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Bradykinin release of TNF-α plays a key role in the development of inflammatory hyperalgesia

S. H. Ferreira, B. B. Lorenzetti, F. Q. Cunha and S. Poole

Faculty of Medicine of Ribeirão Preto, Ribeirão Preto 14049, Brazil

Abstract

Using specific antisera for IL-1β and IL-8, as well as cyclooxygenase inhibitors and propranolol, we have demonstrated that these cytokines are responsible for the prostaglandin and sympathetic components of carrageenin-induced hyperalgesia in the rat paw test. The release of IL-1β and IL-8 is preceded by the liberation of TNF-α. We have also tested in a nociceptive model the effects of bradykinin and a specific bradykinin antagonist, HOE 140, on the hyperalgesia induced by carrageenin and lipopolysaccharide (LPS). Bradykinin-induced hyperalgesia was abolished by HOE 140 and by treatment of the paws with anti-TNF-α antisera. HOE 140 significantly inhibited the hyperalgesia induced by carrageenin and LPS. It is suggested that in these two models bradykinin is associated with the release of hyperalgesic cytokines.

Introduction

It is now becoming clear that in inflammatory responses, the release of hyperalgesic mediators are secondary to the release of cytokines [1–3]. In this context, cytokines seem to constitute a link between cellular injury and/or recognition of non-self and the development of local and systemic inflammatory signs and symptoms, such as cell migration, oedema, hyperalgesia, fever and acute-phase protein release.

We have studied the ability of several recombinant cytokines to cause hyperalgesia and, with the use of specific antagonists, elucidated the mediators which trigger the hyperalgesic process. In addition, by pretreating rat paws with specific antisera, we have defined the sequence of cytokines released. Furthermore, use of cyclooxygenase inhibitors or sympatholytics have helped to identify the cytokines responsible for the release of the mediators ultimately responsible for the development of inflammatory hyperalgesia [1–3]. The first interleukin shown to be involved in inflammatory pain was IL-1β [1, 4]. The hyperalgesic effect of IL-1β was abolished by indomethacin, thus indicating that its effect was mediated via the release of cyclooxygenase products. From the structure of IL-1β we delineated the region which mediated hyperalgesia and developed an analgesic tripeptide analogue of IL-1β (K-DP-T) which antagonised the hyperalgesic effect of IL-1β. With the use of this IL-1β antagonist we demonstrated that in carrageenin-evoked hyperalgesia the release of PGE_2 was preceded by the release of IL-1β.

The hyperalgesia induced by carrageenin in the rat paw test has, in addition to the prostaglandin component, a sympathetic component [5]. We have demonstrated that carrageenin-evoked sympathetic hyperalgesia is also mediated via the release of IL-8. Thus, pretreatment of rat paws with both IL-1β and IL-8 antisera abolished carrageenin-induced hyperalgesia [2]. IL-6 appears to

cause hyperalgesia via cyclooxygenase metabolites but also involves the release of IL-1β since its effect was abolished by local administration of K-DP-T, IL-1 antisera and indomethacin. In carrageenin-induced hyperalgesia, IL-6 antisera also inhibited the prostaglandin-mediated component [3].

A general characteristic of several cytokines is that they induce the release of other cytokines, thus acting as an amplifying mechanism in the inflammatory response. TNFα seems to play a pivotal role in the release of other cytokines [3]. Recently, we showed that TNF-α-induced hyperalgesia was partially inhibited with the use of indomethacin or sympatholytics but abolished by the combination of both drugs. Antiserum neutralising TNF-α abolished TNF-α responses as well as the hyperalgesic response to carrageenin. The combined treatment of a paw with antisera to IL-1β and IL-8 abolished TNF-α-induced hyperalgesia. We concluded that TNFα has a pivotal role in carrageenin-induced hyperalgesia and that its release was essential for the release of the other cytokines [3].

It has been described that the specific bradykinin antagonists, HOE 140, has antinociceptive effects [6] and that bradykinin releases TNF-α and IL-1 from macrophages [7]. In the present study, we investigated whether the hyperalgesic effect of bradykinin in the rat paw test was indirect, i.e. via the release of cytokines. Also we have tested the involvement of bradykinin in hyperalgesia induced by two different inflammatory agents, carrageenin and bacterial lipopolysaccharide (LPS). In these experiments, we have used our modification of the Randall Selitto rat paw test [8].

Results and discussion

Bradykinin injection in the rat paw produced a dose-dependent hyperalgesia which was partially blocked by indomethacin and propanolol, but was abolished by the combination of both drugs (data not shown). Thus, there are at least two components present in bradykinin-induced hyperalgesia that are common to carrageenin-induced inflammation. The hyperalgesic effect of bradykinin was blocked in a dose-dependent fashion by HOE 140 (data not shown). To test the possible participation of cytokines we used a variety of antisera which, in the volumes used, specifically abolish the hyperalgesic effect of certain cytokines.

The left panel of Fig. 1 shows that the hyperalgesia induced by bradykinin was partially inhibited by

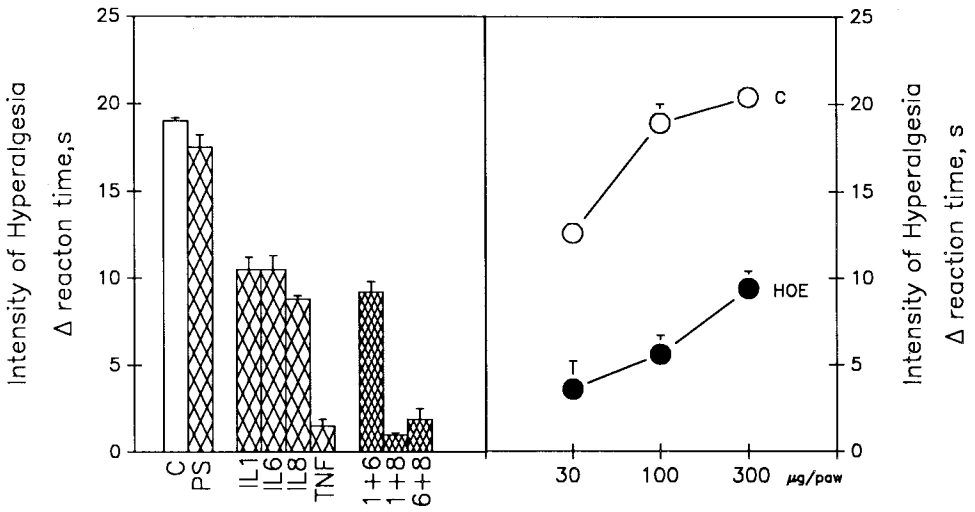

Figure 1
Effect of specific anti-cytokine antisera on bradykinin-induced hyperalgesia (left panel) and HOE 140 on carrageenin-induced hyperalgesia (right panel). In the left panel pre-immune serum (PS) or antisera (50 μl/paw) were administered 30 min before BK challenge (C, 0.5 μl/paw). The right panel shows the effect of HOE 140 (closed circles, 1 mg/kg, sc) administered 30 min before carrageenin challenge (open circles). The data are the mean ± SEM of 5 animals per group measured 3 h after BK or carrageenin injection.

antisera to IL-1β, IL-6 or IL-8 and abolished by anti-TNF-α serum. Abolition of bradykinin-induced hyperalgesia was also observed with the combined pretreatment of the paws with antisera against the cytokines which are responsible for the release of prostaglandins and sympathomimetic mediators respectively (i.e. IL-1β + IL-8, IL-6 + IL-8). There was no additive effect when the paws were treated with a mixture of antisera to IL-6 + IL-1β, because both sera neutralise cytokines which act via the release of prostaglandins.

The right panel of Fig. 1 shows that carrageenin-induced hyperalgesia was antagonized by HOE 140. Taking together our previous demonstration of the sequential participation of cytokines in carrageenin-induced inflammation and the effect of HOE 140, one might reasonably conclude that in this model the release of bradykinin induces the release of TNF-α which is then responsible for the release of the other hyperalgesic cytokines. LPS injected in the rat paw caused a dose-dependent hyperalgesia (0.5–5 µg/paw). The hyperalgesic effect of LPS was also mediated by the release of TNF-α, IL-6, IL-1 and IL-8, as revealed by the use of the neutralising specific antisera. Pretreatment of animals with HOE 140 significantly antagonized the hyperalgesic response to the low doses of LPS but had no effect upon the hyperalgesia induced by 5 µg/paw of LPS. This result indicates that, with lower doses of LPS, bradykinin contributes to the release of the hyperalgesic cytokines. With higher doses, however, it seems that LPS-induced hyperalgesia results from the direct release of cytokines. The mixture of antiserum to IL-1β and IL-8 greatly reduced the hyperalgesic effect of higher doses of LPS.

In conclusion, bradykinin-induced hyperalgesia results from the release of TNF-α, which stimulates the release of the other hyperalgesic cytokines responsible for the generation of cyclooxygenase and sympathomimetic amines. Bradykinin and TNF-α play a pivotal role in the hyperalgesia induced by the inflammatory stimuli, carrageenin and LPS.

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