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The long-lasting sensitization of primary afferent nociceptors induced by inflammation involves prostanoid and dopaminergic systems in mice

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ABSTRACT

In recent years, evidence that sensitization of primary afferent nociceptors is an important event associated with chronic pain has been accumulating. The present study aimed to evaluate the participation of the prostaglandin and sympathetic components in the long-lasting sensitization of nociceptors induced by acute inflammation in mice. The intraplantar administration of carrageenan (100 μg) enhanced the nociceptive response to a small dose of PGE_2 (9 ng/paw) or dopamine (3 μg/paw) up to 30 days later. This long-lasting sensitization is dependent on dopaminergic and prostanoid systems, since the pre-treatment with chlorpromazine (3 μg/paw) or indomethacin (100 μg/paw), but not local (6 μg/paw) or systemic (6 mg/kg) treatment with morphine, prevented its development. In agreement with this idea, the previous intraplantar administration of hyperalgesic doses of PGE_2 or dopamine also induced long-lasting sensitization, which was fully prevented by pretreatment with EP_4 and D_1 antagonists, respectively. In summary, the present work described in mice a long-lasting sensitization of nociceptors, initiated by an acute inflammatory stimulation and dependent on dopaminergic and prostanoid systems. The present data represent new insights on the mechanisms of peripheral sensitization that could contribute to establish the basis of new therapeutic strategies for acute and chronic inflammatory pain.

1. Introduction

While the physiological pain has an important protective function, chronic pain can take on a disease character in pathological states, such as inflammation and neuropathy (Kuner, 2010). When pain persists for years, its broader effects on psychological health and performance of social responsibilities in work and family life can be profound (Turk and Rudy, 1988). Chronic pain not only differs from acute pain in its onset and duration, but also in its underlying mechanisms, and thus it often responds poorly to conventional analgesics. Better treatment of chronic pain will require clear understanding of the mechanisms that contribute to the transition from an acute tissue insult to chronic pain, and testing of pharmacological agents in such settings.

Recently, it has been proposed that chronic pain can result from a persistent sensitization of primary afferent nociceptors, which typically develops as a consequence of tissue insult (Ferreira et al., 1990; Gold and Gebhart, 2010). The clinical consequence of the sensitization of nociceptors is allodynia and hyperalgesia, which may correspond to a reduction in the nociceptive threshold and to an increase in the magnitude of the response to noxious stimulation, respectively (Bessou and Perl, 1969). Although the mechanisms that lead the acute injury to turn into chronic pain are poorly understood, clinical evidence suggests a relationship between the sensitization of nociceptors and pain maintenance (Gold and Gebhart, 2010). Usually, acute insults resolve without persisting pain, suggesting that brief nociceptor sensitizations are generally reversible. On the other hand, during chronic pain states initiated with tissue lesion, the sensitization tends to be irreversible. In addition, there are chronic pain states in which tissue pathology is not obvious, but the peripheral input and nociceptor sensitization are sufficient for the maintenance of pain (Gracely et al., 1992; Price et al., 2006, 2009; Staud et al., 2009; Verne et al., 2003). Considering this point of view, the duration of acute sensitization of nociceptors can be an important determinant to the development of chronic pain.

In fact, in some clinical chronic pain states, following the resolution of an episode of acute inflammation, an increased susceptibility to pain induced by subsequent stimuli is observed (Jacobson et al., 1989; MacIntyre et al., 1995; Melhorn, 1998). In line with this clinical

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observation, it has been demonstrated that acute inflammation, produced by carrageenan administration in the rat hind paw, induces an increased susceptibility to the hyperalgesia mediated by inflammatory mediators, such as prostaglandins (Aley et al., 2000). Indeed, prostaglandins are important inflammatory mediators that contribute to the sensitization of nociceptors (Ferreira, 1972; Ferreira and Nakamura, 1979). In addition to prostaglandins, the involvement of a sympathetic component has been described (Coderre et al., 1984; Cunha et al., 2005; Khasar et al., 1999; Nakamura and Ferreira, 1987; Safieh-Garabedian et al., 2002).

The present work aimed to evaluate the participation of prostaglandin and sympathetic components in the long-lasting sensitization of nociceptors induced by local inflammation in mice, by using the hyperalgesic priming model. This study intended to understand mechanisms of nociceptor sensitization and to identify new molecular targets for pharmacological intervention.

2. Methods

2.1. Animals

Experiments were performed using male Swiss Webster mice (20–25 g, University of São Paulo, Ribeirão Preto, Brazil). Animals were housed at 24 ± 1 °C, under a 12:12 h light–dark cycle (lights on at 07:00 AM), with free access to chow and tap water until the day of the experiment, when only water was made available to them. Animal care and handling procedures were in accordance with the International Association for the Study of Pain guidelines for the use of animals in pain research (Zimmermann, 1983) and the Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and any discomfort. Each animal was used only once and all behavioral testing was performed between 8:00 a.m. and 4:00 p.m. Behavioral tests were done without knowing which experimental group each mouse belonged to.

2.2. Nociceptive mechanical test

Mechanical hyperalgesia was evaluated in mice using a Dynamic Plantar Aesthesiometer (Ugo Basile) test. This apparatus has a pressure transducer coupled to a digital force detector that records the applied force in grams. Mice were placed in acrylic cages with wire grid floors 15–30 min before starting the test to environment adaptation. The test consists of evoking a hind paw flexion reflex using a filament-containing Universal Tip (10 μL, T-300, Axygen) that touches the plantar surface and exerts an upward force (maximal 50 g) on the plantar surface of the mouse hind paw. The end point was defined by the withdrawal of the paw followed by clear flinching movements. With paw withdrawal, the recorded force was automatically displayed. The results are expressed by the intensity of hyperalgesia (in grams) calculated by subtracting the force measured after the treatment from the basal value (Δ force). The nociceptive threshold of mouse paws measured at the first experimental day and before any experimental procedure was considered the baseline value and was used throughout the experimental period.

2.3. Drug preparation and administration

The agents used in this study were: indomethacin (Prodome, Campinas, SP, Brazil); morphine sulfate (Cristalia, Itapira, SP, Brazil); dopamine, chlorpromazine, L741626 (D_2 antagonist), SCH-23390 (D_1 antagonist), L-745870 (D_4 antagonist), AH23848 (EP_4 antagonist) and PGE_2 (Sigma, St. Louis, MO, USA); carrageenan (FMC, Philadelphia, USA). All the drugs were dissolved in 0.15 M NaCl (saline), except for indomethacin, which was dissolved in Tris buffer (Merck, Darmstadt, Germany) and for PGE_2, which was stored in dimethyl sulfoxide 2% (Sigma, St. Louis, MO, USA) and dissolved in saline. All drugs were administrated locally (hind paw, intraplantar — i.pl.) with a 29 G hypodermic needle in a volume of 25 μL per paw, with the exception of morphine, which was also delivered systemically by subcutaneous (s.c.) route.

2.4. Statistical analysis

Results are presented as means ± SEM of measurements made on 6 animals in each group and represent the intensity of hyperalgesia. The curves were analyzed by two-way ANOVA to find interactions between time and treatment. To analyze distinct points of the curves we used one-way ANOVA followed by Bonferroni's pos-test. Differences were considered to be statistically significant at $p<0.05$.

2.5. Experimental protocols

Induction of long-lasting sensitization of nociceptors: in this protocol the effects of a single administration of a pro-inflammatory agent (carrageenan) or two agents known to be released by carrageenan (PGE_2 and dopamine; Nakamura and Ferreira, 1987) were evaluated. In the first experimental day, the nociceptive threshold was evaluated before any treatment. Carrageenan (100 μg), PGE_2 (90 ng), dopamine (30 μg), or saline were administered by intraplantar route, and the intensity of hyperalgesia was evaluated 2–3 h later. Aiming to quantify the long-lasting sensitization, the response to an intraplantar injection of PGE_2 (9 ng or 90 ng), dopamine (3 μg) or saline was evaluated 5 or 30 days after the acute inflammation. The intensity of hyperalgesia was evaluated at different times, as indicated in the figures.

We also tested the effects of pre-treatment with the cyclooxygenase inhibitor indomethacin, the dopamine-receptor antagonist chlorpromazine, and the morphine against carrageenan-induced sensitization. Indomethacin (100 μg/paw), chlorpromazine (100 μg/paw), and morphine (6 μg/paw) were administered 30 min before the carrageenan injection at doses derived from previous studies (Cunha et al., 2005). Morphine (6 mg/kg) was also administered by subcutaneous route 30 min before and 12 h after the carrageenan administration. In addition, the effects of dopamine antagonists (D_2, L741626; D_1, SCH23390; D_4, L745870) and PGE_2 antagonist (EP_4, AH23848) were evaluated in PGE_2- or DA-induced sensitization.

3. Results

In the present study, we describe a long-lasting sensitization of nociceptors induced by local inflammation in mice, which is dependent on the dopaminergic and prostanoid systems. As shown in Fig. 1, the intraplantar treatment with carrageenan (100 μg) in the first experimental day produced a short-term hyperalgesia from which the animals fully recovered, with values similar to normal baseline at experimental days five (Fig. 1a) and thirty (Fig. 1b). Subcutaneous intraplantar injection of a small dose of PGE_2 (9 ng/paw) or dopamine (3 μg/paw), at the same site into which carrageenan had been injected 5 or 30 days earlier, resulted in an increased hyperalgesic response. During this sensitized state, the mechanical nociceptive threshold has returned to pre-carrageenan values (Fig. 1, day 5 and day 30). However, administration of PGE_2 or dopamine, at doses that only induce a slight and brief hyperalgesia in normal conditions, induced an improved hyperalgesic effect (Fig. 1).

Because the carrageenan-induced hyperalgesia in mice is dependent on prostaglandins and sympathetic amines, the contribution of these two components to inflammation-induced long-lasting sensitization was evaluated. The pre-treatment with a cyclooxygenase inhibitor, indomethacin (100 μg/paw), or a dopaminergic antagonist, chlorpromazine (3 μg/paw), partially inhibited the carrageenan-induced acute hyperalgesia, but completely prevented the development of the long-lasting sensitization (Fig. 2a). On the other hand, the systemic pre-treatment with morphine (6 mg/kg/s.c.; Fig. 2b) twice in the first experimental day, fully prevented the acute hyperalgesia induced by