POTENTIATING EFFECTS OF PROSTAGLANDIN E₂ ON BRADYKININ AND CAPSAICIN RESPONSES IN MEDIAL THALAMIC NOCICEPTIVE NEURONS*

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Abstract—Potentiating effects of prostaglandin E₂ (PGE₂) on bradykinin and capsaicin responses were studied in 34 gallamine triethiodide immobilized cats. Single neurons were recorded from the medial thalamus by using stainless steel microelectrodes. The animals were given agents into the femoral artery through a retrogradely inserted cannula. Of the 22 neurons responding to bradykinin, 13 were potentiated by the injection of PGE₂, and the remaining 9 neurons were ineffective. Nine of the 17 neurons responding to capsaicin were also potentiated by PGE₂. PGE₂ significantly shortened the mean latency of bradykinin to fire the neuronal activity without changing the duration, but with the injection of capsaicin, there was no change in latency and duration in the presence of PGE₂. Aspirin suppressed the increased activity of the medial thalamic neurons produced by bradykinin, and this suppression was antagonized by arterial infusion of PGE₂. However, the activity of medial thalamic neurons with capsaicin was scarcely affected by aspirin. These results suggest that the bradykinin-induced activity of medial thalamic neurons may be mediated by PGE₂ and that the mechanism of activation of nociceptive neurons produced by capsaicin is different from that of bradykinin.

It has already been reported that capsaicin, one of the pungent principles of Capsicum annuum L., selectively stimulates peripheral pain receptors (1–3). Recently, it was found that large doses of capsaicin markedly reduce the content of substance P without any effect on the opiate receptor affinity of the dorsal horn of the spinal cord (4). Although cold perception and perception of mechanical stimulation are not suppressed (1, 3), after large doses of capsaicin, there is strong suppression of heat perception and chemical stimulation (1–6), indicating its importance as a releaser of substance P (7). Furthermore, with large doses of capsaicin, changes in the tissue of the rat dorsal horn, associated with abnormalities in glial phagocytes have been observed (8).

We found an increase in rate of firing of nociceptive neurons of the medial thalamic nuclei by administration of capsaicin into the femoral artery in cats (9). The pattern of the response to capsaicin is that of a strong algesic substance (3) and differs from the response to bradykinin. That is,

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bradykinin has been found to increase the frequency of neuronal firing after a latency of 7–9 sec following intra-arterial administration, whereas capsaicin causes increased firing after only 1 sec. Prostaglandins of the E series (PGEs) have been shown to cause potentiating effects on the vocalization (10) and the flexion reflex (11) by bradykinin. Nevertheless, it is still unknown whether PGE2 augments the firing of medial thalamic nociceptive neurons induced by bradykinin or capsaicin. Therefore, we investigated the relationships in nociception between PGE2 and two algesic substances, bradykinin and capsaicin.

**MATERIALS AND METHODS**

**Animal preparations:** Thirty-four adult cats weighing 2.0–4.0 kg were used. Under ether anesthesia, tracheal and vascular cannulations were performed, and all operating wounds were closed with sutures. The animal was placed in a stereotaxic apparatus. The skull was trephined over the medial thalamus (nucleus centralis lateralis, medial part of the nucleus ventralis lateralis and the nucleus medialis dorsalis). The dura was removed for electrode implantation and a metal chamber was mounted with dental cement. Bleeding into the chamber was carefully halted. Wound margins and pressure points were infiltrated with Xylocaine jelly. The animal was then immobilized with gallamine triethiodide and artificially respired. The fact that the animals were not suffering pain was shown by occurrence of spindle bursts and slow waves on the continuously recorded EEG (somatic-sensory area 1). At least, 3 hr were allowed to elapse between the continuation of ether anesthesia and beginning of the experiment. Body temperature was kept at 37.5–38.5 ℃.

**Recording procedures:** To reduce movement by respiration and heart beat, a modification of the “closed-head” technique was used (12). The impulse activity from a single neuron was recorded extracellularly by using stainless steel microelectrodes. The electrode coated with insulated paint had head tip diameters of 1–3 μm and resistances of 1–2 megohms at a test frequency of 1,000 Hz. Neuronal activity was displayed on a cathode-ray oscilloscope, and a spike height discriminator was employed for the identification of single neuron impulse. The impulse was also monitored through a loudspeaker. The electrode was advanced dorsoventrally into the thalamus, according to the stereotaxic co-ordinates of Jasper and Ajimone-Marsan (A; 9.5 to 10.0, L; 2.0 to 4.0, H; +5.0 to +1.0) (13).

The following modes of somatic stimulation were used: the hair bent with a blower (hair-bending), the superficial and deep tissues tapped (tapping) and skin pinched with toothed forceps (pinching). The neuronal impulse sequences were stored in an FM magnetic tape and reproduced through the spike height discriminator and the spike counter for the purpose of data transcription.

**Drugs used:** Anesthetic ether (Sanraku Ocean), xylocaine jelly (Fujisawa), gallamine triethiodide (Teikoku), sodium pentobarbital (Dainippon), aspirin (Yoshida), bradykinin (Protein Research Foundation), capsaicin (synthesized by Takahashi et al.) (14) and prostaglandin E2 (Ono). Aspirin (50 mg/kg or 100 mg/kg) was administered i.p. after suspension in 0.5% Tween 80. Bradykinin was dissolved in Ringer’s solution (50 μg/ml) and capsaicin dissolved in one drop of ethanol and diluted in Ringer’s solution (50 μg/ml), since it is insoluble in water. Bradykinin and capsaicin at a dose of 3–5 μg/cat are administered retrogradely to the right femoral artery. PGE2 was dissolved in Ringer’s solution and infused at a rate of 1.06 μg (3 nmol)/min when continuous
infusion was done and at 5.3 μg (15 nmol)/cat when given together with bradykinin or capsaicin via the same cannula.

Histology: At the end of each experiment, a current of 20 μA was passed through the electrode for 15 sec to mark the electrode tip. The animals were sacrificed under deep pentobarbital anesthesia and perfused with 10% formalin containing 1% potassium ferrocyanide. Frontal serial frozen sections of 25 μm thickness were stained with 0.1% cresyl violet and recording sites were determined.

Criteria for determining the incidence of firing, latency and duration: The latency of the agent effect was determined as the time interval from the immediate time after injection of the agent to the time when spike counts reached over 150% of the mean pre-administration value. The duration was determined as the time interval from the immediate time when firing increased to the time when spike counts returned to pre-administration value, provided it was over 3 sec at least. The rate of increased firing was calculated from the difference between the spike count of the duration period and the mean pre-administration spike counts. That is, the rate of change by PGE₂ in spike frequency was calculated from the difference between spike counts before and after PGE₂ administration (Fig. 1).

RESULTS

Properties of medial thalamic neurons: Investigations were made on 66 individual neurons in the medial thalamus concerning their receptivity to various somatic stimuli. The number of neurons responding to non-noxious stimulation (hair-bending and/or tapping) and noxious stimulation (pinching) was 34. Those responding to only non-noxious or noxious stimulation were 23 and 2, respectively, and the number showing no response to peripheral stimulation was 7. The receptive fields of those neurons activated by some form of peripheral stimulation were located nearly over the entire body (Table 1).

Examination was also made of all these neurons following the administration of bradykinin or capsaicin. Of the 36 neurons responding to noxious stimulation, 27 also responded to bradykinin and 33 to capsaicin. Of the 23 neurons responding only to non-noxious stimulation, 11 responded to bradykinin and 14 to capsaicin. Of the 7 neurons which were not fired by any kind of peripheral stimulation, 1 responded to
Table 1. Responses of medial thalamic neurons in cats

| Stimuli                      | No. of neurons responding to somatic stimuli | No. of neurons excited by bradykinin | No. of neurons excited by capsaicin |
|-----------------------------|---------------------------------------------|-------------------------------------|-------------------------------------|
| Non-noxious+noxious         | 34                                          | 26                                  | 31                                  |
| Non-noxious                 | 23                                          | 11                                  | 14                                  |
| Noxious                     | 2                                           | 1                                   | 2                                   |
| Not driven                  | 7                                           | 1                                   | 1                                   |
| Total                       | 66                                          | 39                                  | 49                                  |

Non-noxious: bending hairs with a blower and/or tapping superficial and deep tissues. Noxious: pinching skin with toothed forceps. Bradykinin and capsaicin were given into the femoral artery at 3–5 μg/cat.

bradykinin and 2 to capsaicin. All neurons fired by bradykinin also responded to capsaicin (Table 1).

The effects of PGE₂: The effects of PGE₂ were investigated in all the 33 neurons responding to bradykinin and/or capsaicin. As shown in Fig. 2, the excitatory action of bradykinin and capsaicin on the firing rate was potentiated by continuous intra-arterial infusion of PGE₂ (1.06 μg/min). Thirteen of the neurons responding to bradykinin showed increased firing by PGE₂. Of the 17 neurons responding to capsaicin, 9 were affected by PGE₂ (Table 2). With regard to the duration of continuous infusion or simultaneous administration, marked increases in firing rate

![Fig. 2](https://via.placeholder.com/150)

Fig. 2. A typical effect of intra-arterial infusion of PGE₂ on the excitation of a medial thalamic neuron induced by bradykinin and capsaicin. Brady: bradykinin, Cap: capsaicin.

Table 2. Effects of continuous and simultaneous infusion of prostaglandin E₂ on activation of medial thalamic neurons induced by bradykinin and capsaicin.

| Agents (3–5 μg) | No. of neurons |
|-----------------|----------------|
|                 | Potentiation   | No effects   |
| Bradykinin      | 13 (59%)       | 9 (41%)      |
| Capsaicin       | 9 (53%)        | 8 (47%)      |
were seen after 5 min of PGE$_2$ infusion (365% for bradykinin and 395% for capsaicin) and after 10 min (695% for bradykinin and 385% for capsaicin). The simultaneous administration of PGE$_2$ with bradykinin or capsaicin produced increased firing of 431% or 209%, respectively (Fig. 3). PGE$_2$ also increased the firing rate which was caused by non-noxious and noxious mechanical stimuli, but PGE$_2$ per se did not cause the increase in firing rate of medial thalamic neurons when a dose of 5.3 µg was administered alone.

We have already reported that the response pattern of medial thalamic neurons to bradykinin and capsaicin is markedly different, especially with regard to the latency of the response (9). In the present study, we have investigated the differences in latency to bradykinin and capsaicin stimulation induced by PGE$_2$ (Table 3). The mean control value for latency of the response to bradykinin was 7.29 sec, while after the infusion of PGE$_2$ for 5 and 10 min or the simultaneous administration of PGE$_2$ (5.3 µg), latencies were shortened to 6.94 sec, 4.47 sec and 4.64 sec, respectively. These shortenings of the latency as compared to control values were statistically significant for both 10 min infusion and simultaneous administration. In contrast, the latency of the response to capsaicin in the presence of PGE$_2$ did not differ significantly from control values, regardless of the way of PGE$_2$ administration (Table 3).

Little change in the duration of the

![Fig. 3. The mean rate of increase caused by PGE$_2$ in unit activity induced by bradykinin and capsaicin.](image)

| Duration (min) of PGE$_2$ infusion | Bradykinin (3–5 µg) mean±S.E. (sec) | Capsaicin (3–5 µg) mean±S.E. (sec) |
|------------------------------------|-------------------------------------|-------------------------------------|
| 0                                  | 7.29±0.51                           | 1.88±0.30                           |
| 5                                  | 6.94±1.60                           | 1.80±0.25                           |
| 10                                 | 4.47±0.71**                         | 1.10±0.19                           |
| 5.3 µg (simultaneous infusion)     | 4.62±0.44*                          | 1.03±0.25                           |

The infusions were made at a rate of 1.06 µg-min$^{-1}$. *P<0.05, **P<0.01 significantly different from 0 min (control) of bradykinin (Student's t-test).
Table 4. Effects of intra-arterial infusion of prostaglandin E$_2$ on duration of neuronal activity induced by bradykinin and capsaicin

| Duration (min) of PGE$_2$ infusion | Duration (3–5 μg) | Capsaicin (3–5 μg) |
|-----------------------------------|-------------------|-------------------|
|                                   | mean±S.E. (sec)   | mean±S.E. (sec)   |
| 0                                 | 13.44±1.13        | 11.67±2.25        |
| 5                                 | 15.20±2.04        | 10.60±2.32        |
| 10                                | 12.83±3.39        | 9.50±3.40         |
| 5.3 μg (simultaneous infusion)    | 17.00±2.34        | 13.33±5.07        |

The infusion were made at a rate of 1.06 μg·min$^{-1}$.

Fig. 4. Effects of aspirin on increased unit activities in medial thalamic single neuron induced by bradykinin and capsaicin. Ordinate: number of spikes per sec. Abscissa: time in sec. On the right are interval histograms constructed from the same neuron. Brady: bradykinin, Cap: capsaicin.

Fig. 5. Continued from Fig. 4. Legends and abbreviations are the same as those of Fig. 4.
response to bradykinin or capsaicin was found (Table 4).

The effects of aspirin: It is evident that aspirin has a suppressive effect upon the algesic effects of bradykinin (15). In the present study, similar results were obtained: between 20 to 60 min after i.p. administration of aspirin (100 mg/kg), there was complete suppression of the bradykinin response. The response returned to nearly control levels 90 min after aspirin administration. In contrast, aspirin exerted almost no effect on the capsaicin response, and no effect on the spontaneous firing rate of these neurons. Typical examples are shown in Figs. 4 and 5. Complete suppression of the bradykinin response by aspirin was seen in all of 4 animals, whereas weak suppression of the capsaicin response was apparent in only 1 of 4 animals. Aspirin suppressed the bradykinin response, but neither the capsaicin response, nor the response of the neurons to non-noxious stimulation or noxious stimulation of pinching.

In 3 animals, the administration of PGE$_2$ results in transient recovery of the bradykinin response suppressed by aspirin (Fig. 6).

DISCUSSION

It is generally acknowledged that bradykinin, which is one of the serum kinins, has strong algesic properties in peripheral sites (16, 17). It has also been reported in cats that bradykinin administered peripherally activates afferent spinocervical thalamic tracts (18), including the lamina V spinal neurons (19, 20), and the thalamic posterior neurons (21). Therefore, the finding of the present study on medial thalamic neurons seems to support the above facts concerning the activation of the nociceptive pathway by bradykinin.

It has been known that PGEs enhances nociceptive responses by bradykinin at peripheral sites (10, 11, 22, 23). In the present study, we also demonstrated that PGE$_2$ greatly potentiates the response of the nociceptive neurons of the medial thalamus to bradykinin.

Several investigators reported that bradykinin facilitates the release of PG in peripheral sites, and this release is suppressed by aspirin-like drugs (24–26) which abolishes the biosynthesis of PGEs (27, 28). Moncada et al. demonstrated that bradykinin induced a dose-dependent pseudoaffective reflex in blood pressure which was reduced, but not abolished, by local administration of aspirin in dogs (29). Although the methodology, administration route and preparation used in the present study is
different from theirs. Systemic administration of aspirin in cats abolished the bradykinin-induced response.

Furthermore, the latency of the response to bradykinin was significantly shortened by PGE₂, and a transient recovery of the bradykinin response was produced by PGE₂ when the bradykinin-induced response was suppressed by aspirin. These results are consistent with the assumption put forth by Lembeck et al. (26) that the algesic effect of bradykinin depends on the relative amount of E-type PG released.

Jancsó et al. reported that inflammatory responses to antidromic electrical stimulation of sensory nerves were completely inhibited by capsaicin (30). This inhibition may be attributed to the inhibition of release of some mediators or modulators (e.g., PG) of inflammation. As shown in Fig. 3, the capsaicin-induced unit activity was enhanced by PGE₂, though this effect of PGE₂ on capsaicin showed a tendency to be weaker than that on bradykinin. However, this augmentative effect of PGE₂ seems to be a non-specific action on chemogenic nociception, since PGE₂ not only enhanced the medial thalamic responses to somatic stimuli (hair-bending and pinching), also those to chemical stimuli. Aspirin failed to suppress the action of capsaicin, but not that of bradykinin. This result is in line with experimental results of Szolcsányi (3) that capsaicin at a threshold concentration caused immediate pain, thus being about 10 times more potent than bradykinin on the blister base.

Molnár et al. demonstrated that the tachyphylaxis of the isolated guinea pig ileum in response to capsaicin was lasting and highly specific without having cross-tachyphylaxis with other compounds including bradykinin (31). It was also found in our recent study that there was no cross-tachyphylaxis between bradykinin and capsaicin (unpublished data). This fact suggests that the algesic mechanism of capsaicin is different from that of bradykinin.

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