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Pharmacological characterization of the effects of 5-hydroxytryptamine and different prostaglandins on peripheral sensory neurons *in vitro*

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Abstract

The excitatory and sensitizing properties of 5-HT and different prostaglandins (PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2 and PGI_2-analogue, cicaprost) were characterized on an *in vitro* preparation of the neonatal rat spinal cord with functionally attached tail. Prolonged (10 min) perfusion of the tail with 5-hydroxytryptamine (5-HT, 0.5–10 μM) or any of the tested prostaglandins (0.1–5 μM) did not evoke an excitatory response recorded from a lumbar ventral root, but significantly enhanced responses of peripheral nociceptors to thermal and chemical (bradykinin, capsaicin) stimuli. PGD_2 did not induce such an enhancement. Following sensitization of peripheral nociceptors with low concentrations of bradykinin or capsaicin, 5-HT (1–10 μM) evoked a ventral root response. Using specific 5-HT-receptor agonists and antagonists, 5-HT-evoked excitation was determined to be mediated via a 5-HT_1-like receptor while 5-HT-induced sensitization involved 5-HT_2 receptors.

Introduction

One characteristic of inflammation and tissue injury is hyperalgesia, defined as an enhanced responsiveness to painful stimuli and a decrease of the pain threshold. In functional studies, chemical products of tissue damage such as prostaglandins [1, 2] and 5-hydroxytryptamine (5-HT) [3, 4] produce hyperalgesia at concentrations which are devoid of any direct algesic properties. Presently, we have characterized the sensitizing and excitatory properties of different prostaglandins and 5-HT on nociceptive neurons in an *in vitro* preparation of the neonatal rat spinal cord with functionally connected tail [5].

The intact spinal cord with tail attached was isolated from 0–2-day-old rats and the most superficial layers of the skin were removed from the tail. The isolated cord and tail were separately superfused (3 ml/min on cord; 6 ml/min in tail) with a physiological salt solution at 23 ± 2 °C and gassed with 95% O_2:5% CO_2. The activation of peripheral nerve fibres was assessed by measuring spinal ventral root potentials (VRP). Recordings were made using a glass micropipette placed in an electrolyte-filled well containing the selected ventral root (L_3–L_5). Conventional methods were used to record and store electrophysiological signals. Peripheral nociceptors were activated by stimuli

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applied at regular intervals but sufficient to avoid tachyphylaxis.

Brief (10 s) perfusion of the tail with heated superfusate (50 ± 2 °C) evoked a VRP which was a reliable measure of maximal responsiveness of the preparation to noxious stimulation. Other responses were, therefore, normalized with respect to the response evoked by noxious heat on the same tissue. Perfusion of the tail for 10 min with 5-HT (0.5–10 μ*M*) or any of the tested prostaglandins (0.1–5 μ*M*) did not evoke a VRP. However, the prolonged exposure of peripheral nerve fibres to 5-HT (5 μ*M*), PGE_1 (500 n*M*), PGE_2 (500 n*M*), $PGF_{2\alpha}$ (500 n*M*), PGI_2 (5 μ*M*) or the PGI_2-receptor agonist, cicaprost (100 n*M*), significantly enhanced subsequent responses to submaximal concentrations of capsaicin (75–350 n*M*) or bradykinin (100–250 n*M*) in a concentration related manner (Fig. 1). PGD_2 had no effect at the highest tested concentrations (0.5–5 μ*M*). Similarly, responses evoked by brief (10 s) exposure to threshold thermal stimulation (36–42 °C) were enhanced by 5-HT and each prostaglandin, except PGD_2 (results not shown). Sensitization by 5-HT or the prostaglandins was not affected by 1–5 μ*M* indomethacin. However, the 5-HT-induced sensitization was blocked by ketanserin (10 n*M*–1 μ*M*; Fig. 1), indicating a 5-HT_2-receptor-mediated effect. The $5\text{-HT}_1/5\text{-HT}_2$-receptor antagonist methiothepin (1 μ*M*) or the $5\text{-HT}_3/5\text{-HT}_4$-receptor antagonist ICS 205-930 (1–10 μ*M*) did not affect 5-HT-induced sensitization. The $5\text{-HT}_{1C}/5\text{-HT}_2$-receptor agonist α-methyl-5-HT (1–5 μ*M*) mimicked the 5-HT-induced enhancement of chemically and thermally evoked responses, but the 5-HT_3-receptor agonist 2-methyl-5-HT (10 μ*M*) and the 5-HT_1-receptor agonist 5-carboxamidotryptamine (5 μ*M*) did not. These data further support that 5-HT-induced sensitization was mediated by 5-HT_2-receptors.

Following continuous perfusion of the tail for 10 min with a threshold concentration of bradykinin (20–25 n*M*) or capsaicin (50–100 n*M*), a brief

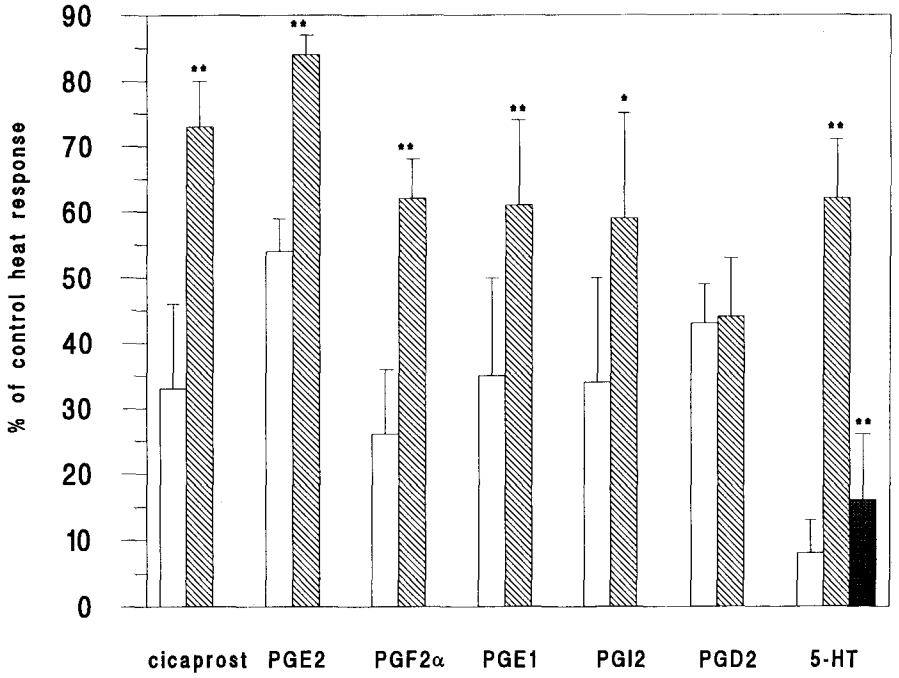

Figure 1

Enhancement of submaximal control responses (open bars) to bradykinin (100–250 n*M*) and capsaicin (75–350 n*M*) by cicaprost ($n=6$), PGE_2 ($n=5$), $PGF_{2\alpha}$ ($n=6$), PGE_1 ($n=6$), PGI_2 ($n=5$), PGD_2 ($n=15$) and 5-HT ($n=4$). Each bar shows the mean ± SEM of the combined responses to bradykinin and capsaicin which were significantly enhanced in the presence of 100 n*M* cicaprost, 500 n*M* PGE_2, 500 n*M* $PGF_{2\alpha}$, 500 n*M* PGE_1, 5 μM PGI_2 or 5–10 μ*M* 5-HT, but not 0.5–5 μ*M* PGD_2 (hatched bar). Ketanserin (1 μ*M*; $n=4$) significantly blocked the 5-HT-induced sensitization (solid bar). Statistical evaluation was performed with paired student's *t*-test; $^{**}p<0.01$; $^{*}p<0.05$.

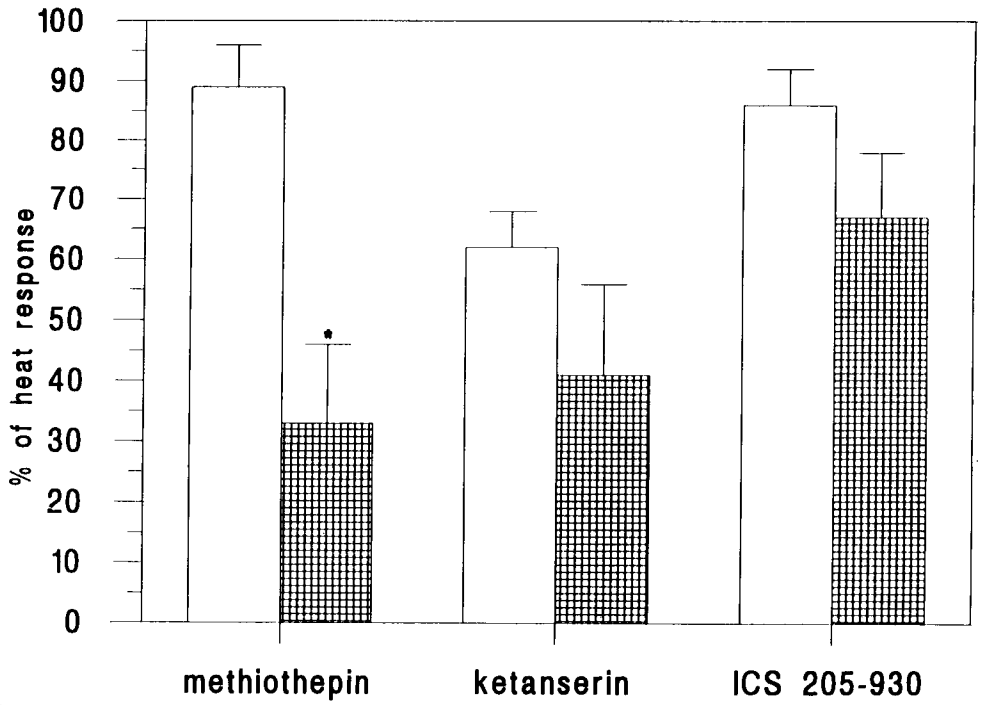

Figure 2
5-HT-evoked control responses (open bars) on sensitized peripheral nociceptors were significantly reduced by 1 μM methiothepin ($n=4$). Similar pretreatment with 1–10 μM ketanserin ($n=4$) or 1–10 μM ICS 205-930 ($n=6$) did not significantly reduce 5-HT-evoked excitation (solid bars).

application of 5-HT (1–10 μM) now evoked a VRP which was reduced by methiothepin (1 μM) but not by ketanserin (1–10 μM), ICS 205-930 (1–10 μM) or indomethacin (1–5 μM), indicating a 5-HT_1-like-mediated effect (Fig. 2). This result was confirmed with 5-carboxamidotryptamine (1 μM) which also evoked a VRP on the sensitized preparation. α-Methyl-5-HT (10 μM) and 2-methyl-5-HT (10 μM) were ineffective in this respect.

In summary, these results show that different cyclooxygenase products as well as 5-HT sensitize peripheral nociceptors at concentrations which are present under inflammatory conditions. 5-HT also excited nociceptors when applied to sensitized tissue. Two types of receptor appear to be involved in the actions of 5-HT. The increased responsiveness of nociceptors involves a 5-HT_2-receptor, whereas 5-HT-mediated activation of nociceptors involves a 5-HT_1-like-receptor. These effects may contribute to peripheral hyperalgesia following inflammation or tissue injury.

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