The role of the sympathetic efferents in endotoxin-induced localized inflammatory hyperalgesia and cytokine upregulation

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Abstract

The sympathetic system (SNS) is considered to be a major component of the neurogenic contribution to inflammation and hyperalgesia. We have investigated the role of the SNS in the local inflammatory pain induced by intraplantar (i.pl) injections of bacterial endotoxin (ET). Treatment of rats with an α-adrenoceptor antagonist (phentolamine, 0.25–1 mg/kg, i.p.), a β-adrenoceptor antagonist (propranolol, 1–10 mg/kg, p.o.) or a sympathetic neuron-blocking agent (guanethedine, 30 mg/kg, s.c.) resulted in a dose-dependent reduction of the thermal hyperalgesia induced by ET. Mechanical hyperalgesia, however, was less sensitive to inhibition by propranolol and guanethedine but significantly inhibited by phentolamine. ET injection produced significant upregulation of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and nerve growth factor (NGF). Treatment with any one of the three sympatholytics abolished the upregulation of NGF and IL-6, while phentolamine and guanethedine also reversed the upregulation of TNF-α. IL-1β was resistant to all of the sympatholytic treatments. We conclude that the SNS can contribute to the local inflammation and hyperalgesia following injection of ET. The resistance to sympatholytics shown by IL-1β, known to play a key role in the inflammatory cascade, suggests that ET can initiate inflammation and hyperalgesia independently of peripheral and central sympathetic mechanisms. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The sympathetic nervous system (SNS) can modulate, under certain circumstances, the functions of somatic afferents, especially those of nociceptors (Baron, 1998). Despite divergent reports, this effect becomes more evident during neurogenic pain of various origins, such as causalgia, reflex sympathetic dystrophy and other syndromes now assembled under the label of complex regional pain syndromes (CRPS) as proposed by the recent nomenclature of pain (for review, see Bonica, 1990; Scadding, 1999).

In animal models of pain due to injury of peripheral nerves, damaged and adjacent intact nerves develop increased sensitivity to catecholamines (Wall and Gutnick, 1974; Ali et al., 1999) and may exhibit abnormal sympathetic innervation (McLachlan et al., 1993). The contribution of the SNS to inflammation-related pain, however, is less evident than its role in neurogenic pain. During infection or in diseases leading to challenges of the hypothalamus-pituitary-adrenal (HPA) axis, the SNS is considered to be a component of the efferent loop attempting to control the immune responses, leading to a reduction in the inflammation and the resulting pain (Chrousos, 1995; Green et al., 1998). Also, the involvement of sympathetic efferents in inflammatory pain has

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been shown to constitute part of the neurogenic contribution to inflammatory reactions (Levine et al., 1986; Nakamura and Ferreira, 1987). This efferent contribution of the SNS to inflammatory pain has been shown to be mediated through either the release of noradrenaline (Drummond, 1995) or the production of prostaglandins (Gonzales et al., 1991) and IL-8 (Cunha et al., 1991).

The aim of the present study was to examine the possible contribution of sympathetic efferents to inflammation-induced hyperalgesia. For this purpose, we employed the model of endotoxin-induced localized inflammation and hyperalgesia as described by Kanaan et al. (1996). We show that treatment of rats with α or β adrenoceptor antagonists or with an inhibitor of peripheral sympathetic transmission prevents the hyperalgesia induced by intraplantar injection of endotoxin (ET). The anti-hyperalgesic effects of these treatments were associated with alterations in the effects of ET on the concentrations of proinflammatory cytokines and nerve growth factor (NGF).

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (200–250 g) were used in all the experiments. The animals were housed under optimum conditions of light and temperature (12 h light and 12 h dark cycle and 22 ± 3°C), with food and water provided ad libitum. All experiments were carried out with strict adherence to National Institutes of Health guide for the care and use of laboratory animals for pain experimentation and were approved by the Institutional Animal Care Committee.

2.2. Behavioral measurements

Thermal and mechanical pain tests were performed for three consecutive days prior to any injections to establish baseline values of the nociceptive thresholds. The paw pressure (PP) test was used to assess mechanical hyperalgesia and the hot plate (HP), paw immersion (PI) and tail immersion (TF) tests were performed for the assessment of thermal hyperalgesia, as described in detail previously (Kanaan et al., 1996). Briefly, in the HP test, animals were placed individually on a HP (52.8–53.4 °C) and the latency to the onset of the first sign of paw licking or jumping was taken as an index of the pain threshold. In the TF test, the tail of each animal was immersed in a beaker of distilled water (T = 48°C) and the latency to onset of paw removal was recorded. Mechanical threshold was assessed by the PP test, by applying a constant pressure of 0.20 kg/cm² alternately to the left and right hind paws, with a 5 min interval between consecutive applications. The pressure was discontinued when the animals displayed a typical reaction characterized by a vigorous flexion reflex (Kanaan et al., 1996).

2.3. Drug administration

To establish inflammatory hyperalgesia, groups of rats (n = 5 each) received intraplantar (i.pl.) injection of a solution (1.25 µg in 50 µl saline) of ET (lipopolysaccharide from Salmonella typhosa, Sigma) in one hind paw. This caused both thermal and mechanical hyperalgesia, restricted to the injected leg, as described previously (Kanaan et al., 1996). Protocol 1: some of these groups received either propranolol alone (0.5 mg/kg in 100 µl saline, i.p.) or were pretreated with different doses of this drug (0.25, 0.5 and 1 mg in 100 µl saline, i.p.) 30 min before injection of ET (1.25 µg in 50 µl saline, i.pl.). Protocol 2: other groups were treated either with propranolol alone (10 mg/kg, p.o.) or with different doses of propranolol, (1, 5, 10 mg/kg) 30 min before ET injection. Protocol 3: other groups of rats were treated with either guanethidine alone s.c. (30 mg/kg) or this drug 1 h before injection of ET. This method of treatment has been shown to produce a block of peripheral sympathetic neurotransmission by preventing the release of catecholamines (Coderre et al., 1984).

The time course of the effects of pretreatment with one dose at least (the middle one in general) from each drug was observed over a period of 24 h, following the injection of either ET preceded by the drug or the drug only. For purpose of comparison, however, the reported results were based on observations made at 9 h after ET injection, which corresponds to the peak of hyperalgesia induced by this toxin as described previously (Kanaan et al., 1996).

The dosages, timing of injections and protocols followed for the observation of the effects of drugs on nociception and cytokines, were based on previously established characteristics of each of these drugs (as reviewed by Borchard et al., 1996; Hoffman and Lefkowitz, 1996). The used dosage of propranolol (inderal-sustained release, Zeneca, UK) was based on previously established data in rats (Levine et al., 1988 and for review see Borchard et al., 1996. The phenolamine (Regitine-Novartis) is known to reach a maximum plasma level within 30–60 min, and to exert long lasting effects (1–4 days) on neuropathic manifestations in rats (Kim et al., 1993). Both propranolol and phenolamine are known for their lipophilicity and their ability to cross the blood brain barrier and to act on the central nervous system.
system. By contrast, guanethidine (monosulfate Sigma), known to act only on peripheral adrenergic synapses, is characterized by its long lasting effect with a peak of action at 9 h post injection with the dosage used (Coderre et al., 1984). Injections of saline (50–100 µl, i.pl.) or each drug alone were shown not to produce significant alteration of the pain thresholds.

2.4. Cytokine and NGF measurements

The experiments in which concentrations of cytokines and NGF were measured, the animals were terminally anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and the entire hind paw skin (from left and right feet) was removed. The tissue samples were weighed, snap frozen on dry ice and stored at −70 °C to be processed for assay of the cytokines and NGF. Tissues were removed from two groups of animals (n = 5 in each group) 4 h after injection of either saline or ET, for the assay of interleukin-1β (IL-1β), IL-6 and NGF, and 1 h after either saline or ET for tumor necrosis factor-α (TNF-α) assay. These time intervals correspond to the peak upregulation of each cytokine after injection of ET as reported previously (Safieh-Garabedian et al., 1997; Kanaan et al., 1998). In other experiments, tissues were removed, as described previously, from six groups of rats (n = 5 in each) treated with phentolamine (0.5 mg/kg), guanethidine (30 mg/kg) or propranolol (10 mg/kg) either alone (three groups) or 30 min before the injection of ET (three groups).

Skin tissue was homogenized in phosphate buffered saline (PBS, pH = 7.4) containing 0.4 M NaCl, 0.05% Tween-20, 0.5% bovine serum albumin, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 KI/ml aprotinin. The homogenates were centrifuged at 12000 × g for 60 min at 4 °C. Two-site Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the cytokine and NGF contents in the supernatants. NGF was measured using an immunoassay kit (Promega) as described by the manufacturer. TNF-α, IL-1β and IL-6 were assayed as described previously (Safieh-Garabedian et al., 1997; Kanaan et al., 1998).

2.5. Data collection and statistical analysis

Values for pain thresholds were averaged for each experimental group. The values obtained following treatment with each of the sympatholytics were compared with either those measured for ET at the same time interval or with the baselines established before the injection. For the concentrations of the cytokines and NGF, the values obtained from animals injected with ET alone, sympatholytic alone or ET+sympathomlytic were compared with those obtained from matched groups injected with saline or from the non-injected (contralateral) legs of the same animals. The degree of significance of differences was measured by ANOVA followed by Bonferroni post hoc test, using the Instat 3 for statistics and Prism 3 for illustrations, (GraphPad, In. CA, USA).

3. Results

3.1. Treatment with phentolamine

Injection of ET (1.25 µg in 50 µl saline, i.pl.) in the hind paws of rats caused a significant decrease in the nociceptive thresholds, measured at 9 h (peak of hyperalgesia), as determined by the PP test (1.10 ± 0.07s vs saline control of 2.07 ± 0.06s, P < 0.001) for mechanical hyperalgesia and PI (1.25 ± 0.05 s vs saline control of 1.90 ± 0.06s, P < 0.001), HP (5.40 ± 0.20s vs saline control of 10.00 ± 0.40s, P < 0.001) and TF (2.50 ± 0.09s vs saline control 3.00 ± 0.06s, P < 0.01) tests for thermal hyperalgesia. Treatment with the phentolamine (0.25, 0.5 and 1 mg/kg) reduced, in a dose-dependent manner, ET-induced hyperalgesia (Fig. 1). With the dose of 1 mg/kg phentolamine, the latencies to onset of the various responses were 2.1 ± 0.07, 1.9 ± 0.7, 8.5 ± 0.4, and 3.15 ± 0.08s for the PP, PI, HP and TF tests, respectively (P > 0.05 for all values, compared with control or phentolamine alone). Injection of ET in the hind paws of rats resulted in a significant (P < 0.001 for TNF-α, IL-1β and NGF and P < 0.01 for IL-6) increases in the concentrations of IL-1β (8411 ± 870 pg/hind paw), TNF-α (412 ± 34 pg/hind paw), IL-6 (2367 ± 517 pg/hind paw) and NGF (5.30 ± 0.38 ng/hind paw) when compared with the concentrations measured in the non-injected paws (IL-1β: 1598 ± 166 pg/hind paw, TNF-α: 250 ± 25 pg/hind paw, IL-6: 305 ± 25 pg/hind paw and NGF: 2.25 ± 0.12 ng/hind paw) or in saline controls (IL-1β: 955 ± 125 pg/hind paw, TNF-α: 245 ± 14 pg/hind paw, IL-6: 215 ± 20 pg/hind paw and NGF: 2.05 ± 0.13 ng/hind paw). Treatment with phentolamine (0.5 mg/kg) abolished the increases in the concentrations of TNF-α, IL-6 and NGF but had no significant effect on the increase in IL-1β concentrations (Fig. 2). Injection of phentolamine alone (0.5–1 mg/kg) had no significant effect on the thresholds of the different pain tests and cytokine concentrations measured in control rats (Fig. 2).

3.2. Treatment with propranolol

Injections of propranolol (1, 5, 10 mg/kg), reduced in a dose-dependent manner, the ET-induced local thermal hyperalgesia as assessed by the PI, HP and TF tests. At the highest dose tested (10 mg/kg), the latencies to onset of the different pain responses returned to control values, except in the PP test in which the latencies recovered to 1.62 ± 0.07s (as compared with a value of
2.07 ± 0.06 in controls, \( P < 0.05 \), Fig. 3). Treatment with propranolol (10 mg/kg) had no significant effect on the increases in the concentrations of IL-1β and TNF-α following the injection of ET (Fig. 4). In contrast, this drug reversed the increases in the concentrations of IL-6 and NGF in the ET-injected paws as compared with the non-injected paws and saline controls (Fig. 4). Propranolol had no significant effect on the nociceptive thresholds or on the concentrations of the different cytokines in the control animals (Figs. 3 and 4).

3.3. Treatment with guanethidine

Chemical sympathectomy, using guanethidine (30 mg/kg, s.c.) prevented the thermal hyperalgesic response
Fig. 3. Dose-dependent attenuation of the ET-induced hyperalgesia by propranolol (inderal). Each bar represents the average ± SEM of measurements performed on a separate group of rats (n = 5) for each indicated treatment. All measurements were made 9 h after the injection of saline (control), ET only or ET preceded by the administration of propranolol. The significance of differences was calculated with reference to the values of either the control group (∗) or the ET group (+).

Fig. 4. Pretreatment with propranolol (inderal, 10 mg/kg) attenuates the levels of IL-6 and NGF increased by ET injection. Each pair of bars represents the determination of the concentration of a cytokine in the skin tissue of the hind paws (injected and non-injected) of a different group of rats (n = 5) for each type of treatment. For more details refer to the legend of Fig. 2 and to Sections 2 and 3.

The mechanical hyperalgesia, however, was moderately affected by this drug (the latency to onset of the PP response was 1.56 ± 0.05s, as compared with a control value of 2.04 ± 0.05s, P < 0.001). As with phentolamine, treatment with guanethidine abolished the increases in the concentrations of IL-6, TNF-α and NGF, but had no significant effect on the increase in the concentrations of IL-1β following the injection of ET (Fig. 6). Guanethidine alone did not cause significant effect on pain thresholds or the concentrations of the cytokines (Figs. 5 and 6).
4. Discussion

TNF-α, IL-1β, IL-6 and NGF have all been reported to have hyperalgesic activity in various models of inflammatory hyperalgesia (Poole et al., 2000) and these observations suggest that the sympathetic efferents contribute to the ET-induced, cytokine-mediated, inflammatory hyperalgesia. A possible contribution of sympathetic efferents to inflammation was suggested by Levine et al. (1986) and was supported by several studies employing various models of inflammation (Nakamura and Ferreira, 1987; Drummond, 1995; Woolf et al., 1996). Other studies, however, have failed to identify a significant contribution of the sympathetic efferents in
inflammatory pain (Lam and Ferrell, 1991; Meyer et al., 1992; Sluka et al., 1994).

In otherwise unstimulated rats, the injection of sympathetic antagonists did not elicit any effects on the thresholds of the acute tests of hyperalgesia or on the concentrations of the various cytokines and NGF. Although treatment with sympathetic antagonists may interfere with thermoregulatory mechanisms through the modulation of peripheral vasoconstrictive sympathetic tone, the dosage of antagonists used in our experiments seems not to be enough to produce such alterations. In fact, the highest doses of phentolamine (1 mg/kg) or propranolol (10 mg/kg, p.o.) used in our experiments were equivalent or below the lowest doses of these drugs, used in other reports (1–5 mg/kg of phentolamine used by Kim et al., 1993; 3×20 mg/day or 100 mg/kg/day of propranolol, used by Levine et al., 1988 and Weiss et al., 1974, respectively). Furthermore, the observed lack of effect of guanethedine on the thresholds of nociceptive tests in naïve rats, correlates well with the results reported by Coderre et al. (1984) on the tail flick test of naïve rats. Therefore, this result implies that the observed attenuation of the hyperalgesic effects of ET is not secondary to changes in skin temperature by sympatholytic treatments.

During the ET-induced inflammation, α and β adrenoceptor antagonists attenuated (dose-dependently) the ET-induced decrease in the latencies to onset of the nociceptive responses (hyperalgesia). All the three antagonists tested had similar effects on the thermal hyperalgesia, but only the α-adrenoceptor antagonist phentolamine reversed completely the mechanical hyperalgesia measured in the PP Test. The order of potency of the sympatholytics tested was: phentolamine > propranolol > guanethedine. The finding that the blocker of peripheral sympathetic synapses, guanethedine, was the least effective in preventing mechanical hyperalgesia might suggest that the anti-hyperalgesic effects of the other sympatholytics were exerted, at least in part, centrally. The contribution of both central and peripheral mechanisms to inflammation-induced hyperalgesia has been suggested (Coderre and Melzack, 1987; Schaible et al., 1987), notably in the ET-induced hyperalgesia (Saadé et al., 1998) and the more pronounced anti-hyperalgesic effect of phentolamine is consistent with its use in the treatment of sympathetically maintained pain (Gonzales et al., 1991; Mansikka and Pertovaara, 1995). However, other studies support a role for β-adrenergic mechanisms in inflammatory pain (Levine et al., 1988) and in mechanical hyperalgesia (Cunha et al., 1991; Khasar et al., 1999).

Intraplantar injection of ET increased the local concentrations of IL-1β, TNF-α and NGF (Safieh-Garabedian et al., 1997; Kanaan et al., 1998) whereas local treatment with IL-1 receptor antagonist and antisera against TNF-α and NGF attenuated the ET-induced thermal and mechanical hyperalgesias (Safieh-Garabedian et al., 1997) as did local treatment with the ‘anti-inflammatory’ cytokines IL-10 and IL-13 (Kanaan et al., 1998). These observations provide evidence for an important role of the cytokines and NGF in the ET-induced hyperalgesia. A role for the SNS in ET-induced, cytokine-mediated, inflammatory hyperalgesia is suggested by our observation that sympatholytics attenuated the ET-induced increases in the concentrations of the cytokines and NGF. All three sympatholytics tested reduced the ET-induced increases in the local concentrations of IL-6 and of NGF, which has actions mediated, in part, by the SNS (Woolf et al., 1996) and reduced by sympathectomy (Andreev et al., 1995). The finding that phentolamine and guanethedine (but not propranolol) reduced the ET-stimulated increases in the local concentrations of TNF-α, whereas ET-stimulated increases in local concentrations of IL-1β were resistant to all three sympatholytic treatments, argues against TNF-α-induced IL-1β production in this model, contrary to that described for another model of inflammatory hyperalgesia, the carrageenin-induced mechanical hyperalgesia (Cunha et al., 1992).

A role of adrenergic mechanisms in the cytokine-mediated inflammatory hyperalgesia was first proposed by Cunha et al. (1991), who showed that the carrageenin-evoked hyperalgesia was attenuated by guanethidine (adrenergic sympatholytic), indomethacin and an anti-IL-8 anti-serum. The same study revealed that IL-8 caused hyperalgesia by a prostaglandin-independent mechanism involving the SNS. More recently, intradermal injections of adrenaline were shown to cause hyperalgesia mediated through β-adrenergic mechanisms (Khasar et al., 1999), a finding consistent with the earlier report by Levine et al. (1988) suggesting contribution of β-adrenergic-mechanisms to experimental arthritis.

Sympathetic involvement (reflex or peripheral) in the ET model could be through one or more mechanisms, which include sensitization of nociceptors, central sensitization or increased local concentrations of cytokines and NGF, through PGE2-dependent and/or independent mechanisms. Each of these mechanisms could be shared, in varying degrees, by irritant induced-inflammation and by neuropathies leading to inflammation (Bennett, 1999; Arruda et al., 2000). The simplest scenario could involve either sensitization of the peripheral nociceptors, leading to central activation (reflex) of the sympathetic efferents, or excessive activation of sympathetic efferents, leading to changes in nociceptor function. In both instances, a ‘vicious cycle’ is established, leading to a cascade of the inflammatory mediators and pain (for review, see Devor and Seltzer, 1999).

The contribution of the sympathetic system to the local inflammation induced by ET may appear in contradiction to the general role played by SNS, as a major efferent component of the HPA axis (Chrousos, 1995;
Green et al., 1998). It is probably appropriate to consider the contribution of the SNS to local inflammation as an element of a local neurogenic loop (Levine et al., 1985). The resulting local inflammation and hyperalgesia ultimately lead to the activation of the HPA axis, which results in a cascade of events involving the neuroendocrine system. The activation of the HPA axis (global loop) is known to control the immune function that leads to the restriction of the inflammatory reaction to the affected area (Green et al., 1995, for review, see Elenkov et al., 2000).

In conclusion, we provide evidence to suggest that the SNS is actively involved in ET-induced inflammatory hyperalgesia. This involvement appears to be mediated by cytokines, NGF and other mediators well known to be involved in modulating the functions of peripheral and central neurons.

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