

THE EFFECTS OF BRADYKININ AND PROSTAGLANDIN E₁ ON RAT CUTANEOUS AFFERENT NERVE ACTIVITY

LORIS A. CHAHL

Department of Physiology, University of Queensland, St. Lucia, 4067, Australia

A. IGGO

Department of Veterinary Physiology, University of Edinburgh, Summerhall, Edinburgh

- 1 The activity produced by intra-arterial bradykinin and prostaglandin E₁ was investigated in multifibre strands dissected from the saphenous nerves of anaesthetized rats.
- 2 Bradykinin (0.5–10 µg) alone produced little activity in nerve strands but produced considerable activity following a 10 min infusion, but not a single injection, of prostaglandin E₁ (5–100 ng).
- 3 Prostaglandin E₁ alone produced a few large height spikes but following several injections of bradykinin smaller height spikes were also produced by prostaglandin E₁.
- 4 It was concluded that the presence of a low concentration of prostaglandin E₁ is required for bradykinin to manifest its action and that bradykinin and prostaglandin E₁ are mutually potentiating in their effects on afferent nerve terminals.

Introduction

Prostaglandin generation has now been shown to take place in many forms of damage to the skin (Greaves & Søndergaard, 1970; Ånggård & Jonsson, 1971). It was suggested by Ferreira (1972) that the prostaglandins sensitize 'pain' receptors to stimulation by other inflammatory mediators, and it has been widely accepted that inhibition of synthesis of prostaglandins constitutes the principal mechanism of action of the non-steroidal anti-inflammatory agents. This paper reports the electrophysiological effects of bradykinin and prostaglandin E₁ both alone and in combination on cutaneous afferent nerve strands of the rat.

Methods

The method used by Fjällbrant & Iggo (1961) was adapted for use in rats. Male albino rats, 220–270 g, were anaesthetized with urethane (1250–1500 mg/kg i.p.). The trachea and jugular vein were cannulated and an incision was made in the skin of the right hind limb overlying the saphenous nerve. A thread was placed under the femoral artery proximal to branching of the saphenous artery and branches other than the saphenous artery were ligated. A cannula was then inserted in a retrograde direction into the femoral artery distal to branching of the saphenous artery. During insertion of the cannula slight tension was exerted on the thread under the femoral artery to prevent blood loss. The cannula was filled with Tyrode solution before insertion and connected to a three-way

tap. Provided there was no leak in the cannula or tap there was no backflow of blood into the cannula and hence heparin was not used in these experiments. The dorsal surface of the right hind limb was then partially embedded in gypsum and a liquid paraffin pool was made over the saphenous nerve. Recordings were made from dissected multifibre strands of the nerve using silver wire electrodes. The signals were amplified and simultaneously displayed on an oscilloscope and recorded on magnetic tape. Drugs in volumes 0.05–0.1 ml were usually injected into the arterial cannula over a period of 30 s and followed by 0.1 ml of Tyrode solution injected over the following 30 seconds. A control injection of 0.2 ml of Tyrode solution was tested at the start of each experiment. Activity in the strands was observed for a minimum time of 10 min following an injection. In some experiments prostaglandin E₁ solution (0.5–1.0 ml) was given as a slow infusion over 10 minutes. In these experiments the response to infusion of a similar volume of Tyrode solution was also tested. The response to mechanical stimulation by stroking the skin was used as an indication of the stability of the recording conditions and the functional state of the strand.

Since it was suggested by Crunkhorn & Willis (1971) that prostaglandins of the E-type produce inflammatory effects by releasing amines from mast cells, some experiments were performed in rats pre-treated with compound 48/80 to deplete mast cells of histamine and 5-hydroxytryptamine. Rats were

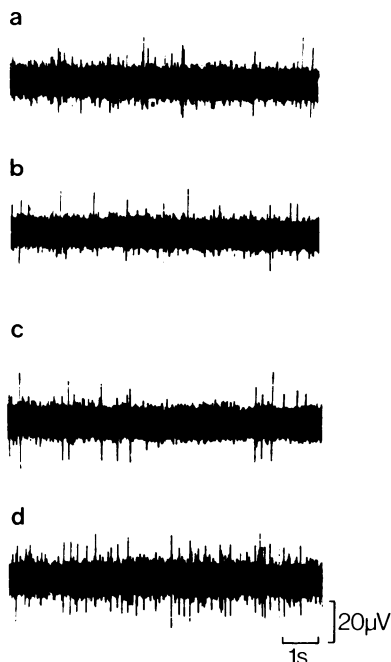


Figure 1 Effect of 10 min infusion of prostaglandin E_1 (5 ng) on response to bradykinin (0.5 μ g). Control responses were recorded in the minute preceding the bradykinin injections. (a) Control; (b) 1 min after injection of bradykinin; (c) 20 min later—control after infusion of prostaglandin E_1 ; (d) 1 min after 2nd injection of bradykinin (note large spikes).

pretreated with twice daily injections of compound 48/80 intraperitoneally for 4–10 days before the experiment. For the first six injections a dose of 0.6 mg/kg was used and thereafter the dose was increased to 1.2 mg/kg. The strain of rats used appeared to be more difficult to deplete than those used by Di Rosa, Giroud & Willoughby (1971) who used a similar regime, since even after several days treatment with compound 48/80 the feet were often swollen on the day of the experiment. Therefore 4 rats were given two intra-arterial injections of compound 48/80 (1 rat—0.1 ml of 10 μ g/ml; 3 rats—0.1 ml of 100 μ g/ml) at the start of the experiment.

Drugs

The following were used: bradykinin triacetate (Sigma), compound 48/80 (Wellcome), 5-hydroxytryptamine creatinine sulphate (Sigma) and prostaglandin E_1 (Upjohn), concentrations and doses being expressed as such.

Results

Bradykinin

Bradykinin was injected intra-arterially at the start of 18 experiments before injection of any other drug, in doses varying from 0.5 μ g to 10 μ g. In 13 of these experiments very little change in activity in the nerve strands was recorded and in the other 5 experiments no change in activity was apparent (e.g. Figure 1). In only 1 experiment was there marked response to bradykinin (10 μ g).

Prostaglandin E_1

Injection of prostaglandin E_1 in doses 5–100 ng initiated low frequency, irregular firing in a very few fibres in strands from 12 rats which had been given no other drugs, but in 3 rats there was no response. The response was slow in onset, usually occurring 2–5 min after the injection started. The response, although slight, was often long-lasting and sometimes present for as long as 20 minutes. The spikes were large in height and in 3 experiments the response was compared with the response of the same strand to 5-hydroxytryptamine (1–3 μ g). It was found that more units were excited by 5-hydroxytryptamine than by prostaglandin E_1 and that more smaller height spikes were produced. The onset of action of 5-hydroxytryptamine was also more rapid than that of prostaglandin E_1 , usually starting within the 30 s injection time of the 5-hydroxytryptamine dose. In 5 experiments, where higher doses of prostaglandin E_1 (1–10 μ g) were tested, no greater response was obtained than with lower doses.

The response to prostaglandin E_1 was still present in 4 out of 5 rats which had been pretreated with twice daily intraperitoneal injections of compound 48/80, 4–10 days before the experiment, and also in 3 out of 4 rats pretreated with two intra-arterial injections of compound 48/80 at the start of the experiment.

Prostaglandin E_1 after bradykinin

Prostaglandin E_1 injections (6 experiments) or infusions (15 experiments) 5–100 ng, were given following an initial injection of bradykinin (0.5–10 μ g). In these experiments the response to prostaglandin E_1 did not obviously differ from the response in those experiments where it was infused before any other drug. However in experiments where 2 further injections of bradykinin were given it was noted that there was potentiation of the response to injection of 10 ng prostaglandin E_1 (Figure 2).

Bradykinin after prostaglandin E_1

In 6 experiments the response to injection of bradykinin, 0.5–10 μ g, was not potentiated following a

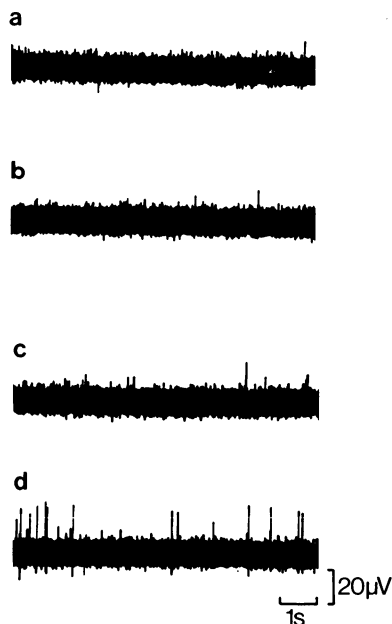


Figure 2 Potentiation of response to injection of prostaglandin E₁ (10 ng) by several injections of bradykinin (10 μg). Control responses were recorded during the minute preceding the prostaglandin injections. (a) Control 40 min after 1st injection of bradykinin; (b) 1 min after injection of prostaglandin E₁; (c) 80 min later—control 20 min after 2 further injections of bradykinin; (d) 1 min after injection of prostaglandin E₁ (note greater number of large and small spikes).

single injection of prostaglandin E₁ or by simultaneous injection of prostaglandin E₁ (5–100 ng). However, marked potentiation of the bradykinin response following a 10 min infusion of prostaglandin E₁ (5–100 ng) occurred in 9 out of 13 experiments (e.g. Figure 1). A 10 min infusion of the same volume of Tyrode solution did not increase the response to bradykinin. The bradykinin response after infusion of prostaglandin E₁ started within 30 s of completion of the injection i.e. during the Tyrode injection. Potentiation of either or both small height spike responses and/or large height spike responses were seen. These differences were probably due to differences in the size of strands as well as in the units contained in the strands. In those experiments where there was no potentiation it was found that the response to mechanical stimulation by stroking the skin was reduced, indicating that recording conditions had altered, probably due to leakage of fluid around the strand. This was a major problem in these experiments because of oedema production by the drugs. The greatest potentiations of bradykinin were found after infusion of 5–10 ng of prostaglandin E₁.

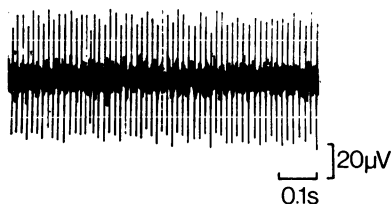


Figure 3 Rapid firing of a unit 1 min after injection of bradykinin (5 μg) preceded by a 10 min infusion of prostaglandin E₁ (5 ng).

The duration of the potentiation was difficult to assess since there was a gradual increase in activity in the strands during the course of the experiments. However it appeared to be relatively short-lived since in 3 experiments where a second injection of bradykinin was given, it was not as effective as the first following the infusion of prostaglandin E₁.

In 2 experiments bursts of activity of a unit with a regular firing rate occurred following the injection of bradykinin after infusion of prostaglandin E₁ (Figure 3). In one experiment the rate of firing of the unit reached a maximum of 170/second. The longest burst lasted for 90 seconds. In this experiment the unit was then found to be responsive to touching a region of the skin stretched to form the paraffin pool. A second infusion of bradykinin 1 hour later (following a second infusion of prostaglandin E₁) also produced firing of this unit but the firing rate was less and the duration was shorter.

Discussion

These experiments show that the action of bradykinin is potentiated by infusion but not by single injection of prostaglandin E₁. Ferreira (1972) using intradermal injections into human skin, and Juan & Lembeck (1974) using the reflex fall in blood pressure of rabbits as an index of the nociceptive action of bradykinin, also found that infusions of prostaglandin E₁ were more effective than single injections in potentiating bradykinin. Prostaglandin E₁ appeared to potentiate the production of small and large height spikes by bradykinin and in 2 experiments marked repetitive firing of a unit, with the characteristics of a slowly adapting mechanoreceptor type II, occurred. One of these units had its receptive field in a region of the skin under stretch to form the paraffin pool and it would seem that bradykinin lowered the threshold of the receptor to stretch.

Bradykinin alone had little action in most of the rat preparations and large doses were necessary to produce any effect. Fjällbrant & Iggo (1961) found that impure bradykinin had little action on C fibres

from the cat saphenous nerve but other workers have shown that doses of bradykinin in the microgram range induced a response in afferent nerves (Beck & Handwerker, 1974; Mense & Schmidt, 1974; Franz & Mense, 1975). The question thus arises as to whether bradykinin is an effective algescic agent in its own right or whether it requires the presence of a low concentration of a prostaglandin or a prostaglandin precursor for its action to be manifest. Marked potentiation of bradykinin (0.5–10 µg) occurred with 5–100 ng of prostaglandin E₁ infused over 10 min but other doses of either bradykinin or prostaglandin E₁ were not used in these experiments. It is possible that much lower doses of prostaglandin E₁ might have been effective in potentiating bradykinin, and that the marked responses to bradykinin of afferent units described by other workers (Beck & Handwerker, 1974; Mense & Schmidt, 1974; Franz & Mense, 1975), and also in one experiment reported here, might have been due to the presence of low levels of prostaglandins released under the conditions of the experiments, including perhaps the use of heparin which in high doses has been shown to release prostaglandins (Damas & Deby, 1974). The potentiating effect of prostaglandin E₁ is probably dose-related since Moncada, Ferreira & Vane (1975) showed in the dog knee joint that bradykinin became more effective in inducing pain as the prostaglandin level increased in the joint cavity. It is significant also that the two observations which established bradykinin as a potent algescic agent were (a) its pain-producing action on the cantharidine blister base (Keele & Armstrong, 1964), an inflamed site probably having high local levels of prostaglandins, and (b) its pain-producing action when injected into the spleen of dogs (Guzman, Braun & Lim, 1962) where a basal resting release of E-type prostaglandins has since been shown to occur (Ferreira, Moncada & Vane, 1973). Nevertheless the possibility remains that the action of bradykinin on small fibres might have been less obvious in the multi-fibre strands used in the present experiments than on the single units used by other workers (Beck & Handwerker, 1974; Mense & Schmidt, 1974; Franz & Mense, 1975). It is also possible that there might be a species difference between cats (used by the previous workers) and rats (used in the present experiments) in

the ability of bradykinin itself to release prostaglandins, an action which bradykinin has been shown to have at some sites e.g. in dog spleen (Ferreira *et al.*, 1973).

The injection or infusion of prostaglandin E₁ produced firing of a few spikes which appeared from their height to be from A fibres. This response differed from that to bradykinin in the presence of prostaglandin E₁, which usually produced many small height spikes and some large spikes, and also from that to 5-hydroxytryptamine which produced many small and some large height spikes. It is possible that some of the larger height spikes were from mechanoreceptors activated by increased interstitial fluid pressure resulting from fluid leakage from the circulation during the course of the experiments. Such a response would be relatively slow in onset and could account for the long latency response to prostaglandin E₁. The response to prostaglandin E₁ did not appear to be due to release of histamine or 5-hydroxytryptamine from mast cells since it was not abolished by pretreatment with compound 48/80.

Although the experiments were not primarily designed to show potentiation of prostaglandin E₁ by bradykinin, it was found that after several injections of bradykinin, prostaglandin E₁ appeared to have a greater effect; obvious firing of both small and large height spikes occurred. In view of the small quantity of prostaglandin E₁ (10 ng) that produced this effect, the possibility must not be discounted that prostaglandin E₁ might be a cause of pain in inflammatory states where bradykinin is also present over a long period.

It is concluded from the results presented here that bradykinin and prostaglandin E₁ are mutually potentiating in their effects on afferent nerve terminals.

Experiments on rats treated with prostaglandin synthetase inhibitors would be of value in determining whether the potentiating action of bradykinin or exogenously applied prostaglandins is due to a release by bradykinin of endogenous prostaglandins or their precursors.

We wish to thank Dr J.E. Pike of the Upjohn Company, Kalamazoo, Michigan for generous samples of prostaglandin E₁. Financial support for part of this work was provided by the Agricultural Research Council.

References

- ÅNGGÅRD, E. & JONSSON, C.-E. (1971). Efflux of prostaglandins in lymph from scalded tissue. *Acta physiol. scand.*, **81**, 440–447.
- BECK, P.W. & HANDWERKER, H.O. (1974). Bradykinin and serotonin effects on various types of cutaneous nerve fibres. *Pflügers Arch.*, **347**, 209–222.
- CRUNKHORN, P. & WILLIS, A.L. (1971). Cutaneous reactions to intradermal prostaglandins. *Br. J. Pharmac.*, **41**, 49–56.
- DAMAS, J. & DEBY, C. (1974). Libération de prostaglandines par la bradykinine chez le rat. *C.r. Séanc. Soc. Biol.*, **168**, 375–378.
- DI ROSA, M., GIROUD, J.P. & WILLOUGHBY, D.A. (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Pathol.*, **104**, 15–29.
- FERREIRA, S.H. (1972). Prostaglandins, aspirin-like drugs and analgesia. *Nature, Lond.*, **240**, 200–203.

- FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1973). Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. *Br. J. Pharmac.*, **49**, 86–97.
- FJÄLLBRANT, N. & IGGO, A. (1961). The effect of histamine, 5-hydroxytryptamine and acetylcholine on cutaneous afferent fibres. *J. Physiol., Lond.*, **156**, 578–590.
- FRANZ, M. & MENSE, S. (1975). Muscle receptors with group IV afferent fibres responding to application of bradykinin. *Brain Res.*, **92**, 369–383.
- GREAVES, M.W. & SØNDERGAARD, J. (1970). Pharmacological agents released in ultraviolet inflammation studied by continuous skin perfusion. *J. invest. Derm.*, **54**, 365–367.
- GUZMAN, F., BRAUN, C. & LIM, R.K.S. (1962). Visceral pain and the pseudoaffective response to intra-arterial injection of bradykinin and other algesic agents. *Arch. int. Pharmacodyn.*, **136**, 353–384.
- JUAN, H. & LEMBECK, F. (1974). Action of peptides and other algesic agents on paravascular pain receptors of the isolated perfused rabbit ear. *N.S. Arch. Pharmac.*, **283**, 151–164.
- KEELE, C. A. & ARMSTRONG, D. (1964). *Substances producing Pain and Itch*. pp. 176–180. London: Edward Arnold.
- MENSE, S. & SCHMIDT, R.F. (1974). Activation of group IV afferent units from muscle by algesic agents. *Brain Res.*, **72**, 305–310.
- MONCADA, S., FERREIRA, S.H. & VANE, J.R. (1975). Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in the dog knee joint. *Eur. J. Pharmac.*, **31**, 250–260.

(Received July 19, 1976.

Revised September 24, 1976.)