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DAILY VARIATION IN AN INTENSITY OF KAOLIN - INDUCED WRITHING REACTION IN MICE

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Summary

The daily variation in an intensity of kaolin-induced writhing reaction was examined in mice kept under conditions of light; 07:00 - 19:00 and dark; 19:00 - 07:00. The number of writhes was counted for 30 minutes after a single intraperitoneal injection of kaolin at 00:00, 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00 and 22:00. The number of writhes showed a daily variation with a peak at 18:00 and a trough at 06:00. The intensity of writhing reaction was significantly reduced by pretreatment with the bradykinin B_1 (Des-Arg^9-[Leu^8]-BK) and B_2 (icatibant) receptor antagonists. Significant daily variation in this parameter was still observed in the group with the B_1 antagonist, but disappeared in the B_2 antagonist-treated group. These results suggest that the kaolin-induced writhing reaction shows the daily variation with a peak at the end of the resting period and a trough at the end of the active period. The B_2 receptor mediated stimuli appears to be involved in this phenomenon.

Key Words: acute pain, bradykinin, icatibant, Des-Arg^9-[Leu^8]-BK

The onset of pain in some chronic diseases such as migraine headache and rheumatoid arthritis shows a daily variation with a peak in the morning and a trough in the afternoon and evening (1). On the other hand, the effectiveness and toxicity of many analgesic drugs which are used for the treatment of these diseases, are also affected by the time of dosing (2). Therefore, chronophathological and chronopharmacological approches are desirable in developing more effective and safer dosage regimens. Analgesic profiles of an agent are usually evaluated using various animal models with pain. However, the chronopathological characteristics of pain observed in some clinical situations (1) are not taken into consideration in these preclinical studies. To use a chronopathological approach, it is important that an animal model with a time-dependent intensity of pain be established. Writhing reactions in mice has been widely used for evaluation of analgesic potency of an agent.

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Several chemical substances including kaolin (3) produce the writhing reactions. This is mediated through elevation of bradykinin (BK) (3).

The present study was undertaken to examine 1) whether the intensity of kaolin-induced writhing reaction shows a daily variation, and 2) the effect of specific BK receptor antagonists on the writhing reaction in mice.

Methods

Male ICR mice (SLC, Shizuoka, Japan) (4-5 weeks old) weighing 20-30g were kept under conditions of light from 07:00 to 19:00 and dark from 19:00 to 07:00 with a temperature of 22 ± 2°C. These animals had free access to food (MF cotaining Na 0.26 g and K 0.75 g/100 g, Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

Kaolin-induced writhing reaction
Writhing reaction was induced by a single intraperitoneal injection of kaolin as previously described (4). In brief, kaolin (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was injected intraperitoneally (i.p.) with 1.25 mg/10 g body weight in 0.9 % NaCl solution (5 mg/ml), and the number of writhes was counted for 30 minutes immeiately after injection. In the first, preliminary study, kaolin was injected i.p. at the end of active (06:00) and resting (18:00) periods in different groups of mice, and the number of writhes was counted every 5 minutes for 30 minutes after injection.

Effect of BK receptor antagonists on the kaolin-induced writhing reaction
Des-Arg^{9}-[Leu^{8}]-BK, a selective B_1 receptor antagonist, and D-Arg-[Hyp^{3}, Thi^{5}, D-Tic^{7}, Oic^{8}]-BK (icatibant), a selective B_2 receptor antagonist (5), were purchased from the Peptide Institute, INC., Osaka, Japan. As the kaolin-induced writhing was greater at 18:00 than at 06:00 in the first preliminary study (Fig. 1), the second preliminary study was performed at 18:00. Five minutes after pretreatment with the B_1 or B_2 receptor antagonist (0, 125, 250, 500 and 1000 nmol/kg in 0.15 ml physiological saline, i.p.), kaolin was injected i.p. in different groups of mice. The number of writhes was counted for 30 minutes. Based on these data, 500 nmol/kg of B_1 and B_2 receptor antagonists was chosen as an appropriate dose and used in the following experiment (Fig. 2) .

Daily variation in an intensity of kaolin-induced writhing reaction
Kaolin was injected i.p. at 00:00, 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00 and 22:00 with or without pretreatment with BK receptor antagonist. Different groups of mice were used at each observation point.

Statistical analysis
Data were expressed by the means ± SE. Statistical analysis was performed using one- and two-way analysis of variance (ANOVA). As a post-hoc test, Fisher's Protected Least Significant Difference test was used. Values of $p<0.05$ were considered as significant.

This experiment was performed in accordance with the Jichi Medical School Guide for

Laboratory Animals in 1993.

Results

Time-course in the number of kaolin-induced writhes (Fig. 1)
The number of writhes reached a peak at 5 - 10 minutes, and almost disappeared at 20 - 30 minutes after the injection of kaolin. This parameter in the 18:00 trial was significantly greater than that in the 06:00 trial (p=0.0004). Total number of writhes was significantly greater in the 18:00 trial [12.5 ± 3.5 (18:00 trial) vs. 1.4 ± 0.7 (06:00 trial) times/30 minutes, p=0.0005].

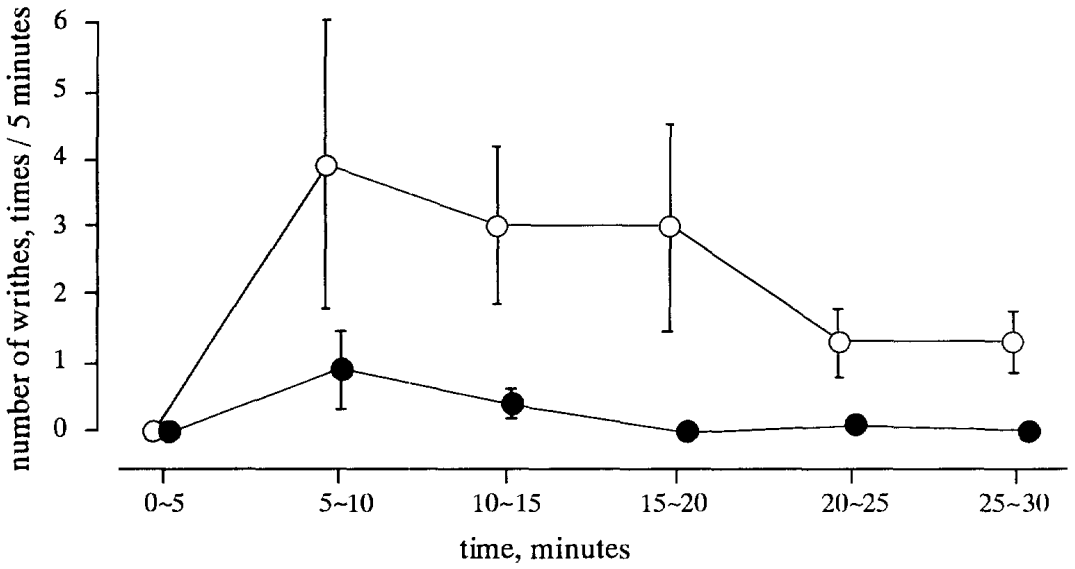

Fig. 1

Number of writhes during every 5 minutes for 30 minutes after kaolin injection at the end of the active (06:00, ●-●) and the resting (18:00,○-○) periods in mice. Mean ± SE, n=10 for each group.

Influence of pretreatment with BK receptor antagonists on kaolin-induced writhing reaction (Fig. 2)
The number of writhes was reduced dose-dependently by pretreatment with the B_1 (p=0.0460) and B_2 (p=0.0006) receptor antagonists. No significant difference was observed in this parameter between the 500 and 1000 nmol/kg pretreatments with each BK receptor antagonist. The number of writhes in the trial with the B_2 receptor antagonist was significantly smaller than that in the trial with the B1 receptor antagonist (p=0.0022) .

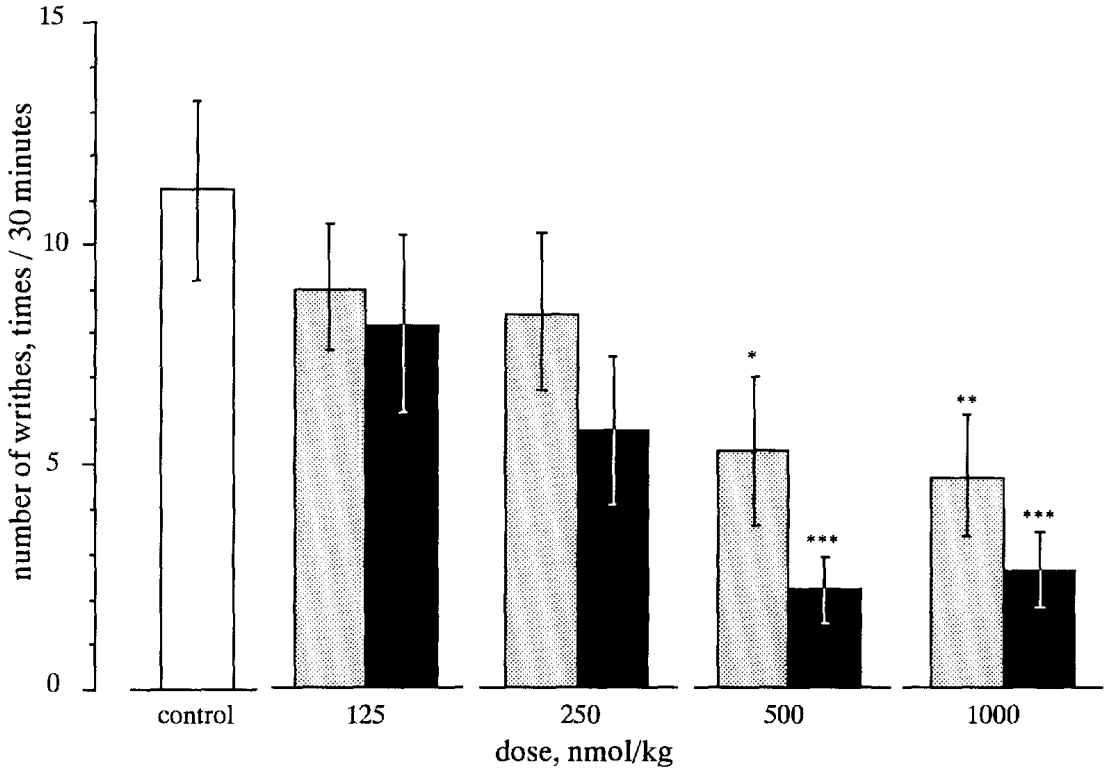

Fig. 2

Influence of pretreatment with the BK receptor antagonists on kaolin-induced writhing reaction in mice. Mean±SE, n=10 for each column. $*p<0.05$, $**p<0.01$, $***p<0.001$ compared with control. ▨:pretreatment with the B_1 receptor antagonist, Des -Arg^9-[Leu^8]-BK, ■:pretreatment with the B_2 receptor antagonist, D-Arg- [Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK. Five minutes after treatment with BK receptor antagonist, kaolin was injected at 18:00, and the number of writhes was counted for 30 minutes.

TABLE 1

Analysis of the time course of the number of kaolin - induced writhes in mice.

group	daily variation	*p* value
control	+	0.0210
pretreatment with B_1 receptor antagonist	+	0.0168
pretreatment with B_2 receptor antagonist	-	0.9604

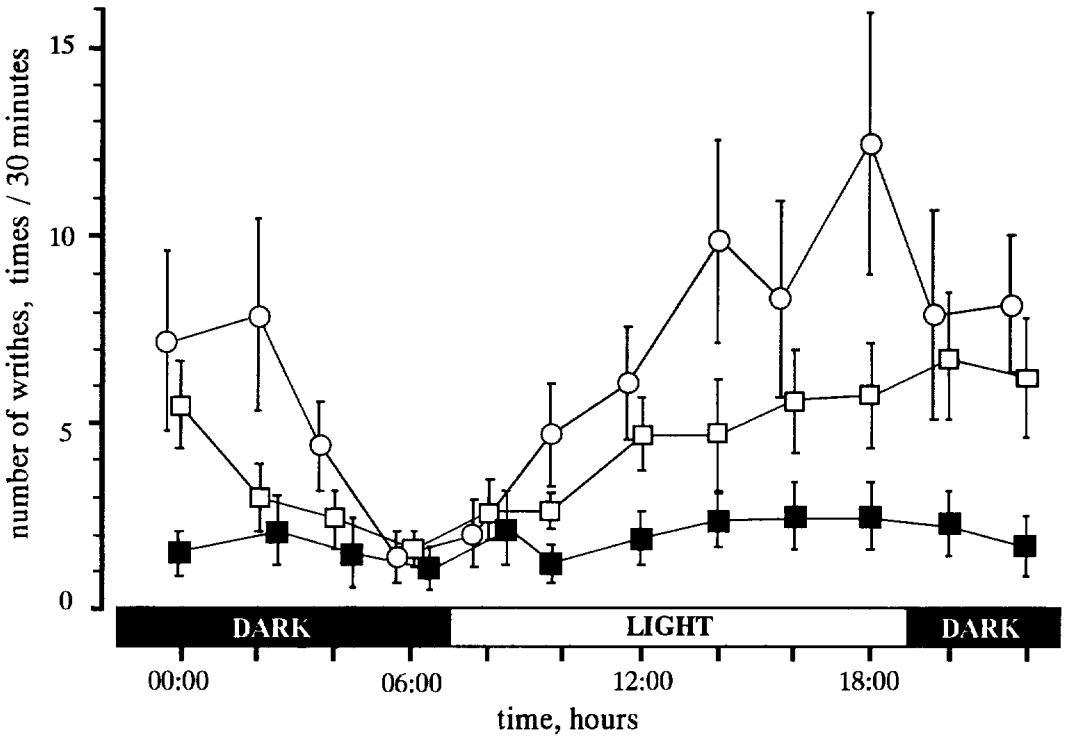

Fig. 3

Daily variation in the number of kaolin-induced writhes and the influence of pretreatment with BK receptor antagonists. Mean±SE, n=10 at each observation point. ○: control, □: pretreatment with B_1 receptor antagonist, ■: pretreatment with B_2 receptor antagonist

Daily variation in the number of writhes induced by kaolin (Table 1, Fig. 3)
The number of writhes showed a significant daily variation with a peak at 18:00 and a trough at 06:00 in the control group of mice. The intensity of writhing reaction was significantly reduced by pretreatment with the B_1 receptor antagonist ($p < 0.0001$). However, significant daily variation in this parameter was still observed. On the other hand, the intensity of writhing reaction was further reduced by pretreatment with the B_2 compared with the B_1 receptor antagonist ($p=0.0001$), and the daily variation in this parameter disappeared.

Discussion

Writhing reaction induced by kaolin, an activator of factor XII, in mice was used in the present study. In general, substances with a negative surface charge such as kaolin and carrageenin activate the kallikrein - kinin system through the activation of factor XII (6). Kaolin is shown to release BK by the activated kallikrein - kinin system (3,7). The

writhing reaction reached a peak at 5-10 minutes, continued for about 10-20 minutes and then disappeared in this study, which are similar to the previous findings (4).

There is increasing evidence demonstrating the linkage between BK and the pathophysiological processes that accompany tissue damage and inflammation, especially the production of pain and hyperalgesia. Several mechanisms have been proposed to account for hyperalgesia including the direct activation of nociceptors and sensitization of nociceptors through the production of prostanoids or the release of other mediators (8). There are at least two types of BK receptors, B_1 and B_2 (5) . Acute and chronic activation of nociceptors are reported to be mainly mediated by the B_2 and B_1 receptors, respectively (9-11). To our knowledge, the roles of the B_1 and B_2 receptors have not been previously determined in the kaolin-induced writhing reaction.

The present study using the specific B_1 and B_2 receptor antagonists demonstrated involvement of both types of BK receptor in this animal model with pain. In addition, as the suppression of the intensity of writhing reaction was significantly greater in the trial with the B_2 receptor antagonist than with the B_1 receptor antagonist, we think that stimuli mediated by the B_2 receptor play a major role in the activation of nociceptors in this model. The present study demonstrated a daily variation in the intensity of the kaolin-induced writhing reaction with a peak at the end of the resting period and a trough at the end of the active period in mice. Previous studies have examined the daily variation in the pain threshold for thermal stimuli in nocturnal rodents (12, 13). They have shown that the threshold was lowest, representing an increased pain sensitivity, at the middle of the active period and highest, representing a decreased pain sensitivity, at the beginning of the resting period. Thus, the profile of the daily variation of pain induced by kaolin is remarkably different from that induced by thermal stimuli.

The daily variation in the kaolin-induced writhing reaction might be explained by one or more of the following mechanisms: 1) daily variation in the sensitivity of nociceptors to BK, and 2) time-dependent difference in the BK production induced by kaolin. To examine the first possibility, several doses of BK (1000 - 6000 nmol/kg) were injected i.p. at 06:00 and 18:00 in this study. However, as reported previously (14), the writhing reaction was very weak and its reproducibility was poor. Therefore, we could not reach any conclusion. Further studies are needed to evaluate the mechanism of this phenomenon.

In the present study, the intensity of the writhing reaction was significantly reduced by the B_1 and B_2 receptor antagonists. The daily variation was completely blunted by the B_2 receptor antagonist while this was still observed in the trial with the B_1 receptor antagonist. Based on these findings, we think that B_2, rather than B_1 receptor mediated stimuli are involved in the daily variation of the kaolin-induced writhing reaction.

The effectiveness of analgesic drugs is usually evaluated using animal models with pain. Most painful events occur during the early morning in some diseases (1). Therefore, it is desirable to use an animal model with a similar daily variation of pain for evaluation of analgesic drugs.

Moreover, such an animal model is helpful for establishing the dosage regimen in clinical practice. In the present study, the intensity of the kaolin-induced pain showed a peak at the end of the resting period which is compatible with the early morning in

human subjects. Thus, kaolin-induced writhing is a useful animal model for evaluating analgesic drugs. These data may prove useful for establishing the dosage regimen for analgesics in diseases with pain which peaks in the early morning.

In summary, the present study demonstrated that the intensity of the kaolin-induced writhing reaction shows the daily variation with a peak at the end of resting period and a trough at the end of active period in mice. The B_2 receptor mediated stimuli may be significantly involved in this phenomenon.

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