it can be readily distinguished from all other known peptides.

Methods

The following isolated tissue preparations were used: portions of the terminal ileum obtained from male Hartley guinea-pigs weighing 300–350 g, rat duodenum,

rat uterus (both oestrus and non-oestrus), spirally cut rat thoracic aorta obtained from both Sprague-Dawley and Osborne-Mendel rats weighing approximately 200 g, and spirally cut rabbit thoracic aorta obtained from New Zealand white rabbits weighing 1,000–1,200 g. The data to be presented are representative of five to ten preparations of each type.

All tissues were suspended in 10 ml baths containing physiological solutions maintained at 37° C and bubbled with 95% O_2–5% CO_2. De Jalon's solution (NaCl, 152 mM ; KCl, 5·6 mM ; $CaCl_2$, 0·4 mM ; $NaHCO_3$, 6·0 mM ; Dextrose, 2·8 mM) was used as the bathing medium for the uterus preparations, and Krebs solution (NaCl, 113 mM ; KCl, 4·7 mM ; $CaCl_2$, 2·5 mM ; KH_2PO_4, 1·2 mM ; $MgSO_4 \cdot 7H_2O$, 1·2 mM ; $NaHCO_3$, 26 mM ; Dextrose, 11·5 mM) was used for all other tissues. The tissues were allowed to equilibrate in the bath for 1–2 h before the experiments were started. An initial tension of 1·5 g was applied to the aortic strips and 1·0 g to all other tissues. Drugs were added to the baths in a volume of 0·05–0·2 ml. Isometric contractions were recorded through Grass transducers and displayed on a Grass recorder.

The following drugs were used: ranatensin (natural and synthetic, prepared in this laboratory), angiotensin (Hypertensin, CIBA), bradykinin (Sandoz), oxytocin (Calbiochem), propranolol (Inderal, Ayerst), phentolamine (Regitine, CIBA), morphine sulphate, hexamethonium bromide, atropine sulphate, lidocaine (Xylocaine, Astra) and mepyramine maleate. All drugs were dissolved in normal saline. Doses are expressed on a molar basis. The agonist concentrations and the order of administration were randomized during the course of the experiments to avoid artifacts due to desensitization.

Results

Preliminary experiments established that the responses of selected tissues to either natural or synthetic ranatensin were identical. Because of the limited supply of natural material, the experiments reported here were performed with the synthetic peptide.

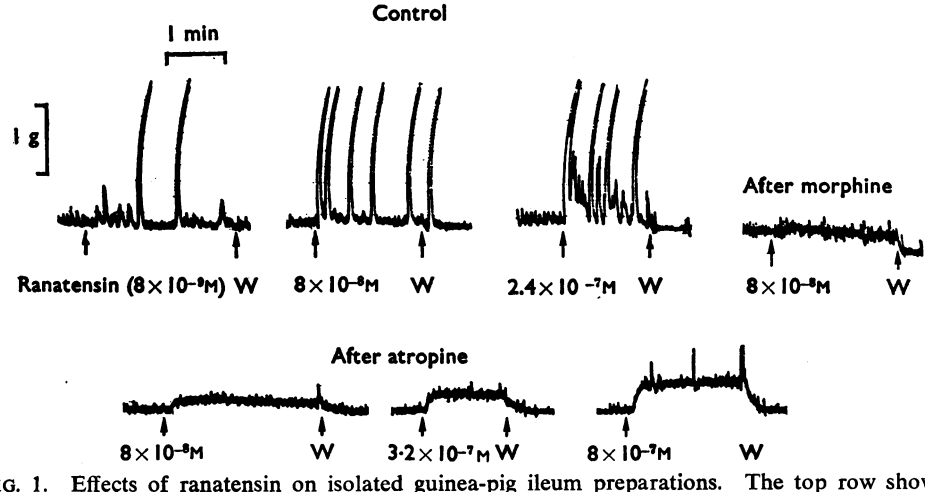

FIG. 1. Effects of ranatensin on isolated guinea-pig ileum preparations. The top row shows responses to ranatensin before and after morphine ($3·6 \times 10^{-6}$M). Responses to ranatensin following treatment of another ileum with atropine ($1·4 \times 10^{-6}$M) are shown in the bottom row. In all of the records, ranatensin was added at the arrow and washed out as indicated (w). The concentrations indicated are final concentrations in the bath.

Guinea-pig ileum

Ranatensin appears to have three distinct actions on the isolated guinea-pig ileum. The primary response consists of a burst of repeated spike contractions (Fig. 1). The threshold for this response, consisting of maximal and submaximal spikes, is $4–8 \times 10^{-9}$M. At higher concentrations, ranatensin produces predominantly maximal spikes and the highest frequency of spiking is seen at $2–4 \times 10^{-7}$M. The β-adrenoceptor blocking agent, propranolol (10^{-7}M), the α-adrenoceptor blocking agent, phentolamine (2×10^{-6}M), the ganglion blocking agent, hexamethonium (10^{-6}M), and the antihistamine, mepyramine ($3{\cdot}5 \times 10^{-6}$M) have no effect on the spike eliciting action of ranatensin. These contractions are, however, blocked by morphine ($3{\cdot}6 \times 10^{-6}$M) and atropine ($1{\cdot}4 \times 10^{-6}$M) (Fig. 1). They are also blocked by the local anaesthetic, lidocaine (5×10^{-3}M) and partially blocked by large doses of mepyramine ($3{\cdot}5 \times 10^{-5}$M).

The other two actions of ranatensin can more easily be observed in the presence of atropine. The peptide produces a sustained contraction which persists until it is washed out of the bath (Fig. 1, lower section). The threshold dose for this effect is about 8×10^{-8}M and the maximum response is seen at 8×10^{-7}M. At this higher dose of ranatensin, repeated spike contractions of low frequency occur superimposed on the sustained contraction. Neither the spikes nor the sustained contraction was blocked by increasing the concentration of atropine.

The response of the untreated guinea-pig ileum to ranatensin is sufficiently different in character from that produced by other peptides to make it difficult to compare potencies. The characteristics of the contractions produced by ranatensin, angiotensin, and bradykinin are shown in Fig. 2. In the atropinized preparation, however, the response to ranatensin is similar to that produced by the other peptides, but ranatensin is approximately 1,000 times less potent than angiotensin and 100 times less potent than bradykinin.

Rat duodenum

Ranatensin produces a relaxation of the isolated rat duodenum which is not altered by atropine, phentolamine or propranolol. Bradykinin also relaxes this tissue, but its action is associated with a decrease in spontaneous activity. Ranatensin does not affect the spontaneous activity. Angiotensin, on the other hand,

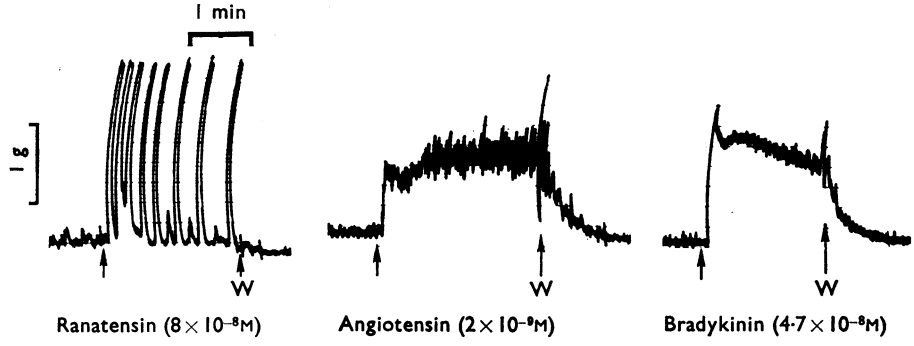

FIG. 2. Comparison of the response of the isolated guinea-pig ileum to ranatensin, angiotensin and bradykinin. The peptides were added to the bath at the arrows and washed out as indicated (w).

contracts and increases the activity of the duodenum. Representative responses to these three peptides are presented in Fig. 3.

Rat uterus

Ranatensin is an extremely potent stimulant of the isolated rat uterus. At low concentrations, it produces short rhythmic contractions ; at somewhat higher concentrations, a sustained contraction occurs which gradually fades into a rhythmic pattern (Fig. 4). The threshold of the rat uterus for ranatensin is approximately 2×10^{-11}M. There is no difference in response between oestrus and non-oestrus uteri. Angiotensin and bradykinin also produce rhythmic and sustained contractions in this preparation. The threshold for angiotensin is about 5×10^{-10}M and that for bradykinin about 1×10^{-10}M in our preparations. The physical appearance of the recordings produced by ranatensin, angiotensin and bradykinin are so nearly identical that we could not distinguish between them. Oxytocin, on the other hand, effects a more prolonged contraction with less frequent rhythmic contractions than any of the other three peptides tested. The threshold for oxytocin was 1×10^{-7}M.

FIG. 3. Response of the isolated rat duodenum to ranatensin, bradykinin and angiotensin. The peptides were added at the arrow.

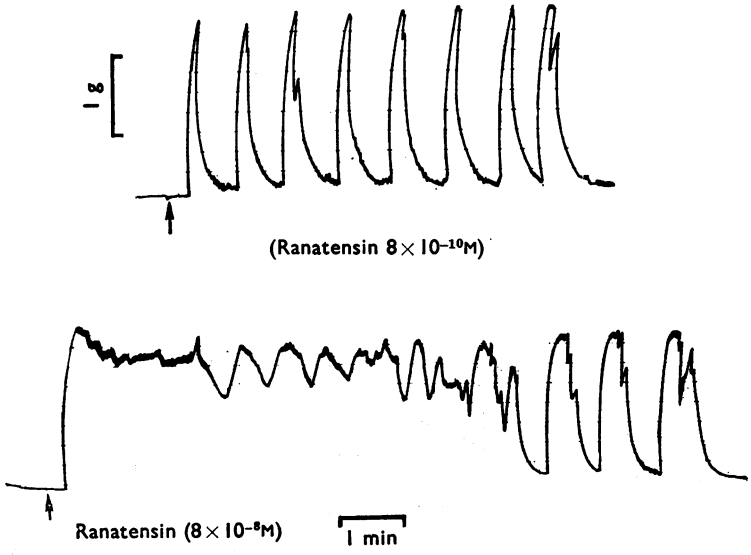

FIG. 4. Response of the isolated rat oestrus uterus to ranatensin. The effect of 8×10^{-10}M ranatensin is shown at the top and the response to 8×10^{-8}M at the bottom. The peptide was added at the arrow.

Intravenous infusion of ranatensin ((0·001–1 μg/kg)/min) produced rhythmic contractions of the rat uterus, *in vivo,* which were similar in appearance to those in the *in vitro* preparation. A single intravenous dose (10 μg) produced a sustained contraction which faded into rhythmic activity. An attempt was made to alter the normal gestation period of rats in the eighteenth, twentieth or twenty-first day of pregnancy. In each animal, either (1 μg/kg)/min of ranatensin was infused intravenously for 2–3 h, or single 10 μg doses were administered every 30 min during this period. Although the *in vivo* pregnant rat uterus contracted in response to ranatensin, the foetuses did not abort.

Rabbit thoracic aortic strip

Ranatensin produces a relatively weak sustained contraction of the rabbit aortic strip. Dose-response curves comparing ranatensin with angiotensin and bradykinin are presented in Fig. 5. Angiotensin was the most potent of the three peptides, followed by bradykinin and then ranatensin. Despite the marked differences in potencies among the three peptides, their threshold concentrations were very similar (8×10^{-8} to 10^{-7}M). The ranatensin response was not affected by atropine, phentolamine or propranolol.

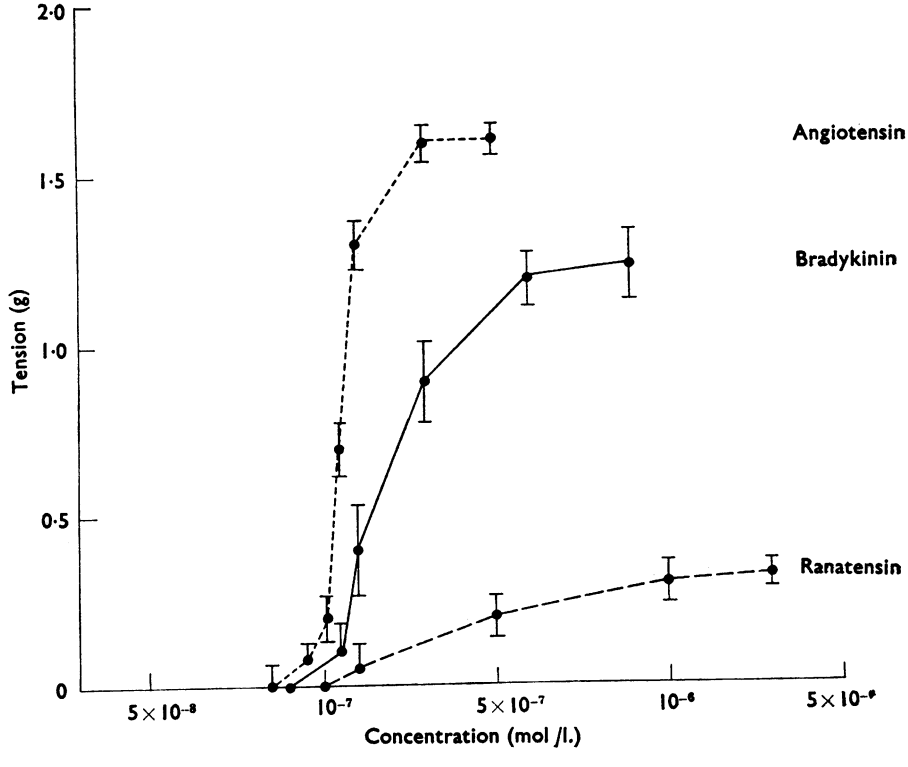

FIG. 5. Dose-response curves for ranatensin, bradykinin and angiotensin in the rabbit thoracic strip. Each peptide was tested individually on five separate strips. The curves represent mean responses for each group of five strips. Standard errors of the mean values are indicated by the vertical bars.

Rat thoracic aortic strip

The rat thoracic aortic strip neither contracts nor relaxes (following serotonin induced contraction) to ranatensin in concentrations up to 2×10^{-6}M.

Discussion

The composite picture presented by the actions of ranatensin on isolated smooth muscle preparations is unlike that of any other known peptide. The many unique actions of ranatensin provide a fairly simple means for pharmacological identification. Table 1 summarizes the primary characteristics of ranatensin and compares them with those of bradykinin, angiotensin and eledoisin-like peptides. It is quite likely that the response of the guinea-pig ileum alone would suffice to identify this peptide. However, this response combined with relaxation of the rat duodenum and an absence of response by the rat aortic strip would make the identification a certainty.

Ranatensin has unique actions on the guinea-pig ileum consisting of repeated, rapid, maximal spike contractions superimposed on a sustained contraction. A possible involvement of acetylcholine in this response was considered. The spike contractions were completely blocked by atropine, suggesting that this activity could be the result of a direct cholinergic action of the ranatensin molecule or an indirect action through the release of endogenous acetylcholine. Morphine, a compound which blocks the release of acetylcholine (Paton, 1957), completely eliminated the repeated contractions. Similarly, compounds having a local anaesthetic action, that is, lidocaine and high concentrations of pyrilamine, also blocked this activity. These observations, although indirect, suggest that ranatensin may release acetylcholine from cholinergic nerves in the intestine. Other compounds release small amounts of acetylcholine in the intestine (Paton & Zar, 1968) but do not produce repeated maximal contractions. The site of action of ranatensin in the ileum is not clear. Apparently ganglionic stimulation is not involved since the response is not affected by hexamethonium.

The sustained contraction produced by ranatensin acting on an atropinized ileum could be a direct non-cholinergic effect of the peptide. Other peptides, including angiotensin, bradykinin, physalaemin (Bertaccini, Cei & Erspamer, 1965) and eledoisin (Erspamer & Erspamer, 1962) also produce a sustained atropine resistant contraction of the guinea-pig ileum. None of these peptides, however, elicits repeated maximal spike contractions. The third action of ranatensin on the ileum, that

TABLE 1. *Responses of isolated smooth muscle preparations to various peptides*

Preparation	Ranatensin	Bradykinin	Angiotensin	Eledoisin-like peptides
Guinea-pig ileum	1. Atropine sensitive maximal spikes 2. Atropine resistant sustained contraction 3. Atropine resistant spikes superimposed on the sustained contraction	Sustained contraction	Sustained contraction	Sustained contraction
Rat duodenum	Relaxation	Relaxation	Contraction	Contraction
Rat uterus	Contraction	Contraction	Contraction	Contraction
Rat aorta	No response	Contraction	Contraction	–
Rabbit aorta	Contraction	Contraction	Contraction	–

is the low frequency, submaximal spikes which become apparent in the atropinized ileum, would also seem to be of a non-cholinergic nature.

Relaxation of the rat duodenum has been considered to be a characteristic identifying feature of kinins such as bradykinin and kallidin (Schachter, 1964). It is of interest, therefore, that ranatensin produced a marked relaxation of this tissue. Yet, unlike a true kinin, ranatensin does not increase capillary permeability and has a hypertensive rather than a hypotensive action in some species (Geller, Govier, Pisano, Tanimura & Van Clineschmidt, 1970).

Thus, ranatensin does not fit conveniently into any of the currently established groups of peptides. It relaxes the rat duodenum, yet it is certainly not a kinin by other criteria. It shows some species specificity in its ability to contract aortic smooth muscle, producing a response in rabbit but not in rat preparations. It would also appear to produce bursts of acetylcholine release in the guinea-pig ileum by acting at some point along the postganglionic nerve fibre. This latter action may have some application in studies of the mechanism of acetylcholine release.

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