withdrawal threshold. Closed circles show data from the ECC group, open circles show the result of the SHAM group. Randall-Selitto test showed decreased pain threshold after ECC. *$p<0.05$, ***$p<0.001$ compared with the value immediately before the day of exercise (-1 day). $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared with the SHAM group on each day after treatment. *B*, Withdrawal threshold for the skin over the exercised muscle measured by von Frey hairs (n=7 for each group). The presentation is the same as in *A*. No significant differences were found between groups on each day, or between -1 day and days after ECC (non-parametric Friedman test followed by Dunn's multiple comparison test). These results suggest that the pain threshold was decreased in the deeper tissue, probably in the muscle.

Figure 3. C-Fos-ir neurons in the ipsilateral dorsal horn of the spinal cord at L4. *A-D*: Camera lucida drawings of representative sections from four groups 2 days after treatment: SHAM group (*A*), ECC group (*B*), SHAM+compression group (*C*), and ECC+compression group (*D*). Labelled neurons are represented as black dots. Note that the number of labelled neurons increased in the ECC+compression group on the second day. This was especially marked in the superficial dorsal horn. *E* and *F*: Photographs with different magnification of the same section as in *D*, showing c-Fos-ir neurons in the ipsilateral dorsal horn of the spinal cord at L4. Calibration bars: 100 μm in E and 50 μm in F, respectively.

Figure 4. Segmental distribution of the c-Fos-ir neurons in the dorsal horn of the spinal cord at L2-L6.

A: Representative camera lucida drawing of the c-Fos-ir neurons in the dorsal horn of the spinal cord at L2-L6 in a rat with ECC+compression. Labelled neurons are represented as black dots. The largest number of labelled neurons was observed in the superficial dorsal horn of L*4* segment, and smaller numbers were seen also in L2 and L3 segments. *B*: Summary of segmental distribution of c-Fos-ir neurons in the spinal dorsal horn (L2-L6). Number of labelled neurons per section in the entire dorsal horn in each segment is shown. The number of animals used was 4 in SHAM, SHAM+compression, and ECC+compression groups, and 5 in ECC group. Note that there is a significant increase in the number of c-Fos-ir neurons at L*4* in ECC+compression group compared with the other three groups (**$p<0.01$). The number of c-Fos-ir neurons at L2 and L3 in ECC+compression group tended to be greater, but differences among the four groups were not significant.

Figure 5. Number of c-Fos-ir neurons in the different areas of the spinal dorsal horn at L*4* (*A*) and suppression of its increase by morphine (*B*).

In *A*, TTL (DH): total number of labelled neurons in the entire dorsal horn, SDH: superficial dorsal horn, PN: proprius nucleus, NDH: neck of the dorsal horn. The number of animals was 4 for SHAM, SHAM+compression, and ECC+compression groups, and 5 in ECC group. A significant increase in the number of c-Fos-ir neurons was observed in the superficial dorsal horn compared with each of other three groups (**$p<0.01$, ***$p<0.001$). The numbers of c-Fos-ir neurons in the proprius nucleus and the neck of the dorsal horn looked somewhat increased in the ECC+compression group, but the difference was not statistically significant. In *B*, black columns show the number of c-Fos-ir neurons per section in the animals that received morphine (10 mg/kg, i.p.) 20

min before compression of EDL muscle 2 days after ECC, and white columns show those in the control group that received saline instead of morphine. Number of animals was 5 for the morphine group, and 6 for the control group. Note that c-Fos immunoreactivity was clearly suppressed in the morphine-treated group (**$p<0.01$, Mann-Whitney test).

Fig. 1

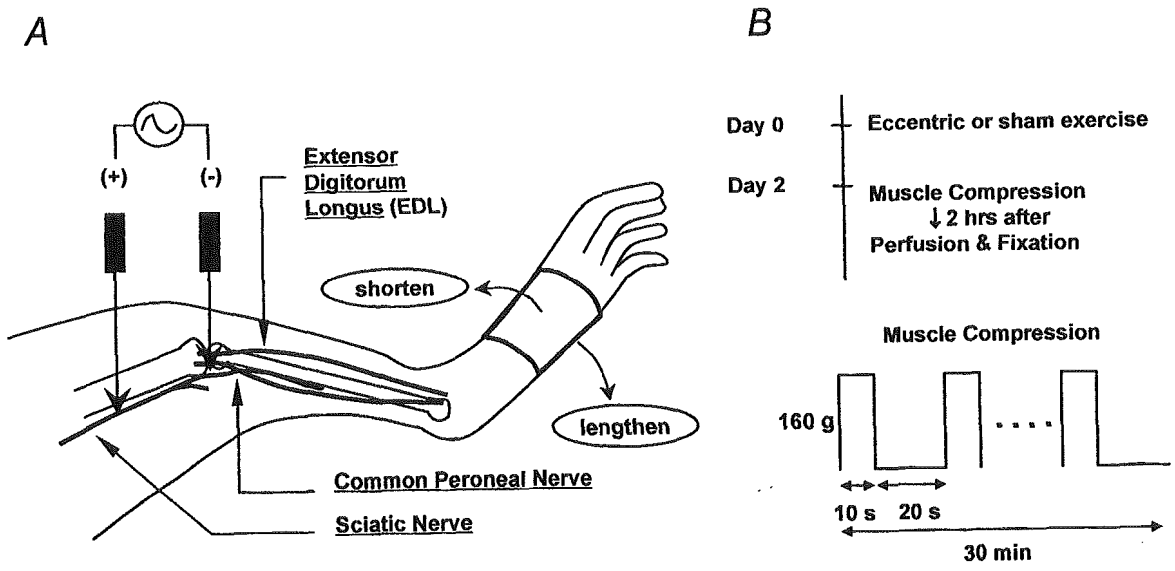


Fig. 2

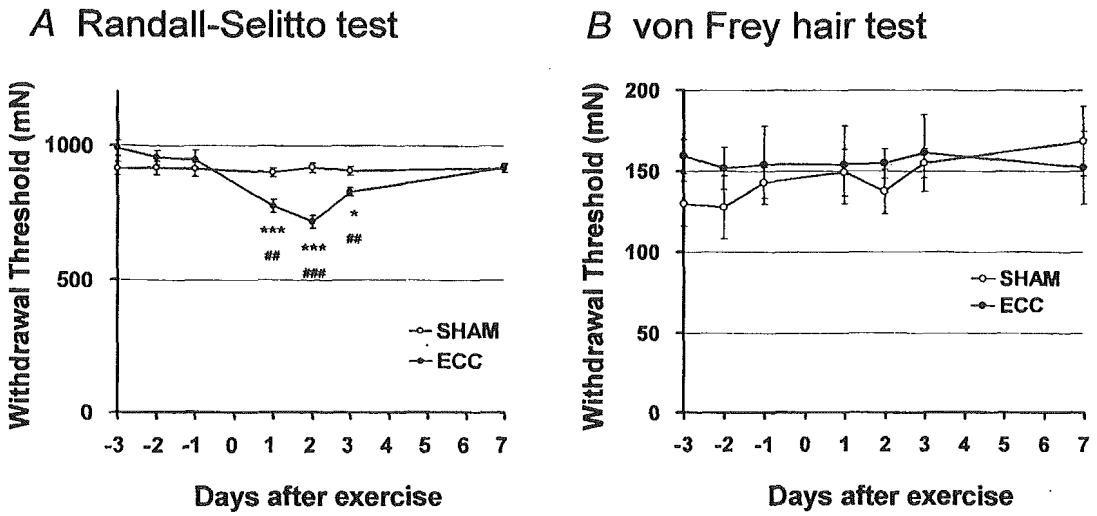


Fig. 3

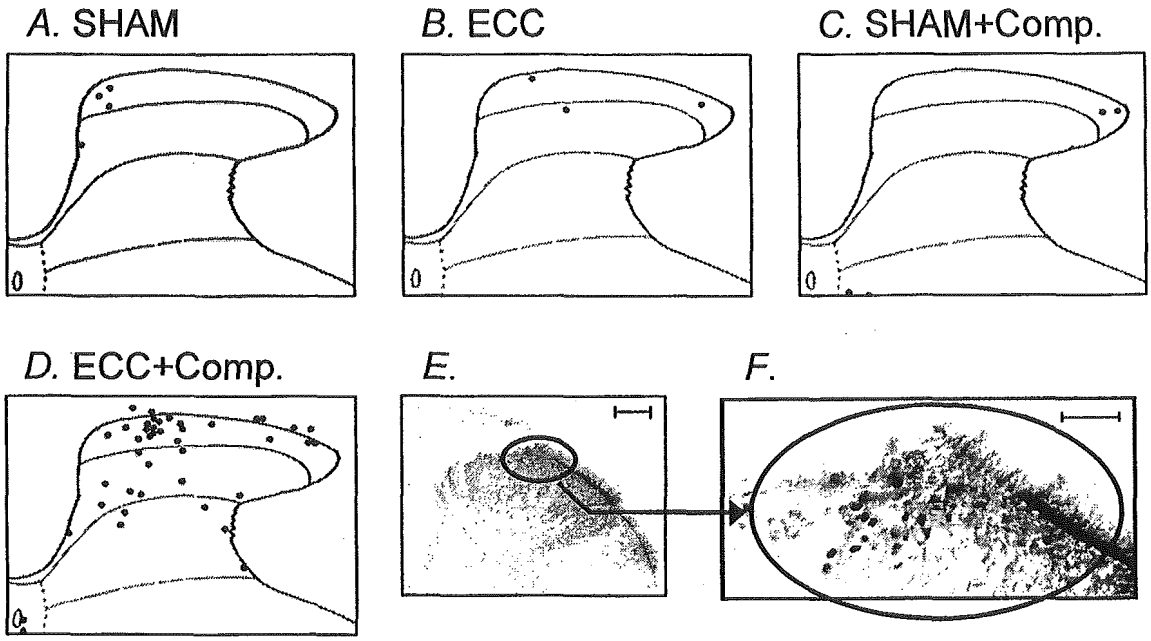

A. SHAM
B. ECC
C. SHAM+Comp.
D. ECC+Comp.
E.
F.

Fig. 4

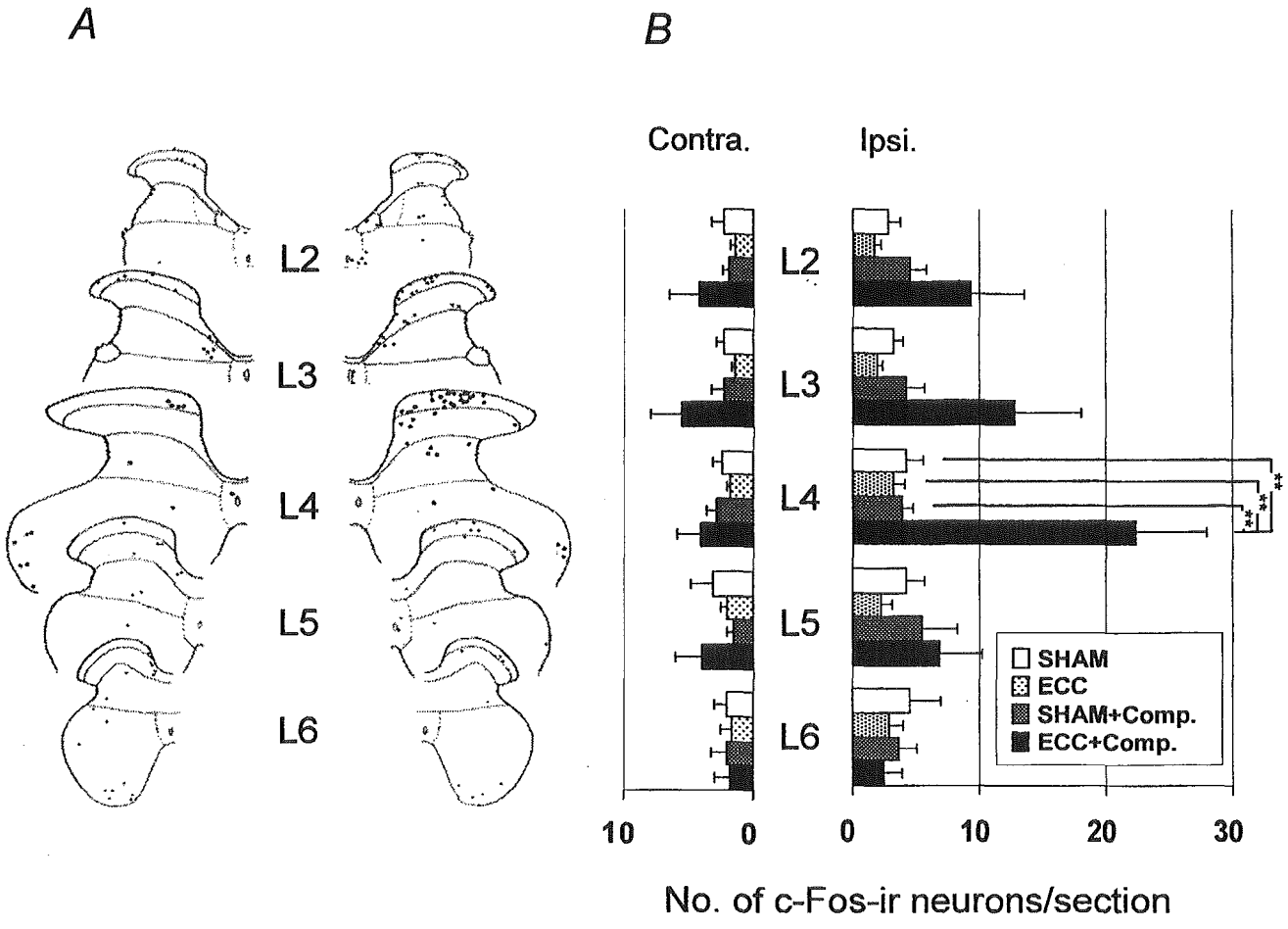
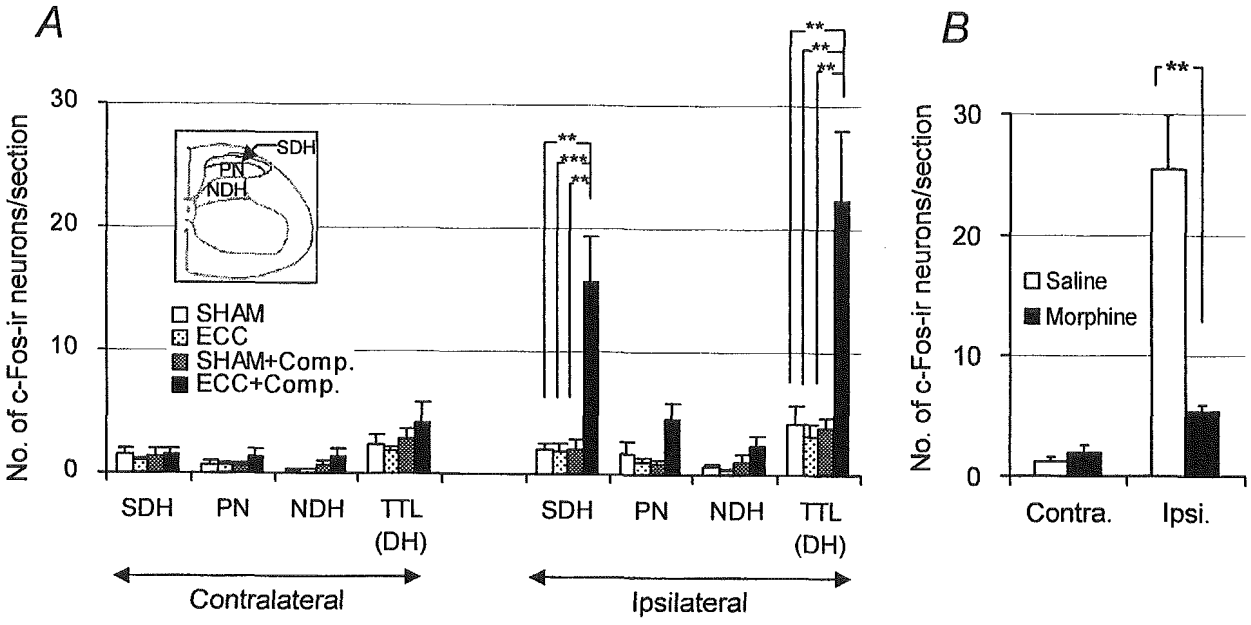

A
B
L2
L3
L4
L5
L6
Contra.
Ipsi.
SHAM
ECC
SHAM+Comp.
ECC+Comp.
10
0
0
10
20
30
No. of c-Fos-ir neurons/section

Fig. 5


A
No. of c-Fos-ir neurons/section
30
20
10
0
SDH
PN
NDH
SHAM
ECC
SHAM+Comp.
ECC+Comp.
**
***
**
**
**
**
SDH
PN
NDH
TTL (DH)
SDH
PN
NDH
TTL (DH)
Contralateral
Ipsilateral
B
No. of c-Fos-ir neurons/section
30
20
10
0
Saline
Morphine
**
Contra.
Ipsi.

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Inhibitory, facilitatory, and excitatory effects of ATP and purinergic receptor agonists on the activity of rat cutaneous nociceptors in vitro

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Abstract

Pathological pain is often associated with changed sympathetic nerve activities. It is known that sympathetic nerve endings release ATP as a co-transmitter of norepinephrine, but the effect of this ATP on the nociceptive system has not been properly studied in that the concentration range used in the previous studies was much higher than is expected in the surroundings of nociceptor terminals. We examined the effects of ATP, especially at low concentration (10^{-5} M or less), on C-fiber polymodal receptor (CPR) activity using a rat skin-nerve preparation in vitro. We found for the first time that ATP inhibited the heat response of CPRs at low concentration (10^{-5} M), but facilitated it at high concentration (10^{-3} M). The former effect was mimicked by a $P2X_3$ agonist, α,β-methylene ATP, at 10^{-5} M, while the latter was mimicked by 2-methylthio ADP (a $P2Y_1$ agonist) or UTP (a $P2Y_2$ agonist) at 10^{-3} M, suggesting that the former is mediated by P2X receptors and the latter by P2Y receptors. After repetitive heat stimuli, ATP-induced CPR excitation was increased (10^{-5} to 10^{-3} M), but none of the purinergic agonists induced CPR excitation in a magnitude comparable to that by ATP. Possible mechanisms for these effects were discussed.

Keywords: Rat; ATP; α,β-methylene ATP; 2-methylthio ADP; UTP; Purinoceptor; Cutaneous nociceptor

1. Introduction

Neuropathic or chronic inflammatory pain in patients is often associated with disorders in sympathetic nerve functions such as a change in skin blood flow or abnormal sudomotor activity in the painful region (Birklein et al., 1997, 1998). This association and the relief of pain by interrupting sympathetic nerve activity to the affected region have led to characterization of these pathological conditions as sympathetically maintained (Roberts, 1986). There are several possible sites of interaction between the sensory and sympathetic nervous systems that may account for these phenomena (Sato and Perl, 1991; Seltzer and Devor, 1979; McLachlan et al., 1993). To clarify the mechanism of peripheral interaction, we have investigated the effects of sympathetic nerve activation on nociceptive fiber activities using the single C-fiber recording technique. Our previous experiments have shown that cutaneous nociceptors (C-fiber polymodal receptors (CPRs)) exhibit abnormal excitation to sympathetic nerve stimulation or norepinephrine (NE) administration in animals rendered neuropathic (Sato and Perl, 1991) or chronically inflamed (Sato et al., 1993), but not in healthy ones. Further, a simulated acute inflammatory state produced by repetitive application of bradykinin caused sensitization of CPRs so that they were excited by exogenous NE (Banik et al., 2004). We therefore consider these alterations in the sensitivity of cutaneous nociceptors to adrenergic stimulation to be significant as a peripheral mechanism for sympathetically maintained pain.

ATP has long been known as an intracellular energy source. It is also known to be stored in vesicles of sympathetic terminals and other nerve terminals in periphery and in central nervous system, and released when sympathetic nerves are excited (Burnstock, 1995; Bodin and

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Burnstock, 2001; Santos et al., 2003). Although the role of ATP released from the sympathetic nerve terminals in pathological pain states as described above is not fully understood, ATP is now known as a pain producing substance: ATP administration into the human skin generates burning pain (Hamilton et al., 2000), and ATP injected into the plantar skin induces a dose-dependent nocifensive foot withdrawal response in rats (Bland-Ward and Humphery, 1997). Amongst ATP receptor subtypes, significant research efforts have been directed to $P2X_3$ receptor as this receptor subtype is expressed specifically in small-sized dorsal root ganglion (DRG) neurons (Chen et al., 1995; Lewis et al., 1995), many of which are cell bodies of nociceptors. It has been shown that α,β-methylene ATP (α,β-met ATP), a $P2X_3$ receptor agonist having also affinity with $P2X_1$, $P2X_{2/3}$ and $P2X_{4/6}$ receptors, injected into the planter skin induced behaviors related to spontaneous pain in rats and hyperalgesia to heat and mechanical allodynia (Tsuda et al., 2000). Further, patch clamp studies on isolated small-sized DRG neurons showed that ATP facilitated the heat response (Kress and Guenther, 1999) and that ATP as well as α,β-met ATP elicited inward currents (Ueno et al., 1999). Recent reports have demonstrated an additional contribution of P2Y receptor subtypes in pain: $P2Y_1$ and $P2Y_2$ receptors were also expressed in small-sized DRG neurons, and nociceptive behavior to heat was facilitated by P2Y agonist (Moriyama et al., 2003). ATP facilitated TRPV1 currents through $P2Y_1$ receptors in a heterologous expression system (Tominaga et al., 2001) and through $P2Y_2$ receptors in DRG (Moriyama et al., 2003). Additionally, ATP- and α,β-met ATP-induced nocifensive withdrawal responses of the hindpaw were facilitated in chronically inflamed animals, and expressions of $P2X_3$ and $P2X_{2/3}$ receptor subtypes in their DRG neurons were increased (Xu and Huang, 2002). $P2Y_1$ receptor in rat DRG neuron was increased after peripheral axotomy (Xiao et al., 2002). In dura mater, ATP enhanced the proton-induced calcitonin gene-related peptide release from sensory nerves through $P2Y_2$ receptors (Zimmermann et al., 2002). This ample evidence supports the hypothesis that ATP may activate and sensitize peripheral nociceptors through activation of $P2X_3$ (or $P2X_{2/3}$) and P2Y receptor subtypes, and thus has an important role in pathological pain. In fact, recent experiments have shown that a proportion of C-fibers in normal rats respond to ATP, and under inflammatory conditions both the proportion of sensitive units to this agent and their response magnitude were increased (Hamilton et al., 2001).

It is worth noting that the experiments described above tested the effects of high concentrations (mM order) of ATP, which mimicked the extracellular ATP level in damaged or inflamed tissues (Cook and McCleskey, 2002). Although it has been hypothesized that ATP acts on $P2X_3$ or $P2X_{2/3}$ receptors on nociceptors to contribute to the initiation and maintenance of sympathetically maintained pain (Burnstock, 2000), the mode of action of ATP released from the sympathetic nerve terminals as a co-transmitter with NE and neuropeptide Y (Burnstock, 2000) has not yet been clearly identified. Our present study was therefore designed to elucidate the role of ATP, especially at the low concentration that is expected to be present in the tissue when sympathetic nerves are activated in conditions of pathological pain. The effects of $P2X_3$, $P2Y_1$ and $P2Y_2$ agonists on CPR activity were also tested to identify putative purinergic receptor subtype(s) involved in the ATP effects.

2. Materials and methods

All experiments were conducted following the ethical guidelines of the Animal Care Committee of Nagoya University, and were performed in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.1. Electrophysiological recording

Male Lewis rats ($n = 42$, Charles River Laboratories, Japan) weighing 250–280 g were used in this study. The experimental procedures for in vitro single fiber recording techniques (Reeh, 1986) were described in a previous report (Banik et al., 2001). Under deep anesthesia (pentobarbital sodium, 55 mg/kg, i.p.), the saphenous nerve and its innervating territory on the hairy hindpaw skin was subcutaneously dissected and excised. After dissection, the rats were killed with an intracardial injection of a lethal dose of pentobarbital sodium. The skin was pinned, out side down, in the perfusion chamber, and superfused (14 ml/min) with a modified Krebs–Henseleit solution (110.9 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2SO_4, 24.4 mM $NaHCO_3$, and 20 mM glucose), which was saturated with a gas mixture of 95% O_2–5% CO_2 maintained at a temperature of 32 ± 0.5 °C. The saphenous nerve was drawn through a hole into a recording chamber, placed on a small mirror and covered with a layer of liquid paraffin. Small filaments of the nerve were repeatedly split with sharpened watchmaker forceps and a thin needle until a single unit activity could be recorded. Action potentials were amplified, filtered, and displayed on an oscilloscope and continuously recorded on videotapes (for off-line analysis). They were later analyzed on a computer with an analog–digital converter and a SPIKE/SPIDI software package (Forster, University of Erlangen-Neurnberg, Germany). Units were first identified by manual probing of the skin with a glass rod with a small round tip (diameter 1.3 mm). Only units responding to this mechanical stimulation were studied in detail. Homemade von Frey hairs (tip diameter 0.5 mm, force strength 83–265 mN) were then used to determine the receptive field size and examine its mechanical response characteristics. Monopolar electrical stimulation was applied to the receptive field. Conduction velocity of a unit was calculated by dividing the distance

between the receptive field and the recording electrode with the conduction delay of the action potential induced by this electrical stimulation. We focused in this study only on those units that showed a slowly adapting response to the von Frey hair stimulation, responded to heat stimulation (described later), and had conduction velocities <1.5 m/s. These units were considered to be the CPRs (Bessou and Perl, 1969).

2.2. Heat and chemical stimulation

A metal cylinder (5.5 mm i.d.; height, 6 mm; volume, 0.4 ml) was placed over the receptive field (ring chamber) to isolate the receptive field against the superfusate. For heat stimulation, solution in the ring chamber was removed and a thermocouple was placed in the chamber to monitor the skin temperature. Heat stimulation was applied at 10 min intervals by a feedback controlled radiation heat stimulator (Thermal Stimulator DPS-701, Dia Medical Co., Tokyo, Japan) at the corium side. The temperature was raised from 32 °C at a speed of 0.65 °C/s up to 50 °C at the maximum (Fig. 1A and B). In the first heat stimulation the temperature was raised until spikes were evoked at 5 Hz (cut-off temperature). The cut-off temperature established in this way was applied in the subsequent stimuli (10 min intervals) for a given unit. Chemical stimulation was carried out by superfusing the ring chamber separately (2.6 ml/min, 32 °C) with drug solutions for 5 min using a push–pull pump. At the end of the drug superfusion, the receptive field was rinsed with warmed (32 °C) Krebs solution. The following drugs were used for stimulation: adenosine 5′-triphosphate lithium salt (ATP), α,β-methylene adenosine 5′-tri-phosphate lithium salt (α,β-met ATP), 2-methylthio adenosine 5′-diphosphate trisodium salt (2-MeS ADP) and uridine 5′-triphosphate trisodium salt dihydrate (UTP). All drugs were obtained from Sigma (St. Louis, MO, USA). Stock solutions (10^{-3} M) of these drugs used were kept frozen (−80 °C) and were diluted to the relevant concentration in the Krebs solution on the day of use.

2.3. Analysis of the heat response and the drug-induced excitation

The effect of ATP or purinergic receptor agonists on the heat response was studied in different groups of CPRs. The control group of CPRs received five to seven trials of heat stimuli delivered at 10 min intervals (control heating protocol); the same protocol was used in the other groups except that after two to four trials of heat stimuli, when the response of a CPR was confirmed to be stable, the ring chamber was superfused for 5 min with one of the above-mentioned chemical solutions, and then the thermal test procedure was repeated.

Two parameters of heat response were measured before and after a 5 min application of these drugs: (1) the heat threshold temperature and (2) the net total number of impulses induced by a heat stimulation. The heat threshold

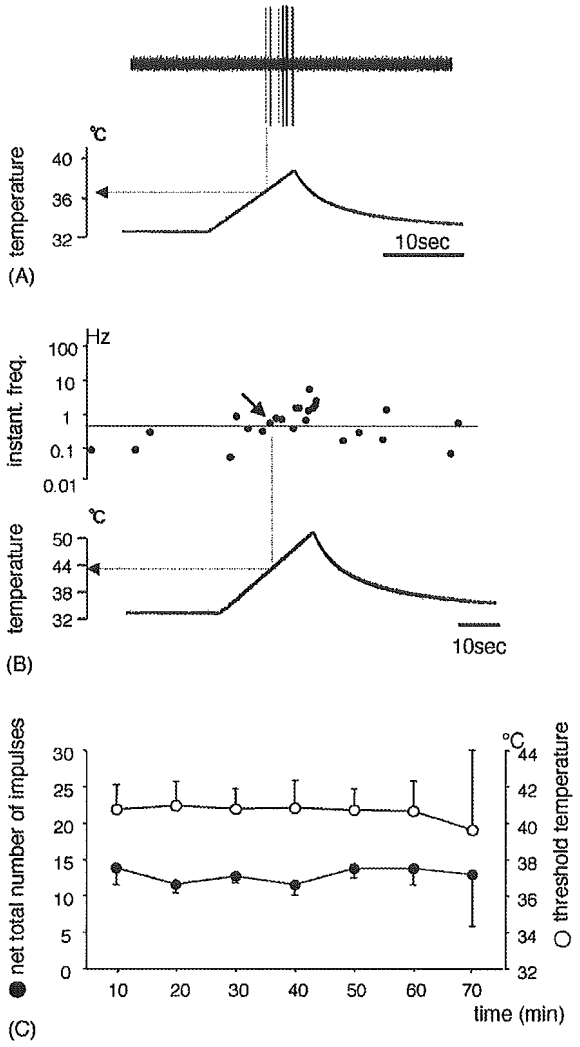

Fig. 1. Responses of C-fiber polymodal receptors to ramp-heat stimulation. (A) Upper panel: original recording of a unit over a period of temperature increase; lower panel: recording of tissue temperature. The stimulus temperature (36.8 °C) that induced the first spike (marked with an arrow and a broken line), was determined to be the threshold of this unit according to the criteria (see methods section). (B) Upper panel: the instantaneous frequency of spikes (in log scale) over the period of temperature increase; lower panel: recording of tissue temperature. Note that this C-fiber polymodal receptor unit has ongoing discharges before the heat stimulation. The spike marked by an arrow was elicited at 43.4 °C, the threshold temperature of this unit according to the criteria (see methods section). The solid line shows the mean basal activity +1S.D. (standard deviation). (C) Average response of C-fiber polymodal receptors to repeated heat stimuli at 10 min intervals with "cut-off temperature". The net total number of impulses (●) and the threshold temperature (○) were obtained in a control group of C-fiber polymodal receptors (mean ± S.E.); $n = 6$ up to 5 heating trials, 4 in the sixth trial and 2 in the seventh trial. Repeated heat stimulation did not alter either response parameter (Friedman test).

was defined as the temperature that evoked the first spike by the heat stimulation (sample shown in Fig. 1A). As previously described (Banik et al., 2001), the majority of CPRs identified in this in vitro preparation had no

spontaneous activity before any stimuli; however, the mechanical search stimulus with a glass rod or thermal stimulation sometimes resulted in ongoing activity of a low frequency in some units. When a unit had spontaneous discharges, the threshold was determined as the lowest temperature at which the instantaneous frequency of two successive spikes exceeded the mean instantaneous frequency during the 30 s control period preceding the heat stimulus by its standard deviation (sample shown in Fig. 1B). Since some fibers had spontaneous discharges and/or after-discharges outlasting the stimulation period, the net total number of impulses induced from the initiation of the heat stimulation to cessation of the response (total response period) was calculated according to the following formula: {(mean discharge rate during the total response period) − (mean discharge rate during the 30 s preceding control period)} × total response period.

Effect of repetitive heat stimuli on the ATP-induced excitation was examined. For this purpose, some CPR units received ATP at a concentration of 10^{-5}, 10^{-4} or 10^{-3} M to their receptive field before any heat stimuli and after the three to four trials of heat stimuli, and the proportion of excited units and the response magnitudes were compared between two ATP applications. Another group of CPR units received one of the purinergic receptor agonists at a concentration of 10^{-5}, 10^{-4} or 10^{-3} M after two to four trials of heat stimuli to compare their response magnitude and discharge pattern with those of the ATP response.

The magnitude of the response to ATP and purinergic receptor agonists at different concentrations was determined by counting the total number of impulses evoked and the peak discharge rate (impulses/s) during the drug superfusion (5 min). To count the total number of impulses induced, spontaneous discharges during the 30 s preceding control period were multiplied by 10 and subtracted from the count during 5 min drug superfusion. If the instantaneous discharge rate of a unit during drug application exceeded that during the preceding 30 s control period by its standard deviation, the unit was scored as having a response to ATP or the purinergic receptor agonists. The latency of the drug response was determined as time from the beginning of the drug application until the instantaneous frequency of two successive spikes exceeded, by its standard deviation, the mean instantaneous frequency during the 30 s control period preceding the drug application.

2.4. Statistical analyses

Data are shown as mean ± standard error of mean (S.E.M.). Statistical analyses were performed using Mann–Whitney *U*-test, Friedman test followed by Tukey test, unpaired *t*-test and Fisher's exact probability test, as appropriate. Difference was considered significant at the $P < 0.05$ level.

3. Results

3.1. General properties of CPRs

Eighty CPR units with an average conduction velocity of 0.50 ± 0.02 m/s (ranging from 0.25 to 0.83 m/s, median: 0.50 m/s) were investigated in the present study. Twenty-two units (27.5%) showed spontaneous discharges ranging from 1 to 4 impulses/30 s before any stimulation other than the search stimulus. No significant difference in the conduction velocity was observed between fibers with or without spontaneous discharges ($P = 0.96$, unpaired *t*-test). In this study all tested CPRs responded to the heat stimulation, and had a single spot-like receptive field. The identified units were randomly assigned for the following experimental series.

3.2. Suppressive and facilitatory effects of ATP on the heat response

In 6 units heat stimulation was applied five to seven times using the heating protocol with the cut-off temperature described in the methods section. We confirmed that CPRs were neither sensitized nor desensitized by this stimulation protocol (Fig. 1C). Based on this finding, we tested the effects of ATP after three to four trials of heat stimuli in another group of units. The last heat response before the ATP application was used as the control heat response, and the difference of the heat responses examined successively after the ATP application from the control heat response was taken as the change of the heat response induced by ATP. Sample recordings of the effects of ATP on the heat response are shown in Fig. 2. In the CPR unit shown in the upper panel (A), ATP at a lower (10^{-5} M) concentration excited the unit to induce low-frequency discharges and clearly decreased the following heat response (P1). This suppression of the heat response disappeared 10 min after the ATP treatment (P2). ATP at the highest concentration (10^{-3} M) used (lower panel (B)), on the other hand, induced higher-frequency discharges and clearly augmented the following heat response (P1). In this unit, such sensitization of the heat response was seen up to 10 min after the ATP treatment (P2). The time course of the sensitizing and desensitizing effects induced by different concentrations of ATP is shown as changes in the net total number of impulses in Fig. 3. On average in 8 units, ATP at 10^{-5} M significantly decreased the heat response just after ATP application (Fig. 3A, $P < 0.05$, Friedman and Tukey tests). Such significant inhibition of the heat response was not observed 10 min later. To the contrary, ATP at 10^{-3} M induced a significant enhancement of the heat response ($n = 5$, Fig. 3C, $P < 0.05$, Friedman and Tukey tests), and this effect was still observed 10 min later with a small tendency to decline. This facilitatory effect was not induced as a result of the smaller control response in this

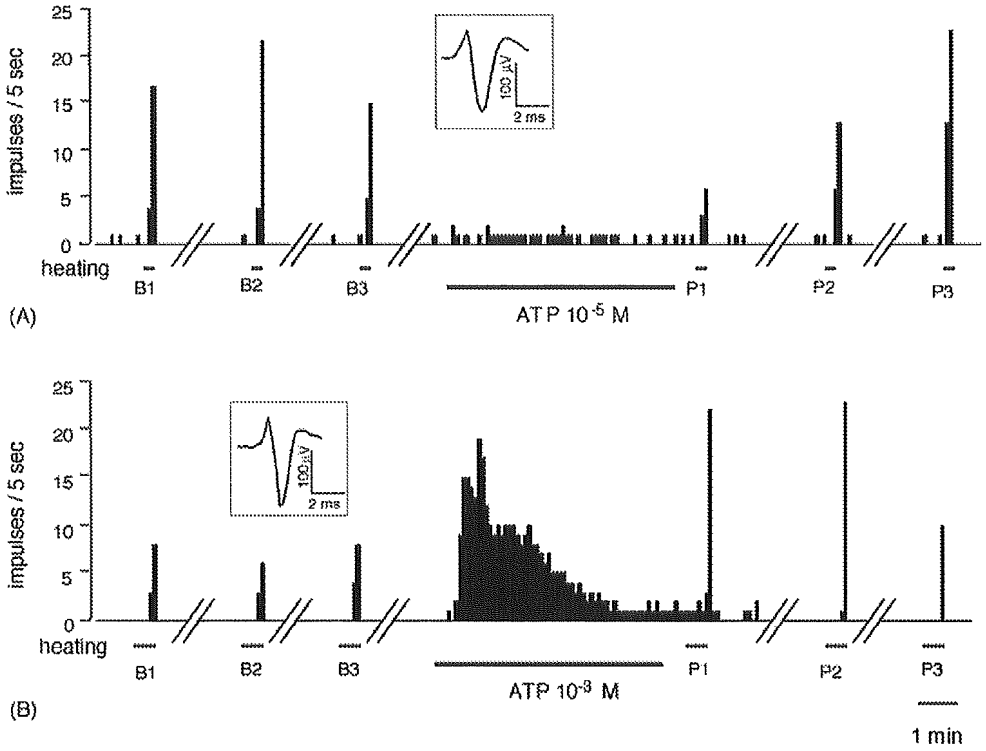

Fig. 2. Examples of bi-directional effect of ATP on the heat responses of C-fiber polymodal receptors. Peri-stimulus time histograms according to the order of testing (1 bin is 5 s). Symbol (//) shows that the recording was interrupted for several min at this point. Lines indicate the time of heat stimulation and ATP application. After three trials of heat stimuli (B1, B2, B3), ATP at 10^{-5} M (A) or at 10^{-3} M (B) was superfused to the receptive field for 5 min. In (A), ATP-induced small numbers of discharges and inhibited the subsequent heat response (P1). In (B), ATP excited the unit strongly and increased the subsequent heat responses (P1, P2). Insets display action potential forms of these nociceptors.

series than that with 10^{-5} M ATP, because we observed such augmenting effect of ATP at 10^{-3} M even in 2 units with larger magnitude of the control heat response (22 impulses and 16 impulses). In all units tested with 10^{-3} M of ATP, observation was carried out further up to 20 min after rinsing, and this declining tendency was confirmed (sample shown in Fig. 2B). For 5 units tested with 10^{-4} M of ATP, 1 unit had a suppressed heat response, while the others were augmented. On average, ATP at this concentration (10^{-4} M) did not induce a significant change in the heat response (Fig. 3B, $P > 0.05$, Friedman test). The threshold temperature tended to increase and decrease after 10^{-5} and 10^{-3} M ATP application, respectively, but these alterations were not statistically significant (data not shown).

In another series of experiments, the influence of even lower (10^{-7} and 10^{-6} M) concentrations of ATP on the heat response was examined in each of 4 CPR units. ATP at either concentration was applied after three to four trials of heat stimuli. ATP at 10^{-7} M did not change the following heat response in any of the 4 units (average net total number of impulses: 29 ± 4.4 impulses before ATP versus 30 ± 5.1 impulses after ATP), while ATP at 10^{-6} M tended to decrease the following heat response of 3 units (from 29 ± 6.7 to 23 ± 4.7 impulses).

3.3. Role of purinergic receptor subtypes in the ATP-induced alteration of the heat response

The result in the previous section showed that ATP has a bi-directional effect on the heat response of CPRs, depending on the concentration. That is, the lower (10^{-5} M) concentration of ATP caused the following heat responses to decrease while the higher concentration (10^{-3} M) augmented them. This result might suggest that different purinergic receptor subtypes are involved in the ATP effects on the heat response at different concentrations. To examine this, we tested the effects of three different purinergic receptor ($P2X_3$, $P2Y_1$ and $P2Y_2$) agonists on the heat response of CPRs. One example of the desensitizing effect of P2X agonist, α,β-met ATP, is shown in Fig. 4 A. α,β-met ATP at 10^{-5} M did not induce any substantial excitation of this unit and clearly decreased the following heat response (P1). This desensitization of the heat response disappeared at 10 min after the treatment (P2). On average in 7 units, α,β-met ATP at 10^{-5} M significantly inhibited the first heat response after the drug, characterized by a decreased net total number of impulses (Fig. 5A, $P < 0.05$, Friedman and Tukey tests). Such inhibitory effect seemed to last for another 10 min, although it was not a significant change. The time course of the desensitizing effect of α,β-met ATP is similar to that of

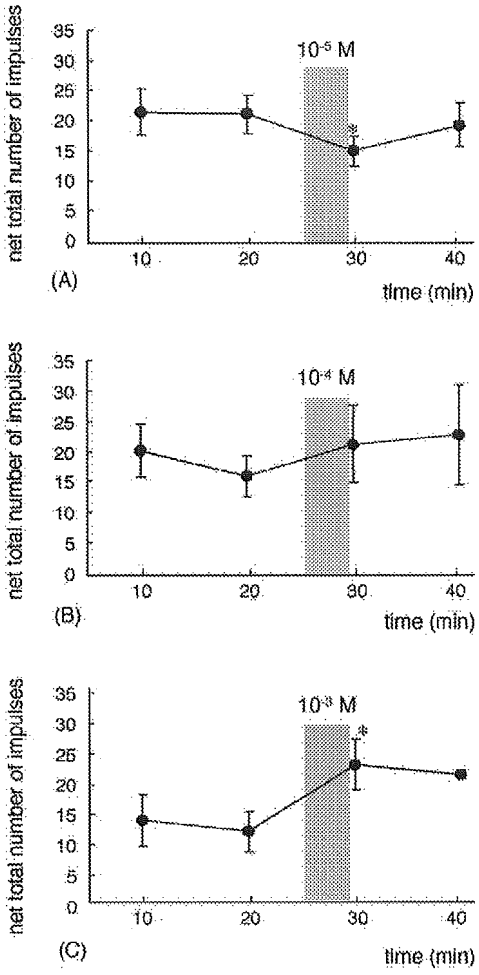

Fig. 3. ATP either suppressed or facilitated the following heat response depending on its concentration (summary). Ordinate: average heat response (net total number of impulses, mean ± S.E.) of C-fiber polymodal receptors. Abscissa: time from the first heat stimulus. Heat stimulation was applied two to four times before ATP application (shadow areas), but only the last two responses are shown. ATP at 10^{-5} M ($n = 8$) significantly inhibited the subsequent heat response, while ATP at 10^{-3} M ($n = 5$) significantly facilitated it. ATP at 10^{-4} M ($n = 5$) did not alter the heat response. $^{*}P < 0.05$, compared with the heat response just before ATP application (Friedman and Tukey tests).

lower (10^{-5} M) concentration of ATP (see Fig. 2A). The threshold temperature rose from 41.4 ± 2.0 to 43.0 ± 1.8 °C after α,β-met ATP at 10^{-5} M; this change was also statistically significant ($P < 0.05$, Friedman and Tukey tests). Higher (10^{-4} M) concentration of α,β-met ATP did not induce significant changes in the total number of impulses (21.7 ± 4.6 impulses before treatment versus 18.6 ± 5.0 impulses after treatment, $n = 8$, Friedman test) or threshold temperature (43.4 ± 2.1 °C before treatment versus 43.6 ± 1.8 °C after treatment, $n = 8$, Friedman test). Neither did the highest (10^{-3} M) concentration of the drug: no changes were observed in the net total number of impulses (16.8 ± 3.1 impulses before treatment versus 16.9 ± 4.7 impulses after treatment, $n = 6$, Friedman test) or threshold temperature (41.4 ± 2.4 °C before treatment versus 41.8 ± 1.5 °C after treatment, $n = 6$, Friedman test) at this concentration. Thus, α,β-met ATP suppressed the heat response of CPRs only at the lowest concentration (10^{-5} M) used.

$P2Y_1$ agonist showed a completely different effect from the $P2X_3$ agonist on the heat response of CPRs. Fig. 4B illustrates an example of the augmenting effect of 2-MeS ADP. In this unit, the highest concentration (10^{-3} M) of 2-MeS ADP excited the unit with rapidly responding and relatively high-frequency discharges, and apparently increased the following heat response (P1). This sensitization of the heat response was seen up to 20 min after the treatment (P2 and P3). On average in 6 units, 2-MeS ADP at 10^{-3} M significantly augmented the heat response just after the drug, characterized by an increased net total number of impulses (Fig. 5B, $P < 0.05$, Friedman and Tukey tests), although the threshold temperature was not significantly changed (39.9 ± 1.3 °C before treatment versus 39.3 ± 1.2 °C after treatment, Friedman test). Lower concentration (10^{-5} or 10^{-4} M) of 2-MeS ADP neither suppressed nor facilitated the subsequent heat responses of CPRs: no changes were observed in the net total number of impulses (10^{-5} M: 16.7 ± 2.6 impulses versus 17.4 ± 5.8 impulses, 10^{-4} M: 12.7 ± 2.6 impulses versus 13.6 ± 2.9 impulses, $n = 6$ for 10^{-5} M and $n = 8$ for 10^{-4} M, Friedman test) or the threshold temperature (10^{-5} M: 41.0 ± 0.9 °C versus 38.8 ± 1.1 °C, 10^{-4} M: 42.3 ± 1.3 °C versus 42.0 ± 1.5 °C, $n = 6$ for 10^{-5} M and $n = 8$ for 10^{-4} M, Friedman test). Thus, 2-MeS ADP augmented the heat response only at the highest concentration (10^{-3} M) used.

We next tested the effects of $P2Y_2$ agonist, UTP on the heat responses of 20 CPR units. Fig. 4C shows an example of single CPR recording. In this unit, UTP at 10^{-3} M itself induced small excitation and increased the subsequent heat response (P1). This sensitizing effect disappeared at 10 min after the treatment (P2). On average in 8 units, UTP at 10^{-3} M significantly augmented the heat response just after the drug, characterized by an increased net total number of impulses (Fig. 5C, $P < 0.05$, Friedman and Tukey tests). The threshold temperature was significantly lowered after application of the highest (10^{-3} M) concentration of UTP (43.7 ± 0.9 °C before treatment versus 40.7 ± 1.1 °C after treatment, $P < 0.05$, Friedman and Tukey tests). Lower (10^{-5} or 10^{-4} M) concentration of UTP did not influence the subsequent heat responses of CPRs, as neither increases nor decreases were observed in the net total number of impulses (10^{-5} M: 10.4 ± 3.4 impulses versus 11.3 ± 4.1 impulses, 10^{-4} M: 16.2 ± 3.8 impulses versus 19.7 ± 4.0 impulses, $n = 7$ for 10^{-5} M and $n = 6$ for 10^{-4} M, Friedman test) or threshold temperature (10^{-5} M: 41.7 ± 1.9 °C versus 40.5 ± 1.6 °C, 10^{-4} M: 41.1 ± 1.1 °C versus 40.9 ± 1.1 °C, $n = 7$ for 10^{-5} M and $n = 6$ for 10^{-4} M, Friedman test). Thus, UTP augmented the heat response only at the highest concentration (10^{-3} M) used. This effect was similar to that of $P2Y_1$ agonist, 2-MeS ADP.

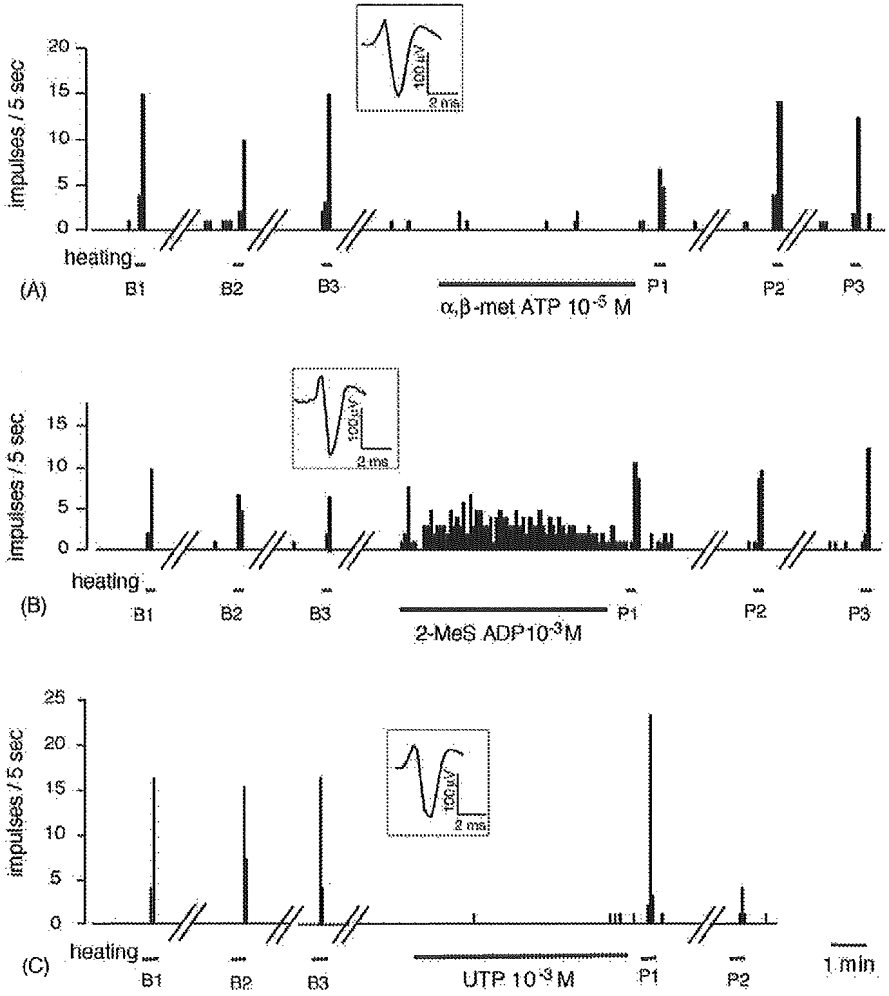

Fig. 4. Examples of different effects of purinergic receptor agonists on the heat responses of C-fiber polymodal receptors. Presentation method is similar to Fig. 2. After three trials of heat stimuli (B1, B2, B3), α,β-met ATP at 10^{-5} M (A), 2-MeS ADP at 10^{-3} M (B) or UTP at 10^{-3} M (C) was superfused in the receptive field for 5 min. In (A), the drug did not excite the unit and inhibited the subsequent heat response (P1). In (B) and (C), the drug excited the unit and increased the subsequent heat responses (P1, P2, P3). Insets display action potential forms of these nociceptors.

3.4. Preceding heat stimuli increased ATP-induced activity of CPRs

Since a bi-directional effect of ATP on the heat response was clearly observed, we next addressed the question whether heat stimulation has any effect on ATP sensitivity. Our previous data (Yajima et al., 2000) showed that NE (10^{-5} M) did not excite any CPRs before heat stimulus, but did excite some (30%) of them after three to four heat stimuli trials. Thus, we applied ATP before any heat stimuli and after three to four heat stimuli. In the present experiment, some CPRs responded to ATP even before any heat stimuli: 2/9 at 10^{-5} M, 5/9 at 10^{-4} M, and 6/9 at 10^{-3} M ATP (Fig. 6A). As shown in this figure, an increased proportion of CPRs seemed to be sensitive to ATP after a few heat stimuli compared with those of before the heat stimuli: 4/8 responded at 10^{-5} M, 3/5 at 10^{-4} M, and all of the 5 units at 10^{-3} M ATP, although this increment is not statistically significant (Fisher's probability test). Fig. 6B shows the concentration–response relationship for the ATP-induced excitation both before and after the heat stimuli. More clearly than the change in proportion of exciting units (Fig. 6A), CPRs displayed increasing net total number of impulses as a function of the concentration of ATP. A significant increase in the number of ATP (10^{-3} M)-evoked impulses was observed after heat stimuli compared with those before the heat stimuli ($P < 0.05$, unpaired t-test). The latency of ATP response was influenced by the concentration of the drug; that is, the higher the concentration, the shorter the latency (10^{-5} M: 119.5 ± 24.5 s, $n = 2$; 10^{-4} M: 63.0 ± 29.8 s, $n = 5$; 10^{-3} M: 40.9 ± 12.5 s, $n = 6$), although this difference was not statistically significant (Kruskal–Wallis test). After heat stimulation, the latency of ATP-induced excitation at different concentrations tended to be shortened (10^{-5} M: 49.9 ± 40.0 s, $n = 4$; 10^{-4} M: 29.0 ± 12.4 s, $n = 3$; 10^{-3} M: 21.7 ± 9.5 s, $n = 5$), again

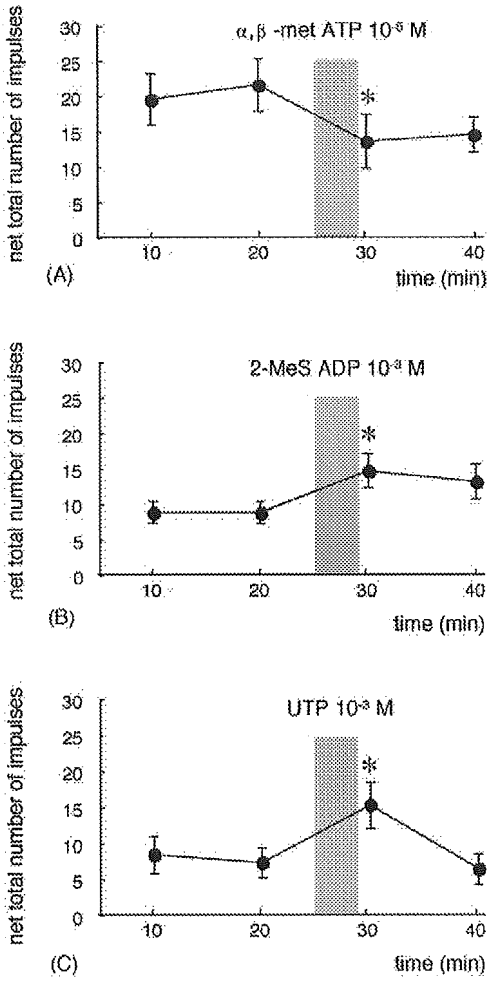

Fig. 5. Purinergic receptor agonists changed the heat response (summary). The method of presentation is the same as in Fig. 3. In (A), α,β-met ATP at 10^{-5} M ($n = 7$) significantly inhibited the subsequent heat response, while in (B) and (C), 2-MeS ADP at 10^{-3} M ($n = 6$) and UTP at 10^{-3} M ($n = 8$) significantly facilitated it. $^{*}P < 0.05$, compared with the heat responses just before drug application (Friedman and Tukey tests).

this change was not statistically significant (Kruskal–Wallis test). Fig. 6C shows averaged discharge patterns evoked by 10^{-3} M of ATP both before and after the heat stimuli. After repetitive heat stimulation, the discharge pattern of the ATP excitation was changed from the "low frequency and slowly increasing" type to "high frequency and rapidly increasing" type. ATP at even lower concentrations (10^{-6} and 10^{-7} M) excited half of the units tested (each $n = 4$) with a small number of impulses (10^{-7} M: 4 and 4 impulses, 10^{-6} M: 3 and 10 impulses).

3.5. Purinergic receptor agonists induced CPR excitation

CPRs were challenged by the different concentrations of $P2X_3$ and P2Y agonists after the heat stimulation. A dose-dependent tendency for the proportion of exciting units (Fig. 7A) and their response magnitude (Fig. 7B) was observed. This dose-dependency in the CPR excitation to the purinergic receptor agonists was similar to that of ATP responses described above. However, the number of discharges induced by these agents was smaller than that of ATP (see Fig. 6B), and the response pattern consisted with "slowly increasing" discharges to the highest (10^{-3} M) concentration of the agonists even after the heat stimulation (Fig. 7C). This was different from ATP, which induced "high frequency and rapidly increasing" discharges at the high concentration (10^{-3} M) after heat stimulations (Fig. 6C, sample shown in Fig. 2B).

4. Discussion

The present study demonstrated that the heat response of cutaneous C-fiber polymodal receptors (CPRs) was inhibited by low (10^{-5} M) concentration of ATP, while the response was facilitated by high (10^{-3} M) concentration of the drug. The study of effects of purinergic receptor agonists showed that the former effect can be mimicked by the low (10^{-5} M) concentration of α,β-met ATP ($P2X_3$ agonist), while the latter was mimicked by the high (10^{-3} M) concentration of 2-MeS ADP ($P2Y_1$ agonist) or UTP ($P2Y_2$ agonist), suggesting that the former effect was mediated by P2X receptors and the latter by P2Y receptors. However, none of the purinergic agonists could induce a magnitude of excitation in CPRs similar to that induced by ATP. In addition, this study revealed that CPR excitation by ATP occurs at a greater magnitude after repetitive heat stimulations.

4.1. Mechanism of inhibition and facilitation of the heat response by ATP

Comparative evaluations of the effects of purinergic receptor agonists suggested that the P2X receptor is involved in the inhibitory effect of ATP at the low concentration and the $P2Y_1$ and/or $P2Y_2$ receptors are involved in the augmenting effect at the high concentration. With respect to the augmenting effect of ATP at high concentration, the present observation is in agreement with a recent publication that ATP, through $P2Y_2$ receptor activation, facilitated capsaicin-induced currents of a heat-sensitive ion channel (vanilloid receptor 1 or TRPV1) in sensory neurons (Moriyama et al., 2003). $P2Y_1$ activation can also facilitate the TRPV1 currents in a heterologous expression system (Tominaga et al., 2001).

This is the first report that ATP at a low concentration inhibited the heat response of CPRs, possibly through activating P2X receptors. Several reports have examined the effects of ATP and $P2X_3$ receptor agonists on nocifensive behavior, but none reported a suppression of the behavioral heat response by these agents, but rather its facilitation: intracutaneous injection of ATP or α,β-met ATP augmented nocifensive paw withdrawal response to heat stimuli in rats

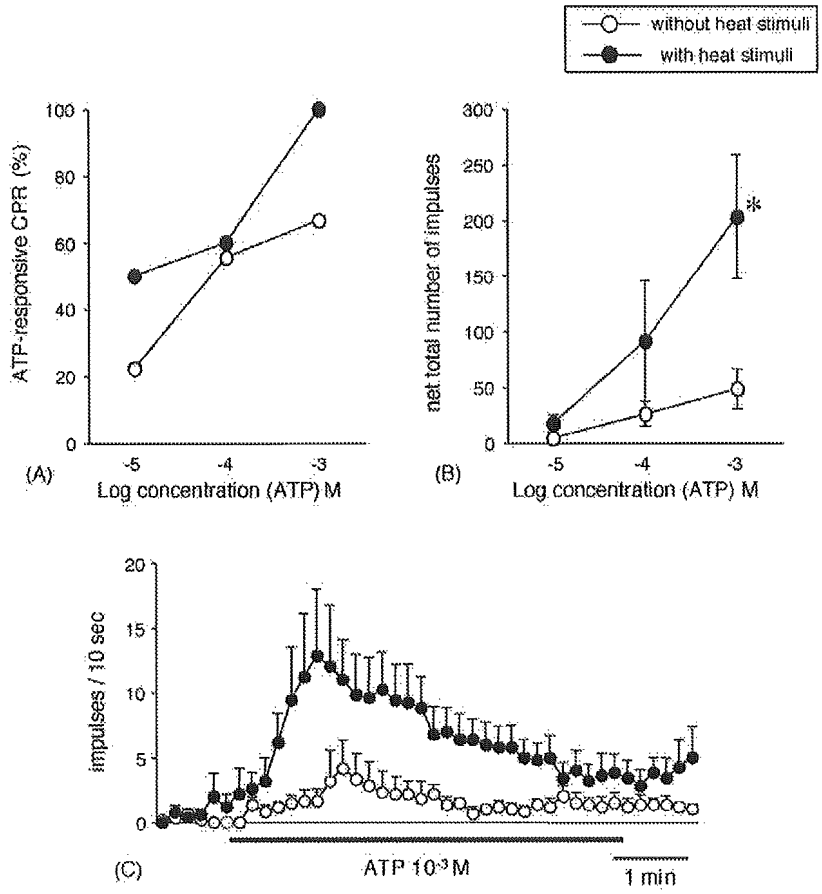

Fig. 6. Repetitive heat stimuli increased ATP-induced excitation of C-fiber polymodal receptors. In (A) and (B), concentration–response curves of C-fiber polymodal receptors for ATP stimulation are shown. There is a tendency for increase in (A), the incidence of ATP sensitive units, and significant increases in (B), the mean net total number of impulses to this drug with heat stimulation ($^*P < 0.05$, unpaired t-test; $n = 9$, without heating and 5–8, with heating). See methods section for criteria of "responsive unit". (C) shows the averaged discharge patterns evoked by 10^{-3} M ATP both without ($n = 9$) and with the heat stimuli ($n = 5$) (1 bin = 10 s, mean ± S.E.).

(Hamilton et al., 1999; Tsuda et al., 2000; Waldron and Sawynok, 2004). However, in these experiments high concentrations of ATP or α,β-met ATP (10–100 mM, calculated from the injected dosage and volume) were used (Hamilton et al., 1999; Waldron and Sawynok, 2004). In the present experiment, $P2X_3$ receptor agonist did not facilitate the heat response even at the highest concentration (10^{-3} M) used, although ATP at 10^{-3} M did facilitate the heat response, in agreement with the findings in the above papers on nocifensive behavior. Thus, P2X receptor-mediated facilitation of the nocifensive behaviors in rats may be mediated by other types of nociceptors than CPRs. It is not known, however, whether these types of receptors are sensitized to heat by low concentration of ATP in rats. Hilliges et al. (2002) recently reported that ATP (5×10^{-3} M) did not induce heat or mechanical sensitization in mechano-insensitive, heat sensitive C-fibers of human skin, and, different from our result, that even mechano-heat responsive (polymodal) C-fibers were not sensitized to heat by ATP. Another possibility is A-delta fiber mechano-heat sensitive nociceptors. Alternatively, the difference between our present result and the behavioral experiments might suggest that some tissue or blood-born factors are involved in α,β-met ATP-induced augmentation of nocifensive behavior. Further studies are needed to answer this question.

As for the mechanisms for suppression of the heat response, at least two possibilities can be considered. Firstly, in the present experiment, the rapid breakdown of ATP by the extracellular ATPases may have resulted in production of adenosine, which might have inhibited the heat response (Sawynok, 1998). This possibility, however, is highly unlikely, since the ring chamber was continuously superfused with the drug when the receptive field was stimulated, and thus the concentration of ATP must have been kept at a constant level. In addition, α,β-met ATP, which is more stable than ATP, also induced suppression of the heat response. Secondly, extracellular ATP at a lower concentration may indirectly inhibit CPR response to heat through activating P2X receptors expressed on other cells/nerve

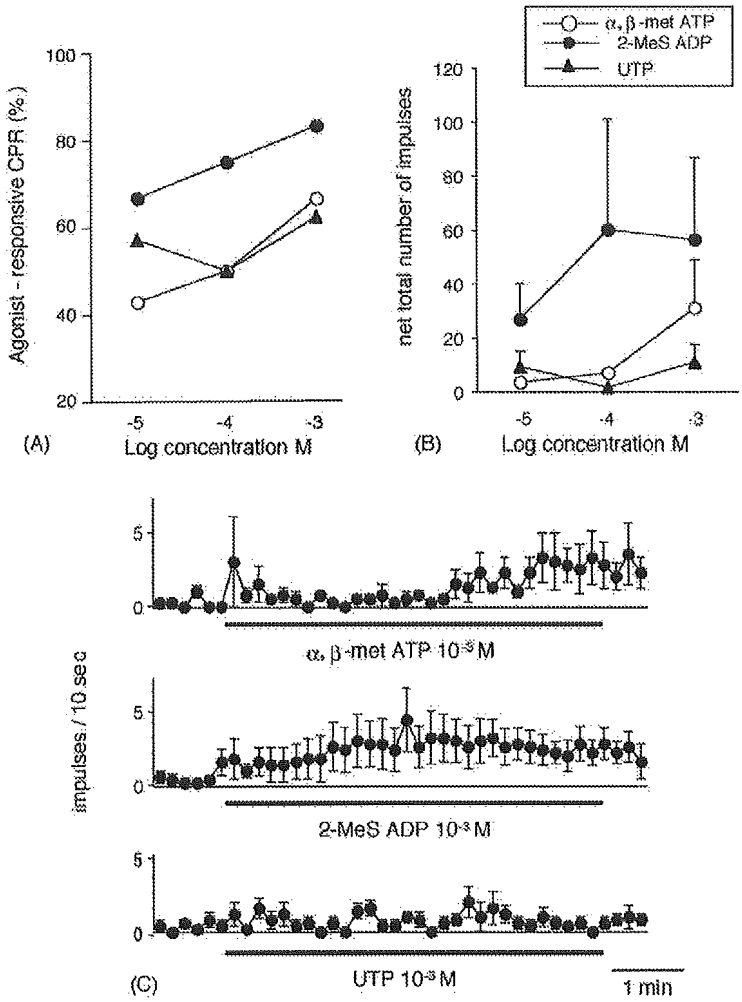

Fig. 7. Response of C-fiber polymodal receptors to the purinergic receptor agonists given after a few heat stimuli. In (A) and (B), concentration–response curves of C-fiber polymodal receptors for three purinergic agonists are shown. In (C), the averaged discharge patterns evoked by the purinergic receptor agonists (10^{-3} M) after the heat stimuli are shown (1 bin = 10 s, mean ± S.E.). The presentation method is similar to Fig. 6. Except for UTP, there is a weak tendency for dose-dependency in (A), the incidence of drug sensitivity, and in (B), the mean net total number of impulses.

fibers. ATP might act on the P2X receptors in the sympathetic nerve terminals and induce release of NE (Boehm, 1999), and this released NE might then inhibit the heat response of CPRs. This hypothesis is supported by our previous observation that endogenous NE inhibited the heat response of CPRs in normal conditions, irrespective of the presence or absence of the NE-induced excitation (Yajima et al., 2000).

What is the physiological role of this inhibitory effect from low concentration ATP? It is known that traumatic injuries during athletic competitions or combat are often reported as being relatively painless (Melzack and Wall, 1982). The inhibitory modulation of pain in the central nervous system (descending inhibition or endogenous opioids and so on) has been used to explain this phenomenon (Fields and Basbaum, 1999). Our present observation might suggest that pain can also be inhibited in the periphery by sympathetic nerve activity in some conditions.

4.2. ATP-induced excitation

ATP excited a proportion of CPRs before heat stimuli in a concentration-dependent nature. As ATP increased in concentration, CPRs were more frequently excited and the magnitude of the response to the drug was increased. Excitation of nociceptors induced by ATP has been reported (Hamilton et al., 2001, Hilliges et al., 2002), but the concentration range used in these experiments was higher than that used in the present experiment. The excitation induced by ATP at a low concentration, such as might exist in the surroundings of nociceptors when ATP escapes from the sympathetic-effector junction, was quite small before any heat stimuli. This might indicate that ATP released from

the sympathetic terminals has a negligible role in induction of excitation of nociceptors except in conditions wherein the sympathetic terminals are located adjacent to nociceptors, e.g. sympathetic sprouting around dorsal root ganglion neurons after nerve injury (McLachlan et al., 1993).

The $P2X_3$, $P2Y_1$ and $P2Y_2$ receptor agonists excited a proportion of CPRs in a roughly concentration-dependent manner. These results were similar to those in the ATP-induced excitation of CPRs, however, the magnitude of the response elicited by these agonists was considerably smaller than that with ATP at the same concentration and in the same condition (namely, after a few heat stimuli). This observation is at odds with the report that α,β-met ATP was more potent in inducing nocifensive behavior than ATP (Hamilton et al., 1999). Although they also examined α,β-met ATP-induced excitation in the same excised skin-nerve preparation in vitro as we did, they did not determine whether α,β-met ATP was more potent than ATP. The difference of our present result from those of the behavioral experiments might suggest that some tissue or blood-born factors are involved in α,β-met ATP-induced nocifensive behavior.

A previous report showed that the effects of α,β-met ATP on $P2X_3$ receptor and 2-MeS ADP on $P2Y_1$ receptor are both more potent than that of ATP (Grubb and Evans, 1999), and UTP is as potent as ATP on $P2Y_2$ receptors. CPR excitation induced by ATP, therefore, cannot be mediated through either $P2X_3$, $P2Y_1$ or $P2Y_2$ receptor alone. Alternatively, in view of the presence of $P2X_{1-6}$ and $P2Y_{1-2}$ in DRG neurons (Burnstock, 2000), there is also a possibility of additional or concerted involvement of a purinergic receptor subtype other than $P2X_3$, $P2Y_1$ and $P2Y_2$ receptors in the CPR excitation.

4.3. Mechanism of facilitation of ATP-induced excitation after heat stimuli

After repetitive heat stimuli, ATP-induced excitation tended to be facilitated at all concentrations used. This augmenting effect of repetitive heat stimulations on the ATP-induced excitation was similar to that by NE, which excited CPRs after a few heat stimuli in normal animals (Yajima et al., 2000). Our results suggest that some P2 receptors may be dormant under normal conditions, and recruited or sensitized after repetitive heat stimuli. Hamilton et al. (2001) reported that mechano-heat nociceptors were sensitized to α,β-met ATP in short inflammatory states by caraggeenan. Our observation was done in vitro; therefore, the sensitizing substance is unlikely to be blood-born.

4.4. Possible interaction of ATP with NE

ATP is a co-transmitter of NE at sympathetic nerve terminals. ATP-induced excitation was small, especially so at low concentration. If NE acts together with ATP, a larger magnitude of the ATP response might be induced. Our previous studies demonstrated a novel action of NE in such a pathological condition: NE sensitized the response to bradykinin of nociceptors (Banik et al., 2004). Recently Waldron and Sawynok (2004) reported that spontaneous pain behavior was augmented by injection of α,β-met ATP and NE. Although in this experiment they employed a high concentration of α,β-met ATP, their results suggested cooperation between ATP and NE. If ATP and NE acted in concert on nociceptors, ATP in damaged tissue or released from the sympathetic terminals might have more potent effects, either inhibitory or facilitatory, on nociceptors. This issue remains open for future study.

Acknowledgement

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■特集「筋・骨格系の痛み研究の最近の話題」

痛み受容器をめぐる最近の話題
—痛み受容器における受容変換機構と筋肉痛モデルにおける痛み受容器活動の変化—

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要旨　カプサイシン受容体（TRPV1）のクローニング以来，その修飾機構が一部明らかにされ，また各種の温度感受性イオンチャネルが次々と発見され，痛み受容器終末における刺激の受容・変換機構についての理解が深まった．その概略を紹介する．次に，筋炎，遅発性筋痛などの筋肉痛のモデルを紹介し，これらのモデルにおける痛みを伝える細径線維受容器活動の変化を紹介する．

Abstract　Since cloning of the capsaicin receptor (TRPV1), demonstration of its modifying mechanism through intracellular signals and cloning of several thermo-sensitive ion channels were done quite rapidly. In this review I will briefly introduce the present understanding of transduction mechanism in nociceptors promoted by these findings. In addition, I will introduce muscle pain models and changed thin-fiber activities in these models.

Key words：痛み受容器（nociceptor），筋肉痛モデル（muscle pain model），受容変換機構（transduction mechanism）

はじめに

肩こり，腰痛などの筋に関連した痛みは，有訴者率が最も高い部類に入り，医療機関に通院している主訴のなかでも上位を占めている．しかし，他の領域の痛みに比べその神経機構についての研究は少ない．その研究を進め理解を深めるうえでは，他の領域で明らかにされた成果を知ることがぜひとも必要である．最近の痛み領域の大きな進歩は，痛み刺激の受容体がいくつもクローニングされ，その細胞内情報伝達・修飾機構の理解が進んだことである．また，病的な痛みの神経機構には，健常な組織に痛み刺激を与えて起こる痛みの神経機構とは別の機構が働いていることもわかってきた．つまり，筋の痛みの研究には，できるだけ臨床的な筋の痛みに近いモデルをつくることが必須であるということになる．本稿ではまず，最近の痛み受容体分子のクローニングの成果を基に，痛み受容器にお

Current topics on nociceptors: Transduction mechanism in nociceptors and changed nociceptor activities in muscle pain models

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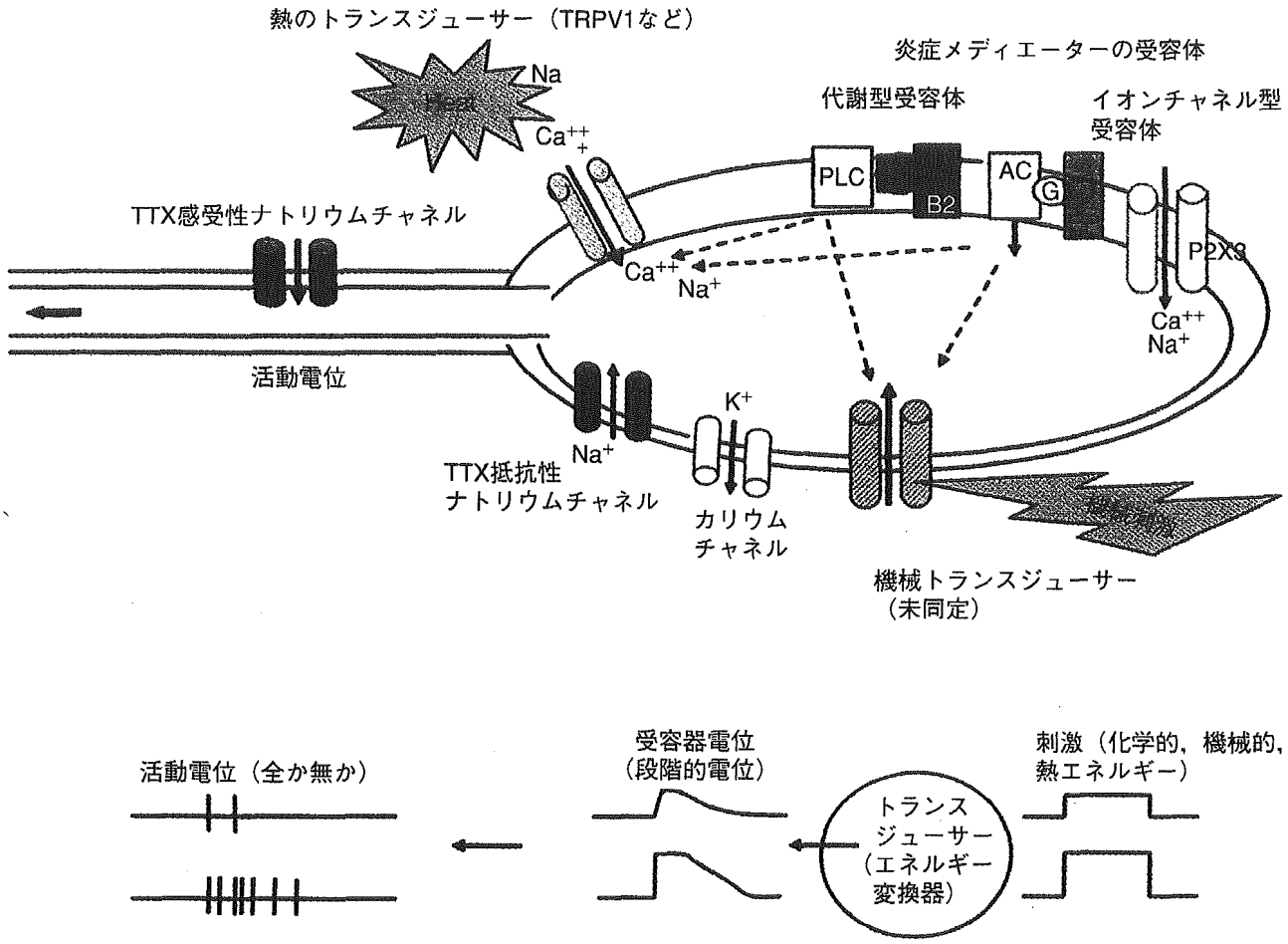

図1 侵害受容器終末における刺激の神経信号への変換（受容変換）の模式図
B2：ブラジキニンB2受容体，EP：Eタイププロスタグランジン受容体，G：Gタンパク質，AC：アデニル酸シクラーゼ，PLC：フォスフォリパーゼC

いて痛み刺激がどのようにして神経の活動電位に変換されるかについて述べ，その後，筋の痛みモデルを紹介して，現在までの理解を概説する．

受容変換—痛み刺激が神経インパルスに変換されるまで(図1)—

痛みを起こす刺激（侵害刺激）には，強度の機械的な刺激（強い圧迫など），熱・冷刺激（約43℃以上または10℃以下），発痛物質（ブラジキニン，酸，ATPなど）がある．これらはすべて，痛み受容器（侵害受容器）終末において神経の信号（電気的な変化）へと変換される．熱刺激によって開いて痛み受容器終末に脱分極を引き起こすチャネルとして最初にクローニングされたのはTRPV1（カプサイシン受容体，以前はVR1と呼ばれた）である．これは唐辛子の辛みのエッセンスであるカプサイシンに感受性があり，かつ熱でも開くチャネルである[7,16]．それに引き続いて，これよりも高い温度で開くチャネル（TRPV2），これよりやや低い温度で開くチャネル（TRPV3，TRPV4），さらに冷却により開くチャネル（TRPM8，TRPA1）などが発見，クローニングされた[16]．

一方，機械刺激で開くチャネルにはまだこれだという確定的なものはない．発痛物質に対しては，それに反応して開くイオンチャネ

ル型の受容体（たとえば酸に対する受容体のASICs，ATPに対する受容体のP2X3など）と，細胞内情報伝達系を活性化する代謝型受容体（たとえばブラジキニンに対するB2受容体，ATPに対するP2Y受容体，プロスタグランジンに対するEP受容体など）がある．後者の場合は生じたセカンドメッセンジャーが何らかのイオンチャネルを開かせて，痛み受容器終末に脱分極を引き起こす．このチャネルの候補の一つがTRPV1である．TRPV1はP2Y受容体やB2受容体が活性化した結果生じるprotein kinase Cの活性化によってリン酸化され[16]，チャネルが開く温度が体温レベル以下にまで下降することが示された[12]．つまり，ブラジキニンやATPが存在すればTRPV1は外から熱をかけないでも開き，脱分極を起こしうるということである．

痛み受容器には数種類あり，それぞれの痛み受容器はそれぞれがもつ反応性に関連した受容体やトランスジューサーチャネルをもっていると想定されている．たとえば，熱にも機械刺激にも各種の発痛物質にも反応するポリモーダル受容器の場合には，熱，機械トランスジューサー，各種発痛物質の受容体をもっていると考えられる（図1）．また冷侵害受容器は強度の冷却刺激で開くTRPA1をもっていると考えられている．

このようにトランスジューサーチャネルや受容体イオンチャネルが開くことによって生じる脱分極の大きさは，刺激の強さに比例している．終末部の脱分極は遠くへは伝わらない（減衰する）ので，末梢神経の中を長い距離にわたって脊髄まで伝達されるためには，さらに活動電位へと変換されなければならない．活動電位の発生には電位依存性のナトリウムチャネルとカリウムチャネルが必要であり，それらは受容器終末に続く軸索に備わっている．終末部における脱分極の大きさは活動電位の頻度へと変換される．つまり刺激が強ければ大きな脱分極が生じ，それは高い頻度の活動放電を生じる．この過程を模式的に図1に示した．

筋の細径線維受容器とその活動による中枢・全身性効果

病的状態で痛みが生じる機構は，実験的に急性的に外から刺激を与えて生じる痛みの機構とはかなり異なることがわかってきている．たとえば，1）通常では痛みを生じないような軽度の機械的な刺激（触刺激）によって痛みが生じたり（機械アロディニア），2）通常では活動電位を生じないような部位（活動電位を伝える軸索）で自発的に興奮が生じたり（異所性興奮），3）同じ濃度の発痛物質に対して大きな反応を生じる，などが挙げられる．

1）が起こるのは脊髄における痛み伝達経路の可塑的な変化（興奮性の増大，求心神経の脊髄内におけるsproutingなど）によると考えられており[3]，2）は活動電位を生じるために必要な電位依存性Naチャネルの種類が変化するためではないかと考えられている．3）はC線維受容器の発痛物質受容体upregulationによると考えられている．

慢性的な筋の痛みも，健常筋に急性的に刺激を与えて生じる痛みの機構とは異なっている可能性があるが未知である．健常状態では皮膚と同様，筋の痛みも細径線維（Aδ，C線維，または筋の場合にはIII群，IV群線維ともいう）受容器によって伝えられる．筆者らはイヌの筋細径線維受容器活動を調べ，多くが熱にも機械刺激にも発痛物質にも反応するポリモーダル受容器であることを示した[8]．

筋細径C線維受容器は筋の痛みを伝える