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Effects of prostaglandin E_2 on the intensity of bradykinin-evoked pain from skin and veins of humans

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Abstract

Prostaglandin E_2 increases bradykinin-induced spike activity from polymodal nociceptors of the skin and deep tissues in animals, suggesting sensitization of these receptors. To see whether these neurophysiological observations in animals correspond with increased pain intensity in humans, and whether also vascular nociceptors are sensitized, we studied in humans the effects of prostaglandin E_2 on the intensity of pain evoked by bradykinin via the nociceptive systems of skin and veins. In seven healthy subjects, bradykinin was injected into the skin and into a vascularly isolated hand vein segment, prior to and after local application of prostaglandin E_2. Subsequent pain intensity was recorded continuously with an electronically controlled visual analogue scale. Prostaglandin E_2 alone never elicited pain, but without exception increased the intensity of bradykinin-induced pain in a concentration-related manner at concentrations from 10^{-9} to 10^{-6} M, both in skin and veins. Thus, bradykinin is more painful after pretreatment with prostaglandin E_2, suggesting sensitization of nociceptors of the skin, but also of hand veins in humans.

Keywords: Prostaglandin E_2; Bradykinin; Pain; Nociceptor; Vein

1. Introduction

Prostaglandins are thought to cause hyperalgesia by sensitization of nociceptors. This conclusion rests on observations in animals, in whom prostaglandin E_2 increased bradykinin-induced spike activity from nociceptors of the skin (Martin et al., 1987), and also from polymodal testicular nociceptors (Mizumura et al., 1987). Consistent with these neurophysiological observations, in different species, prostaglandin E_2 also increased behavioural pain responses, i.e. in the paw-withdrawal test in rats (Ferreira et al., 1978), after intraarticular application into dog knee joints (Ferreira et al., 1978), and in acetic-induced writhing in mice (Uda et al., 1990). Prostaglandin E_2 also enhanced reflex responses in dogs after stimulation of cardiac afferents with bradykinin (Staszewska-Woolley and Woolley, 1990).

In humans, intracutaneous application of prostaglandins increased pain intensities after mechanical and chemical stimulation (Ferreira, 1972). In most studies with direct application of prostaglandins in humans, however, only inflammatory responses of the skin were evaluated, but not pain (Wallengren and Hakanson, 1992), and pain intensity/concentration relations were not established. It is still unclear, whether and to what extent also nociceptors of deeper body tissues, i.e. the vasculature, are sensitive to prostaglandins, although this is of interest in the case of painful inflammatory vascular diseases. Therefore, the effect of prostaglandin E_2 was determined on the intensity of pain evoked by the algogen bradykinin in the skin, and in vascularly isolated hand vein segments, which are innervated afferently by polymodal nociceptors only (Arndt and Klement, 1991). It will be shown that prostaglandin E_2 enhances bradykinin-induced pain from skin as well as from veins, suggesting sensitization of cutaneous and venous nociceptors in humans.

2. Materials and methods

In accordance with the recommendations of the declaration of Helsinki and with the approval of the

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Committee of Medical Ethics of the Heinrich-Heine-University, experiments were performed in seven healthy volunteers. The subjects were studied on two different days depending on the route of drug administration; agents were applied either intracutaneously, or into vascularly isolated hand vein segments.

2.1. Preparation of drugs

Bradykinin (Clinalfa, Switzerland) was freshly dissolved in saline 0.9% (at physiological pH and osmolality) on each day of experimentation, and diluted to a standard of 100×10^{-6} M, of which all further dilutions were made. Prostaglandin E_2 (Minprostin E_2, Upjohn, Germany) was also freshly diluted with saline 0.9% to the wanted concentrations.

2.2. Experimental approach

Intracutaneous injections

A 27-gauge stainless steel cannula was inserted strictly intracutaneously into marked areas at the volar side of the forearm.

Vascularly isolated vein segments

The technique of the vascularly isolated hand vein segment has been described in detail elsewhere (Arndt and Klement, 1991). Briefly, a vein segment on the dorsum of the hand was punctured between two valves with teflon cannulae (Venflon, Viggo, Sweden; outer diameter 2.0 mm), which were advanced until their openings were 15–20 mm apart. With external foam rubber pads, the segment was isolated from the systemic circulation. A catheter (Cavafix, Braun-Melsungen, Germany; outer diameter 0.9 mm) was advanced via one cannula, until its tip just entered the vein segment. This way, a blood-free perfusion of the segment with solutions of defined concentrations is possible via the cannulae, and via the catheter, algetic agents may be injected directly into the segment, thereby precluding dilution by the perfusate. This approach excludes systemic effects of the agents.

2.3. Measurements

Pain intensity was recorded on chart continuously with the help of an electronically controlled visual analogue scale (VAS) between pain threshold (0%) and individual tolerance maximum (100%).

2.4. Programme of experimentation

Intracutaneous injections

Prostaglandin E_2 (50 μl, 10^{-9}–10^{-6} M) was injected into the skin of the volar forearm at random order, and single blind, at intervals of 10 min. For control, 50 μl of saline 0.9% was injected intracutaneously. After each injection, pain intensity was recorded.

Ten minutes after each of the former injections, a painful concentration of bradykinin (50 μl; $1–10 \times 10^{-6}$

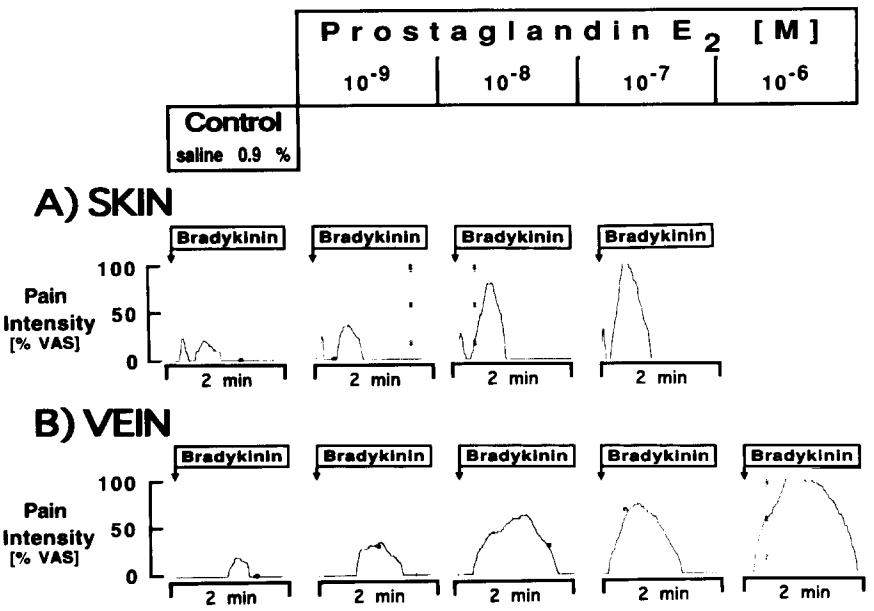

Fig. 1. Time course and intensity of pain on intracutaneous (panel A) and intravenous (panel B) injection of bradykinin prior to and after pretreatment of skin and vein with prostaglandin E_2. Original recordings from one subject. Bradykinin evoked pain on intracutaneous and intravenous application, the intensity of which mounted consistently after pretreatment of skin and vein with increasing concentrations of prostaglandin E_2. This is consistent with the view that prostaglandin E_2 sensitizes cutaneous and venous nociceptors in humans. Note the first, short-lived pain peak on intracutaneous injections, which is caused by the mechanical distension of the skin.

M, depending on individual sensitivity) was injected at the same sites, i.e. into the weals produced either by saline (control), or by prostaglandin E_2, and pain intensity was recorded after each injection.

Perfusion experiments

The vein segment was rinsed free of blood by continuous perfusion of saline 0.9% (2 ml × min^{-1}) for 10 min. Thereafter, prostaglandin E_2 was perfused in increasing concentrations (2 ml × min^{-1}, 10^{-9}–10^{-6} M, each concentration for 10 min), and pain intensity during perfusion was recorded. At the end of each perfusion period, the same painful concentration of bradykinin (200 μl, 1–10 × 10^{-6} M) was injected into the segment via the catheter, and pain intensity following the injection was recorded.

2.5. Data evaluation

From the original recordings the maximum pain intensity for each bradykinin injection was derived and plotted against the corresponding prostaglandin E_2 concentration to show the prostaglandin E_2 concentration/pain intensity curves for skin and veins.

3. Results

Prostaglandin E_2 itself never evoked pain in any subject, neither in the skin, nor in veins, but caused intense itching and erythema on intracutaneous injection. Bradykinin evoked pain on intracutaneous as well as on intravenous injections in all subjects, the intensity of which always increased with prostaglandin E_2 concentration.

The typical time course of pain is demonstrated in Fig. 1 by an original recording. After all intracutaneous injections (i.e irrespective of the applied drug or concentration), an early pain peak immediately on injection was observed. This first pain in all likelihood results from mechanical distension of the skin after the injection. Following the injection of bradykinin, the first pain was followed by a second more pronounced pain peak. This second pain was consistently described as hot or burning, which is typical for bradykinin (Kindgen-Milles et al., 1994). With increasing concentrations of sensitizing prostaglandin E_2 (10^{-9} to 10^{-6} M), the intensity of bradykinin-evoked pain increased (from 21% to 100% VAS), latencies to the onset of pain decreased (from 16 to 8 s), while duration of pain increased (from 26 to 42 s).

On intravenous injections, an initial pain peak was not observed. The intensity of bradykinin-induced pain increased in the same way as on intracutaneous injections, i.e. from control (20% VAS) to the maximum (100% VAS) with a prostaglandin E_2 concentration of

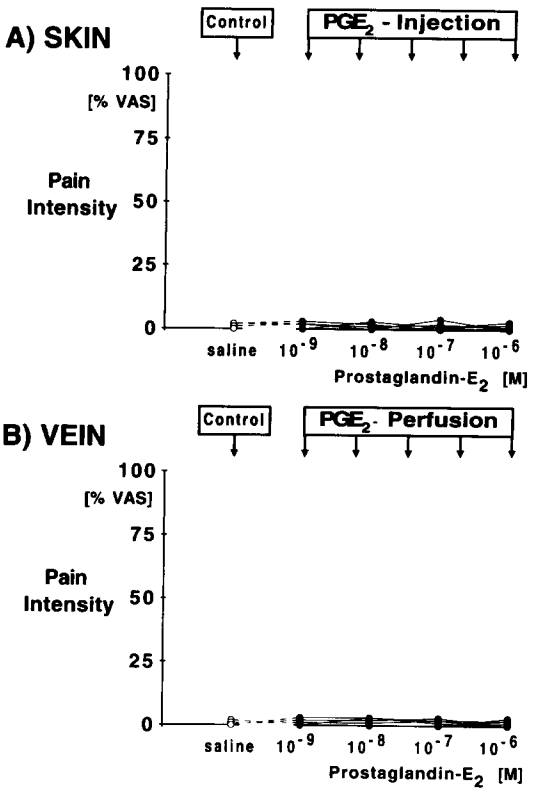

Fig. 2. Maximum pain intensities after intracutaneous (panel A) and intravenous (panel B) application of saline 0.9% (control, open symbols) and prostaglandin E_2 (filled symbols) in seven subjects. Prostaglandin E_2 itself does not evoke pain, neither from skin, nor from veins of humans.

10^{-6} M. Latencies in general were longer than on intracutaneous application, which in all likelihood is caused by a slightly longer diffusion distance to the nociceptors. Nevertheless, as pain intensity increased, latencies consistently decreased (from 46 to 8 s), while duration of pain increased (from 22 to 104 s).

From these original recordings, the maximum pain intensity/concentration effect curves were derived. Fig. 2 shows these curves for all intracutaneous and intravenous applications of prostaglandin E_2 in all subjects. Neither injection of saline 0.9% into the skin or its perfusion through a vein segment (control), nor intracutaneous injection or intravenous perfusion of prostaglandin E_2 evoked pain in any subject.

Fig. 3 shows the maximum pain intensity/concentration curves for all bradykinin injections in all subjects, i.e. prior to and after pretreatment with prostaglandin E_2. In panel A, maximum pain intensities after intracutaneous injections of bradykinin are shown. Bradykinin evoked pain from 21–44% VAS under control conditions (i.e. after preinjection into the skin of saline 0.9%), which mounted with increasing prostaglandin E_2 concentrations to the tolerance maximum (66–100% VAS with $10^{-5/6}$ M prostaglandin E_2).

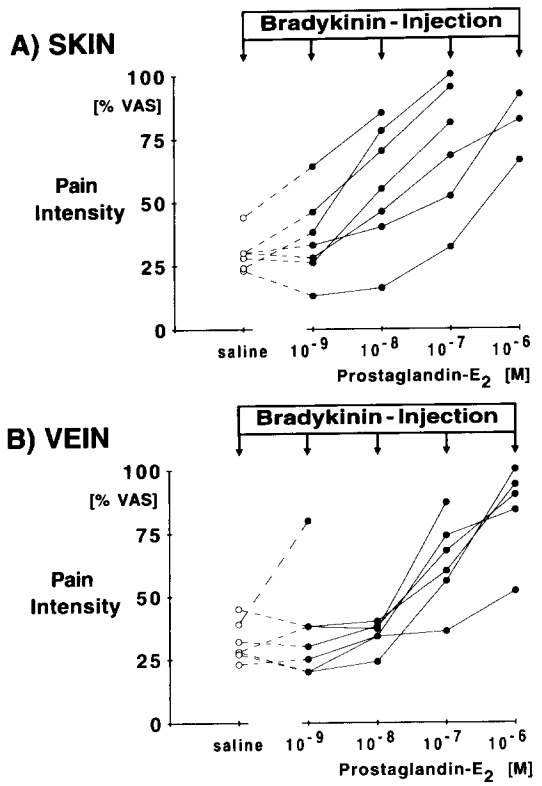

Fig. 3. Maximum pain intensities after intracutaneous (panel A) and intravenous (panel B) injection of bradykinin prior to (open symbols) and after (filled symbols) pretreatment of skin or vein with prostaglandin E_2. Data from seven healthy subjects. Regardless of the application site, bradykinin caused pain in all subjects, the intensity of which increased with prostaglandin E_2 concentration. Thus, the nociceptors of skin as well as of human hand veins are sensitized by prostaglandin E_2 to the endogenous algogen bradykinin. Note that the effective concentration ranges for prostaglandin E_2 are similar both for the skin and the vein.

Similarily, after perfusion of vein segments with prostaglandin E_2, pain intensities increased after application of bradykinin from 23–45% VAS (control) to 52–100% VAS, also with $10^{-5/6}$ M of prostaglandin E_2.

Thus, while prostaglandin E_2 itself never evoked pain, regardless of the application site, bradykinin-evoked pain intensities were strictly dependent on prostaglandin E_2 concentration in every subject.

4. Discussion

In our experiments, prostaglandin E_2 increased at concentrations from 10^{-9} to 10^{-6} M bradykinin-induced pain from skin and veins of humans, suggesting sensitization of the respective nociceptors.

As shown recently, injection of bradykinin into the skin as well as into vascularly isolated hand vein segments evokes pain within nearly identical concentration ranges and with almost identical slopes of the pain intensity/concentration relations so that both the nociceptive systems of skin and veins of humans share the same bradykinin sensitivity (Kindgen-Milles et al., 1994). Therefore, the endogenous algogen bradykinin is well suited as a nociceptive test stimulus to study prostaglandin sensitization of these systems.

Our conclusions concerning the effects of prostaglandin E_2 rest on the premise that with our routes of drug application, bradykinin and prostaglandin E_2 did reach the nociceptors of skin and veins, and that the actions of the drugs were confined to these structures. This is certainly so with intracutaneous injections, because the drugs are applied almost directly to the nociceptors so that the diffusion distance is neglegible.

With perfusion of vascularly isolated vein segments, prostaglandin E_2 without doubt reached the polymodal nociceptors of the vein, which according to electron microscopy are situated just beneath the endothelium (Floel and Staubesand, 1978) and thus only a few μm distant from the application site.

The exposure time of 10 min was long enough for prostaglandin E_2 to reach these receptors, since it has been shown that drugs of quite different lipophilicity, which is the major factor determining tissue penetration, all exert their maximum effects within a few minutes (Klement and Arndt, 1991a,b; Kindgen-Milles et al., 1994). The drugs applied this way reached and stimulated venous nociceptors only, because systemic effects are excluded by the vascular isolation of the segment. Furthermore, pain caused by intrasegmental stimulation is independent of the intactness of the innervation of the overlying skin, so that also cutaneous afferents as possible transmitters of pain are excluded (Arndt and Klement, 1991).

In the case of the intracutaneous injections, the first pain peak is an unspecific side effect of the injection technique. In all likelihood, it is caused by the mechanical distension of the skin after the injections, since it occurs in a similar way after application of saline 0.9%, bradykinin, and prostaglandin E_2 into the skin. It was also observed after intracutaneous injection of nitric oxide (Holthusen and Arndt, 1994), i.e. it occurs irrespective of the drugs applied. Furthermore, pain intensity does not depend on drug concentration, thus underlining that it is not a drug-specific effect. On intravenous injections, an initial pain peak was not observed, obviously because the vein was not overdistended by the application of the small aliquots of 0.2 ml. Pain on distension of hand veins usually starts with venous diameters of 3.5 mm (Arndt and Klement, 1991), which were not reached in our experiments. Furthermore, pain on distension occurs without delay immediately with the start of the stimulation (Arndt and Klement, 1991), while pain on intravenous application of bradykinin shows a longer delay (Kindgen-Milles et al., 1994). Thus, since in all our experiments, pain

after application of bradykinin was described as hot or burning, which is typical for bradykinin-induced pain, and since it also showed the typical time course, with exception of the first pain peak after intracutaneous injections, the pain observed here is a bradykinin-specific drug effect, and not a side effect of our techniques of drug application.

As shown in other studies with cutaneous or venous pain evoked by various agents including bradykinin, the maximum pain intensity is particularly useful to compare pain intensity/concentration relations of different nociceptive systems (Arndt and Klement, 1991, Kindgen-Milles et al., 1994). This is so because the effective drug concentrations at the receptor sites depend on the diffusion rate from the site of application to the receptors as well as on the rate of drug degradation in the tissue, these two processes having attained in all likelihood equilibrium when pain has reached its maximum.

In our experiments, neither injection of prostaglandin E_2 into the skin, nor its perfusion through isolated hand vein segments caused pain in any subject. This does not mean that the concentrations applied were not effective, i.e. too low. With perfusion of vein segments, it is feasible to apply constant and defined drug concentrations to the inner vein wall, since in the absence of blood, dilution and degradation of the agents is avoided. The exact prostaglandin E_2 concentrations at the receptor sites, however, are unknown in these experiments. Nevertheless, because gross dilution is avoided also with intracutaneous injections, the effective concentrations in skin and vein must be close to those injected or perfused, and for certain run in parallel with them.

The painlessness of prostaglandin E_2 found in our experiments corresponds with neurophysiological observations in animals, where prostaglandin E_2 up to concentrations of 10^{-5} M did not excite canine testicular polymodal nociceptors (Mizumura et al., 1987), nor rat cutaneous nociceptors, even if exposure time was prolonged to 15 min (Lang et al., 1990).

That the prostaglandin E_2 concentrations applied in our experiments were effective, is shown by the observation that prostaglandin E_2 increased the intensity of bradykinin-evoked pain both in skin and in veins in a concentration-dependent manner, without exception, and with nearly identical pain intensity/concentration relations. These results are in accordance with neurophysiological observations in animals, where pretreatment with prostaglandin E_2 increased afferent nerve activity from polymodal visceral nociceptors in dogs after stimulation with bradykinin (Mizumura et al., 1987), as well as from cutaneous nociceptors in rats (Lang et al., 1990). Furthermore, also behavioural pain responses like bradykinin-induced writhing in mice (Uda et al., 1990), pressure-induced paw withdrawal reactions in rats (Taiwo and Levine, 1990), and reflex responses after intra-articular injections of bradykinin in dogs (Ferreira et al., 1978) all were increased by prostaglandins, or inhibited by cyclooxygenase inhibitors (Moncada et al., 1975). Thus, in animals, neurophysiological observations as well as pharmacological evidence point to prostaglandin sensitization of various pain receptors.

Our observations in humans show that prostaglandin E_2, which by itself does not evoke pain, within similar concentration ranges sensitizes nociceptors of skin and veins to the endogenous algogen bradykinin.

In conclusion, the nociceptive systems of both skin and veins are similarily responsive to prostaglandin E_2 sensitization, which thus might play a role in hyperalgetic states of inflammatory origin not only of the skin, but also of veins of humans.

Acknowledgements

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