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Increased Response of Hypogastric Nerve Fibers to Bradykinin by Mustard Oil-Induced Uterine Inflammation in the Rat

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It is well known that the inflammation of somatic tissues, bladder and colon can alter the sensitivity of primary afferents innervating these tissues. To see if uterine afferents also show altered sensitivity, we examined their responses to the algesic agent bradykinin before and after induction of uterine inflammation. Inflammation was induced by injecting the mustard oil into the uterine lumen of adult female rats. After induction of inflammation, the response latency to bradykinin did not change, but the duration and peak of the response and integrated impulse discharges during the response period increased significantly. Furthermore, after inflammation, the level of resting discharges of the afferents was much higher. These results are consistent with the idea that the inflammation can sensitize the uterine afferents.

Key Words: Bradykinin, Inflammation, Hypogastric nerve, Uterus, Mustard oils

INTRODUCTION

The uterus is a very unique female organ for reproduction. It is regulated by various hormones and autonomic nervous systems, sympathetic and parasympathetic (Mustafa et al, 1987; Zoubina et al, 1998). For neural regulation, there is another component such as afferent sensory supply.

After Bower's reports (1959, 1966) about afferent fibers of the uterus in the rabbits, Abraham & Teare (1969), Floyd et al (1976) & Berkley et al (1988, 1990) provided the evidence that the hypogastric nerves in the cat and rat contain similar afferent nerve fibers. These studies showed that the afferent fibers in the hypogastric nerve responded to the mechanical stimulation of the uterus and the broad ligament. They are slowly conducting thinly-myelinated or unmyelinated fibers. Berkley et al (1990) & Hong et al (1993) have suggested that some mechanoceptive fibers in the hypogastric nerve have polymodal properties responding to algesic chemical stimulation such as bradykinin, prostaglandins and capsaicin. So these afferent fibers should participate in nociception and relay the information of uterine pathological states to the spinal cord (Berkley et al, 1993).

Previous studies, employing various animal models have suggested that inflammation is accompanied by hyperalgesia, a decrease of pain thresholds to several kinds of noxious stimuli (Reeh et al, 1986; Kocher et al, 1987; Schmidt, 1996; Mizumura, 1997). As the neural bases of inflammatory hyperalgesia, wind-up phenomena in the spinal cord and the sensitization of peripheral nociceptors have been indicated (Woolf, 1983; Schmidt, 1996). However, the evidence for the peripheral sensitization is relatively weak particularly for the female reproductive organs.

Mustard oil is widely used for the induction of inflammation (McMahon & Abel, 1987; Habler et al, 1988; Schmidt, 1996). The primary action of mustard oil is thought to cause substance P release from peripheral nerve terminals (Inoue et al, 1997).

Bradykinin is implicated in multiple physiological processes such as control of blood pressure, contrac-

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tion and relaxation of smooth muscle and inflammatory responses (Caloxto et al, 2000). Also bradykinin may play a role as pain mediator. It is known as a potent painful stimuli (Steranka et al, 1988).

The present study was performed to obtain evidence that uterine inflammation sensitizes uterine afferent nerve fibers.

METHODS

General procedures

The study was carried out on 10 female rats weighing 200~300 g. The animals were anesthetized by subcutaneous injection of 25% urethane (0.5 ml/100 g body weight). A cannula was inserted into the jugular vein, and an infusion pump was connected to the cannula for infusion of urethane at 0.1 ml/h to maintain the anesthesia. All animals were immobilized by pancuronium bromide injection and artificially ventilated. Blood pressure, end-expiratory CO_2 and body temperature were monitored and maintained at physiological levels.

Uterine preparation

A fine polyethylene tubing was inserted into a branch of the uterine artery through the femoral artery for the injection of bradykinin into the uterine circulation. A proper cannulation was ascertained by the injection of Evan's blue (Sigma, T-1824, USA) (Fig. 1A).

To inject mustard oil into the uterine lumen, a small incision was made in the ovarian end of the uterus.

Recording and identification

Electrophysiological signals were recorded from the hypogastric nerve (Fig. 1B). In the pool formed using skin flaps and filled with mineral oil, an isolated nerve strand was laid on the platinum electrode connected to DAM-80 amplifier (W.P.I, USA) and the signals from the uterus were displayed on a storage oscilloscope (Tektronix, 5113, USA) after appropriate amplification and filtering (Tektronix, AM 502, USA). The signals from the nerve fibers were taken up with a window discriminator (W.P.I., window discriminator 121, USA), and the outputs from the window discriminator were analyzed using a specialized computer program (C.E.D., Spike 2, England). All impulse activities were also recorded on VCR tapes for further analysis (Fig. 2).

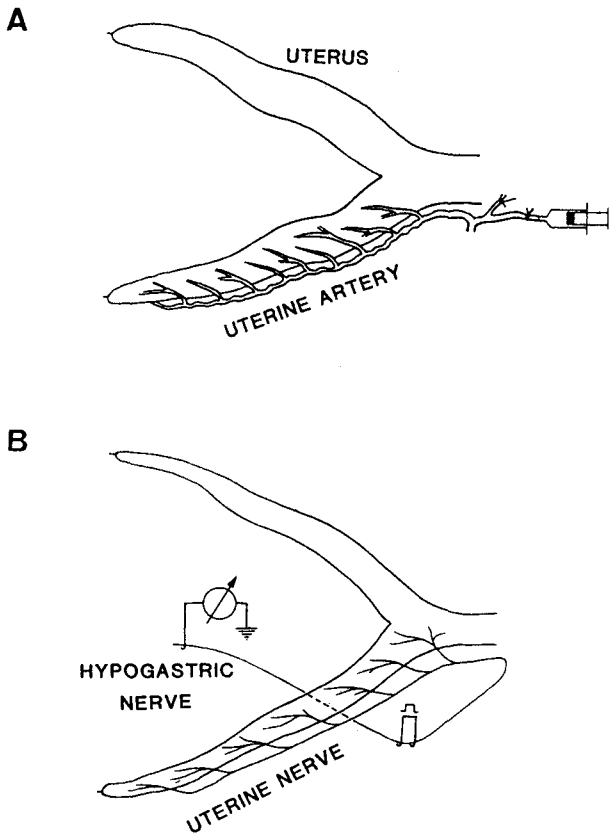

Fig. 1. Schematic drawings of experimental setup. The setup for intra-arterial injection of chemicals is shown in A. The uterine afferent unit (hypogastric nerve) recording setup is shown in B.

Experimental protocol

The signals from the uterus were identified by mechanical stimulation of the uterine body with glass rod. Three to four control responses to bradykinin injections (10 ng in 0.1 ml) were recorded; the intervals of bradykinin injections were more than 10 min. At these intervals, desensitization did not occur. Mustard oil (30% in mineral oil) was then injected into the uterine lumen. After 1 hour, the response of uterine afferents to bradykinin was recorded again to make comparison with the control responses.

Histological examination

To determine whether or not inflammation was

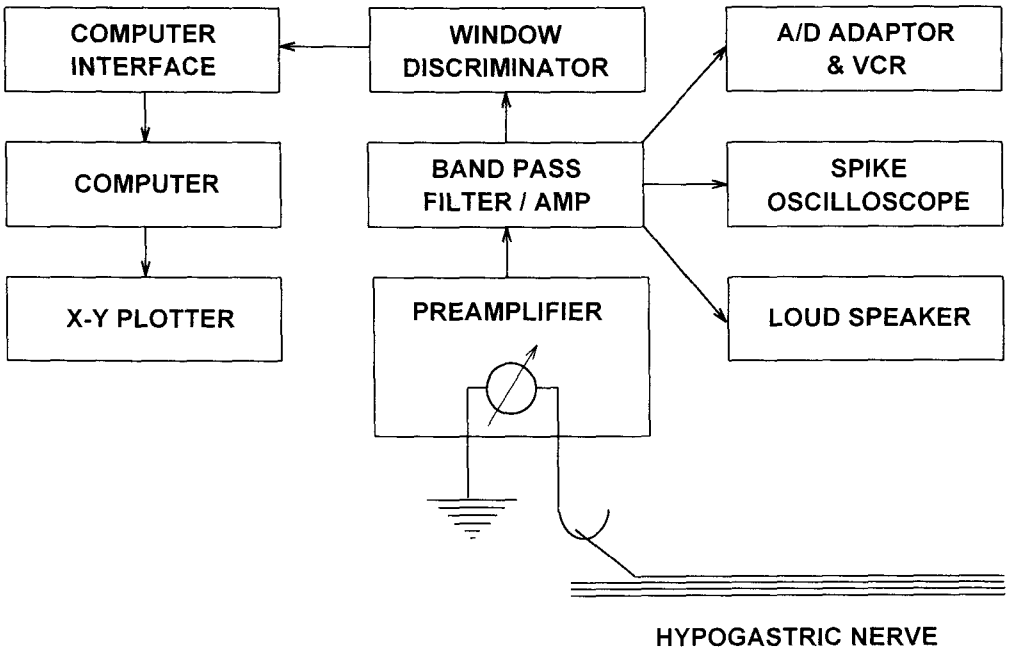

Fig. 2. Schematic drawing of experimental setup for nerve recording and data analysis.

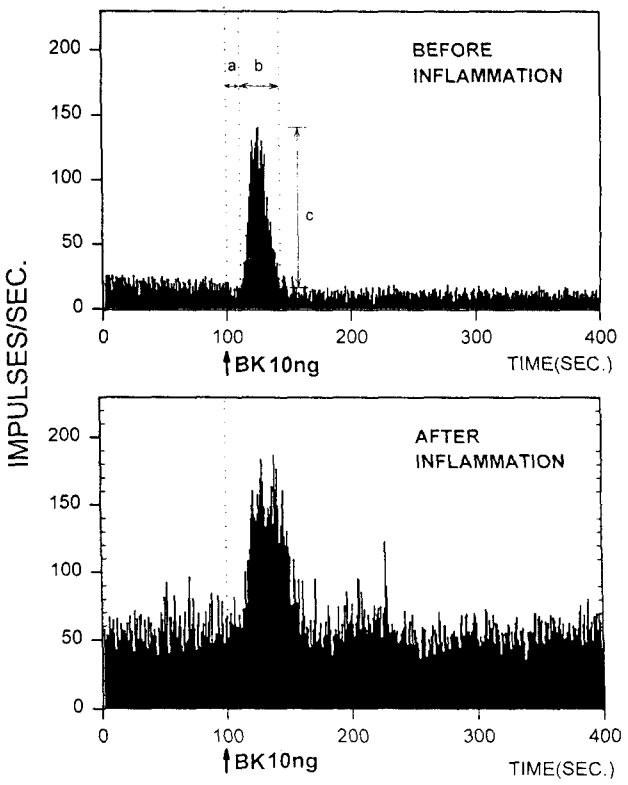

Fig. 3. Changes in the response of uterine afferents to injected bradykinin (BK) before and after inflammation (a: latency of bradykinin response, b: duration of bradykinin response, c: peak amplitude).

induced, the uterus was excised after experiment and processed for Hematoxylin-Eosin staining.

Data analysis

Baseline recordings of the resting discharges (bins/sec) of nerve fibers were made for more than 100 seconds before bradykinin treatment. The latency and peak amplitude of the response to bradykinin and duration were determined. Also, integrated impulse discharges during the response were counted (Fig. 3).

The numerical data derived from each bradykinin response were compared by Wilcoxon Paired test.

RESULTS

Multiunit recordings showed that mustard oil-induced uterine inflammation severely altered the activities of hypogastric nerve fibers. The baseline discharges were much higher after the mustard oil treatment than before (from 271.6 ± 66.4 to 1023.2 ± 510 impulses for 100 seconds, mean$\pm$S.E., n=11, $P < 0.01$) (Fig. 4). Also the responses to bradykinin were significantly different 1 hr after mustard oil treatment. Although the latency of the response to bradykinin was not different (from 23.1 ± 4.7 to 22.0 ± 6.0 sec)

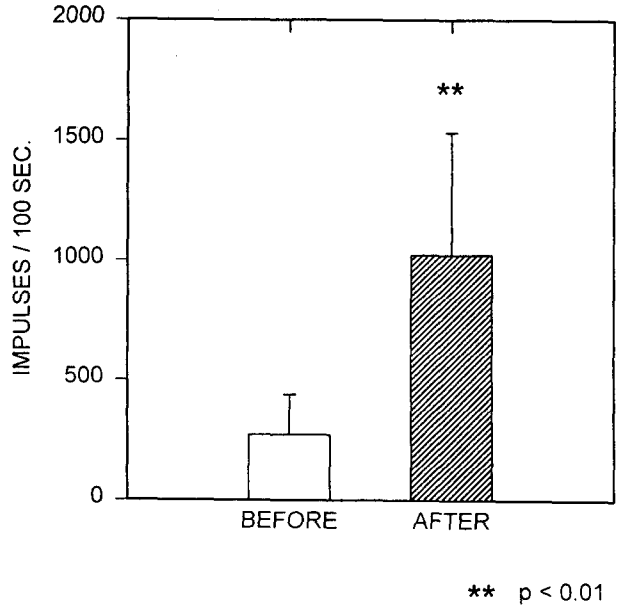

Fig. 4. Changes in resting discharges of hypogastric nerve before and after inflammation (mean±S.E., n=11).

(Fig. 5A), the duration and peak of the response and total impulse discharge during the response were significantly increased by mustard oil treatment. The response duration was increased from 17.6±3.4 sec to 48.4±7.7 sec ($P<0.01$) (Fig. 5B), and peak discharge and total impulse discharges were increased from 13.2±3.3 to 17.0±2.8 impulses/sec ($P<0.05$) and 193.3±23.8 to 276.3±52.3 impulses ($P<0.05$), respectively (Fig. 5C, D).

The histological examination of the uterus after electrophysiological recordings indicated the inflammatory signs, especially the migrating of leukocytes out of blood vessel in the endometrium and myometrium (data not shown).

DISCUSSION

The uterus treated with mustard oil showed the inflammatory signs in the histological observation. The concentration of mustard oil in this study is

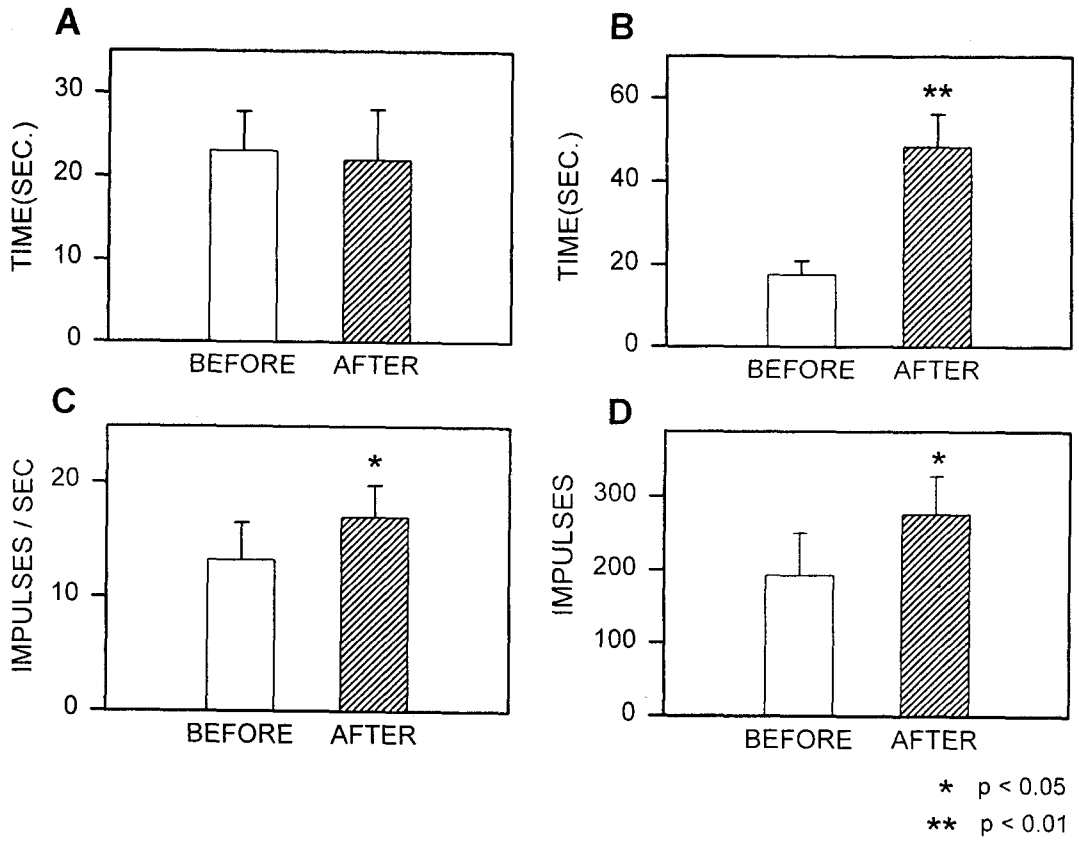

Fig. 5. Changes in responses to bradykinin before and after inflammation. A: latency, B: duration, C: peak amplitude of the response and D: integrated impulses during the response (mean±S.E., n=11).

higher than that used in previous studies with other visceral organs like the rectum and urinary bladder (Habler et al, 1988, 1990) because the thickness of the endometrium seems to be resistant to noxious materials.

The treatment of mustard oil immediately increased the impulse frequencies of the afferent nerve fibers of the uterus. Mustard oil appears to activate some afferent fibers directly. Other reports suggested that mustard oil could elicit a direct response of substance P-containing neurons and that the increase of substance P release in the peripheral tissue should be a major cause of the mustard oil-induced inflammation (Lembeck et al, 1992; Wilsoncroft et al, 1994; Damas et al, 1996; Inoue et al, 1997). This might be one of the reasons for the increase of baseline activities of uterine nerve fibers. Substance P acts as an algesic agent and sensitizes the afferent fibers through increase of inflammatory mediators or nerve growth factor (Hepplemann & Pawlak, 1997; De Felipe et al, 1998; Inoue et al, 1999), so the response of afferent fibers to the innate tension or inflammatory mediators might be augmented although their concentrations are not increased. Furthermore inflammatory mediators including bradykinin were observed to increase after mustard oil treatment in other tissues (Habler et al, 1990). Therefore the increase of spontaneous activities of primary afferent fibers innervating the uterus can be explained by the afferent nerve sensitization and the increase of chemical stimulants such as inflammatory mediators. It is not certain which mediators cause the sensitization. Since continuous treatment of bradykinin induces the tachyphylaxis to the agent, the increase of bradykinin might be excluded from possible candidates (Schmidt, 1996; Mizumura, 1997).

The response of uterine afferent fibers to bradykinin was abrupt and intense, and increased after mustard oil treatment. The increase in the duration and peak of the response and in total impulse discharge during the response may be explained by an increase of substance P as described above or by the changes of bradykinin receptors from B2 to B1. Bradykinin plays an important role in the pain transmission directly acting on Aδ and C fiber primary sensory neurons (Steranka et al, 1988). Bradykinin acts through two kinds of receptors on the plasma membrane, B1 and B2 receptors (Rhaleb et al, 1991). In normal tissues, B2 receptor is expressed and serves as bradykinin receptor. The activation of B2 receptors leads to extravasation, an increase of prostanoids and the release of inflammatory mediators from mast cells. But in pathological states like inflammation, B1 receptor is induced and plays a role in nociception and hyperalgesia (Dray & Perkins, 1993, 1997; Marceau & Bachvarov, 1998).

In this study, no change in the latency of response to bradykinin seems to reflect that bradykinin might act on the same kind of receptor, B2 receptor, before and after mustard oil treatment. Recently it was reported that B1 and B2 receptors had different mechanisms for pain. Boyce et al (1996) observed that mice lacking the B2 gene showed reduced thermal hyperalgesia induced by carrageenan confirming that B2 receptors should participate in nociception. Pesquero et al (2000) also reported, by the use of B1 knockout mice, the role of B1 receptors in the development of central sensitization, known as "wind-up". The time point of 1 hr after mustard oil treatment observed in this study appears too fast to induce the B1 receptors in the tissues. Recently Frode-Saleh et al (1999) reported that substance P-induced inflammatory response in the mouse pleural cavity was inhibited by B2 receptor antagonist but not by B1 receptor antagonist within 4 hr-following substance P treatments. So the response of peripheral nerve fibers to bradykinin might be mediated through B2 receptor.

The increase of response to bradykinin after mustard oil treatment can be from the increase of bradykinin in the peripheral tissue. Bradykinin acts as a potent mediator of inflammation, including vasodilation, plasma extravasation and cell migration (Hall & Morton, 1997). Bradykinin is found to sensitize primary afferent neurons through interactions with most inflammatory mediators, including prostaglandins, and cytokines (Dray & Perkins, 1997) and through the production of diacylglycerol and activation of protein kinase C (Levine et al, 1993; Cesare et al, 1999; Khasar et al, 1999).

To summary, we observed that uterine inflammation sensitized the primary sensory fibers supplying the uterus through the hypogastric nerve. This sensitization may contribute to the development of inflammatory hyperalgesia.

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