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Rat Visceral Polymodal Receptors and Suppression by Zaltoprofen of Their Responses to Bradykinin In Vitro

Jin YU, Hisashi KODA and Kazue MIZUMURA
Department of Neural Regulation
Division of Regulation of Organ Function
Research Institute of Environmental Medicine
Nagoya University, Nagoya 464-8601, Japan

Abstract: The aim of this study was to examine visceral afferent characteristics in rats and investigate the effect of zaltoprofen on their responses to bradykinin. Single fiber recordings were performed using rat spermatic nerve preparations in *vitro*. More than 90% of units tested with thermal, mechanical and chemical (bradykinin, pH, etc) stimulations responded to all of them, and thus were identified to be polymodal receptors. The responses of these receptors to bradykinin (10^{-9} M to 10^{-5} M) showed concentration dependency. There were almost no fibers with receptive fields on the testis, unlike the canine and feline spermatic afferents. In addition, zaltoprofen (10^{-5} M), a non-steroidal anti-inflammatory drug, suppressed the bradykinin response of these receptors.

Key words: visceral afferents, polymodal receptors, bradykinin dose response, zaltoprofen, rats

Analysis of the response characteristics of nociceptors and the mechanisms by which they change in pathological conditions are key factors to understanding the pain mechanism. Many efforts have been made for this purpose using cutaneous, muscular and visceral preparations in *vivo*. Drawbacks of using in *vivo* preparations are the difficulty in controlling the drug concentration and undesired secondary effects through vascular changes. One preparation that has no such drawback is the canine testis-spermatic nerve preparation: the testis and epididymis with spermatic cord attached can be excised without damaging/contaminating the receptive field, the receptive field is mainly located near the surface, and drugs are easily delivered to the receptive field. The concentration and temperature of the drugs are therefore controlled, and no secondary effects are induced through vascular changes because of the absence of driving pressure. Using this preparation we demonstrated that many inflammatory mediators sensitize polymodal receptors to heat and mechanical stimulations (Mizumura and Kumazawa 1996, for review). They also clarified receptor subtypes for these mediators and the intracellular messengers active in sensitization to heat (Mizumura and Kumazawa 1996). For detailed analysis on the altered responses of nociceptors in inflamed animals and on the channels modified, small rodents are more advantageous animal models. We thus examined visceral afferent characteristics in rats in the present study. In addition, we examined the effect of zaltoprofen on the bradykinin response of polymodal receptor units. Zaltoprofen is a non-steroidal anti-inflammatory drug (NSAID) and claimed to be specifically effective in suppressing bradykinin-induced nociceptive behavior in animals (Hatori and Kokubun, 1998: Kawai, 1998).

Methods and Materials

Single fiber activities were recorded in *vitro* using testis-spermatic nerve preparations excised from adult male Sprague-Dawley (SD) rats (350–600 grams). The method was essentially the same as that used for canine preparations (Kumazawa et al, 1987). Animals were anesthetized with sodium pentobarbital (60 mg/kg, i.v.). The testis and epididymis with spermatic cord were excised and suspended in a test pool with modified Krebs-Henseleit solution (in mM: 110.9 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.4 $NaHCO_3$, and 20 glucose, PH 7.4, 34 ± 0.5°C). The spermatic cord was held in a pool with liquid paraffin where single fiber activities were recorded from the spermatic nerve. Conduction velocity was estimated using a conventional method. The mechanical threshold was measured by means of calibrated von Frey-type nylon hairs (39.2 mN to 264.6 mN) with a tip of 0.5 mm in diameter. The threshold was determined by the lowest intensity that induced excitation to the receptor.

Chemical (bradykinin, low pH solution), cold and heat stimulations were carried out by replacing the Krebs solution in the test pool with chemical solution, or with the Krebs solution prewarmed or cooled to 45°C, 50°C or 10–20°C for 60 s. The units responding to all stimuli, mechanical, chemical and heat, were identified as polymodal receptor. Bradykinin solution was prepared by dissolving bradykinin in the Krebs

Reprint request to: Dr. K. Mizumura at the above address.

solution at 10^{-9} M to 10^{-5} M and warmed to 34°C; low pH solution was made by dissolving acetic acid in the Krebs solution to make the pH of the solution 6 to 3 and warmed to 34°C. The stimulation sequence was mechanical stimulation, bradykinin, low pH solution, cold, and then heat.

Zaltoprofen, which was dissolved in Krebs solution at 10 μM and warmed to 34°C, was applied to units during the period starting 10 min before the first bradykinin stimulation until the end of the bradykinin experiments. Bradykinin was obtained from Peptide Institute Inc. (Mino-shi, Osaka, Japan), and Zaltoprofen was a kind gift from Nippon Chemiphar Co., Ltd (Tokyo, Japan).

The net mean discharge rate (NMDR, impulses/s) was calculated by subtracting the mean discharge rate (MDR) obtained during the pre-stimulation control period (30 s-1 min) from the MDR measured during the stimulation period. Total discharge number was also calculated. Results are expressed as the mean of observations ± S.E.M. Effects of zaltoprofen treatment were statistically analyzed by Bonferroni multiple comparisons, with $P<0.05$ considered to indicate a significant change.

Results

1. General characteristics of rat visceral polymodal receptors.

Sixty-nine units were recorded in total. All units responded to mechanical and chemical stimulations. Fourteen of 15 units in which heat stimulation was applied were excited by the heat (93%). Thus, most of the units are identified to be polymodal receptors (Table 1). A representative recording of responses to various kinds of stimulation in a polymodal receptor unit is shown in Fig. 1. Conduction velocities (CV) fell between 0.5 m/s and 2 m/s (average 0.57 ± 0.12 m/s), and only 1 unit had a CV of more than 2 m/s.

The receptive fields identified with a glass rod or VFH were located in the fatty portion of the pampiniformis (fatty portion), epididymis, or membranous structure between the testis and epididymis (membrane portion) (Fig. 2). Thirty eight percent of the spermatic polymodal receptors were found in the fatty portion, 46% in the epididymis and 16% in the membrane portion. We could not find any unit with receptive fields in the testis itself. The absence of receptive fields in the testis clearly contrasts with the canine (Kumazawa et al, 1987) and feline (Leng et al, 1994) spermatic receptors. The median mechanical threshold for the receptor in the fatty, epididymis and membrane portions was 72 ± 39, 141 ± 28, and 72 ± 25 mN, respectively. There was no significant difference in CV among the units in the different parts ($P>0.05$, Mann-Whitney test).

Heat responses were examined in 15 units. Fourteen units responded to heat, and the heat threshold ranged between 40 and 50°C (Fig. 1). The response showed temperature dependency. Only one unit tested showed no response to heat.

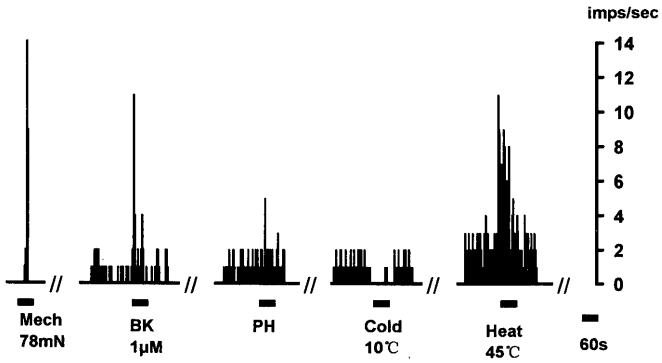

Fig. 1 Responses of a polymodal receptor to various kinds of stimulation
Post-stimulus time histograms are shown. The stimulation period is indicated by a thick line under the histogram.

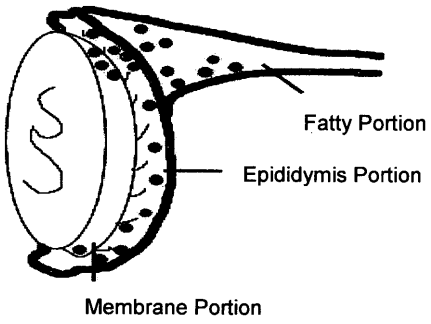

Fig. 2 Distribution of the receptive flied
The receptive field distribution identified by VFH. Units in the epididymis seem to have higher mechanical threshold than those in membrane and fatty portions. There were no receptive fields in the testis.

Table 1 Numbers of units responded to various kinds of stimulations

Items	Mechanical	Bradykinin (10^{-9}M–10^{-5}M)	Heat (45–50°C)	Cold (10°C)	Proton (6–3)	Mech & heat & chemical
Number of units tested	69	69	15	19	8	15
Number of units responded	69	69	14	5	8	14
Percentage (%)	100	100	93	26	100	93

Nineteen units were examined with cold stimulation down to 10–20°C, of 14 units exhibited suppression of their on-going activities (Fig 1). Only five units were excited (10°C).

Stimulation by low pH Krebs solution (range from 6 to 3) was carried out in 8 units, and all units responded to this stimulation (threshold pH 5). The proton response also showed concentration dependency: NMDRs were 0.09 ± 0.04, 0.49 ± 0.40, 0.62 ± 0.27, and 1.23 ± 0.26 to pH6, 5, 4, and 3, respectively ($P<0.05$, Bonferroni multiple comparisons). Fig. 1 shows an example of recording.

2. Response to bradykinin and effect of zaltoprofen

Eleven units were tested for response to 10^{-9} M to 10^{-6} M bradykinin without zaltoprofen. Nine units were tested for response to 10^{-8} M to 10^{-5} M bradykinin and an additional 3 units to 10^{-9} M to 10^{-5} M bradykinin in the presence of zaltoprofen. As seen in Figs. 1 and 3, the response to bradykinin in rat epididymis preparation began several to several 10s of seconds after the start of bradykinin application, and usually ended before the bradykinin was washed out. This discharge pattern was different from canine and feline preparations in which bradykinin response lasted long after the end of bradykinin application (Kumazawa et al, 1987: Leng et al, 1994). The NMDR increased concentration-dependently from 0.12 ± 0.08 imps/s for 10^{-9} M to 0.91 ± 0.14 imps/s for 10^{-6} M, and the responses for the concentrations higher than 10^{-7} M were significantly different from that for 10^{-9} M. The mean latency for bradykinin 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M was 45 ± 16 s, 38 ± 10 s, 19 ± 2 s, and 10 ± 1 s, respectively.

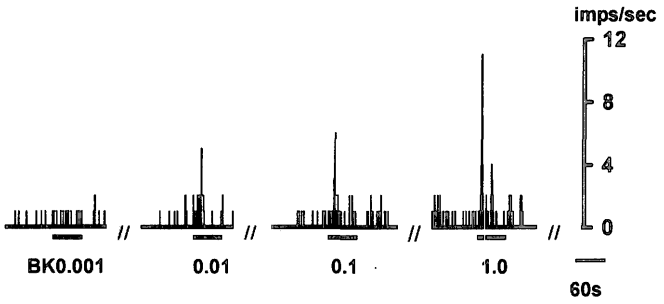

Fig. 3 A example of bradykinin concentration-response relationship. Bradykinin response during 60 s application. Ordinate: discharge rate, abscissa: bradykinin concentration. // indicates that there is several-minute interval

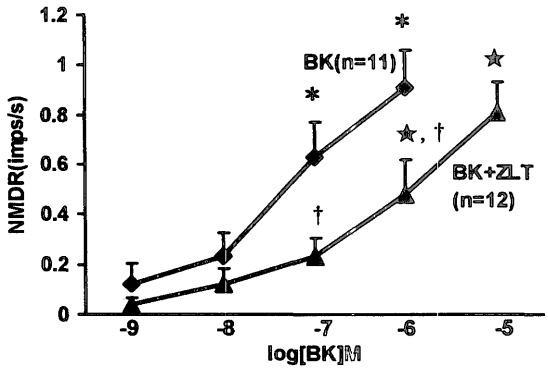

Fig. 4 Zaltoprofen suppressed the bradykinin concentration-response relation.
Zaltoprofen shifted the bradykinin concentration-response curve to the right. Eleven units were tested by 10^{-9} to 10^{-6} M bradykinin, 3 units by 10^{-9} to 10^{-6} M bradykinin, and 9 units by 10^{-8} to 10^{-5} M bradykinin after pre-perfusion with zaltoprofen (10^{-5} M). The net mean discharge rate (NMDR) of bradykinin concentration-response was significantly decreased by zaltoprofen. *: significantly different ($P<0.05$) from bradykinin 10^{-9} M in the absence of zaltoprofen, ★:significantly different ($P<0.05$) from bradykinin 10^{-9} M in the presence of zaltoprofen. †: significantly different ($P<0.05$) from the bradykinin response without zaltoprofen at the same concentration of bradykinin. (Student t-test).

Effects of zaltoprofen were examined in 12 units. Zaltoprofen was applied 10 min before the first bradykinin application and continued throughout the experiment. Bradykinin also induced excitation in epididymis receptors in the presence of zaltoprofen (Fig. 4): the response over 10^{-6}M was significantly different from the concentrations below this level. However, zaltoprofen reduced the response significantly, resulting in shift of the bradykinin concentration-response curve to the right. The latencies for 10^{-8} M bradykinin to 10^{-5} M are 25 ± 4 s, 14 ± 2 s, 12 ± 1 s, and 11 ± 1 s, respectively; no significant difference was found for the latency of the bradykinin response without zaltoprofen.

Discussion

In this experiment we found that polymodal receptors exist in the spermatic nerve of rats. However, none of them had a receptive field on the testis, which is a striking difference from dog (Kumazawa et al, 1987) and cat (Leng et al, 1994) spermatic nerve. This observation is also contrary to findings from an horseradish peroxidase (HRP) retrograde transport study: Injection of HRP to the testis resulted in labeling of a substantial number of dorsal root ganglion neurons (Jin et al, 2001). The reason for this discrepancy is not clear. However possible reason might be that receptors in the testis are inactive under normal conditions (Schaible and Schmidt, 1988).

Sensitivity to heat and weak sensitivity to cold are consistent with canine spermatic afferents. Other differences are, first, that most of canine units are A-delta fibers while those of rats are C-fibers. Second, the response to bradykinin of canine polymodal receptors lasted a long time after washing out bradykinin, whereas the rat polymodal receptors responded for only a short period, and stopped firing before the application ended. The activity of bradykinin degrading enzyme might be high in rats, thus degrading bradykinin quickly.

The effects of bradykinin on canine testicular polymodal receptors have been documented by Mizumura and Kumazawa (1996): Bradykinin at 10^{-8} M and higher excites polymodal

receptors in the canine spermatic nerve. Rat polymodal receptors had similar sensitivity to bradykinin and responded to it at 10^{-7} M and higher. Zaltoprofen, a non-steroidal anti-inflammatory drug, has been found to have long-term efficacy and tolerability in rheumatoid arthritis patients (Hatori and Kokubun, 1998) and a low risk of gastro-intestinal side effects owing to its high COX2 selectivity (Kawai, 1998). It is of note that zaltoprofen also has a relatively high efficacy in blocking the pain behavior induced by intra-arterial injection of bradykinin. In this time we showed for the first time that zaltoprofen effectively suppressed bradykinin-induced discharges in rat visceral afferents. The mechanism for this might be the selective blocking of cyclooxygenase activity to reduce PG production, which is known to occur after bradykinin application, and facilitation of the bradykinin response. The existence of an additional mechanism(s) remains a question open to further study.

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