EXCITATORY EFFECTS OF DIHYDROCAPSAICIN ON NOCICEPTIVE NEURONS IN THE MEDIAL THALAMUS*

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Abstract—Effects of capsaicin and dihydrocapsaicin, which are pungent substances contained in Capsicum annuum L., were studied in 32 gallamine triethiodide immobilized cats. Single units were recorded from the medial thalamus using stainless steel microelectrodes. Of the 56 neurons recorded, 28 were responsive to both noxious (pinching) and non-noxious (hair and/or tapping) stimuli, while 16 were activated only by non-noxious stimuli. The remaining 12 neurons did not respond to any natural stimulus. Somatic receptive fields of nociceptive and non-nociceptive neurons were found to be widely distributed over the whole body. Twenty-six of 28 nociceptive neurons were activated by an intra-arterial administration of bradykinin, capsaicin and dihydrocapsaicin. Fourteen of 16 non-nociceptive neurons were not activated by these substances. The mean latency and duration of bradykinin were 8.7 and 12.5 sec, those of capsaicin were 1.2 and 6.1 sec and those of dihydrocapsaicin were 1.3 and 5.0 sec, respectively. The increase of firing frequency produced by capsaicin and dihydrocapsaicin was inhibited by morphine and this inhibition was antagonized by naloxone. However, the activity of medial thalamic neurons with non-noxious stimuli was not affected by these drugs. These results suggest that the pain-conducting fibers were selectively activated by capsaicinoids as well as by bradykinin.

Pörszasz and Jancsó (1) suggested that capsaicin acts mainly on the pain sensory receptor through action potentials of the saphenous and auricular nerves. It was also reported that the desensitization produced by subcutaneous administration of an increasing dose of capsaicin abolishes impulse generation in the cutaneous pain receptor by noxious stimuli, whereas the response to non-noxious stimulation is unaffected (2). We previously reported that capsaicin selectively activates the nociceptive neurons in the medial thalamus (3).

Thus, it was interest to investigate whether other capsaicinoids, e.g. dihydrocapsaicin contained in Capsicum Annuum L., activate selectively pain-conducting fibers. In the present investigation, we compared the effects of dihydrocapsaicin with those of capsaicin.

MATERIALS AND METHODS

Animal preparation: Thirty-two adult cats weighing 2.5–4.0 kg were used. With the animals under ether anesthesia, tracheal and vascular cannulations were performed, all operating wounds were closed with sutures, and the animal was placed in a stereotaxic...
apparatus. The skull was trephined over the medial thalamus (nucleus centralis lateralis, 
medial part of nucleus ventralis lateralis and nucleus medialis dorsalis), the dura removed 
for electrode implantation and a Lucite chamber mounted with dental cement. Bleeding 
into the chamber was carefully halted. Wound margins and pressure points were infiltrated 
with xylocain jelly. Following this surgery, the animal was immobilized with gallamine 
triethiodide and artificially respired. The animal was not in pain as evidenced by the 
ocurrence of synchronized waves in the continuously recorded EEG (somatic sensory area 
I). At least 3 hours were allowed to elapse between the discontinuation of ether anesthesia 
and the beginning of the experiment. Body temperature was kept at 37.5–38.5°C.

Recording procedures: To reduce movement due to respiration and heart beat, a modifi-
cation of the “closed-head” technique was used (4). The impulse activity from a single 
neuron was recorded extracellularly using stainless steel microelectrodes. The electrode 
was coated with cashew paint with 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 impulses. The impulse was also monitored through a loudspeaker. The electrode 
was advanced dorsoventrally into the thalamus according to the stereotaxic co-ordinates 
of Jaspar and Ajimone-Marsan (A; 9.5 to 10.5, L; 2.0 to 4.0, H; +4.5 to +1.0) (5).

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

Drug administration: Bradykinin, synthetized by Protein Research Foundation (Osaka), 
was dissolved in saline (50 μg/ml). Three to five μg of bradykinin were rapidly injected 
through a polyethylene cannula which had been inserted retrogradely into the right femoral 
artery with the tip reaching the bifurcation where the abdominal artery branches off to the 
right external iliac artery. The bradykinin was then infused into the abdominal, common 
iliac and left iliac artery. Since the capsaicinoids synthetized by Takahashi et al. (6) are 
insoluble in saline, they were dissolved according to the method of Fukuda and Fujiwara 
(7): capsaicin and dihydrocapsaicin (1 mg) were dissolved in one drop of ethanol, then diluted 
to 20 ml with saline, and injected in the same manner as bradykinin. To prevent tachy-
phylaxis, each injection was given at 3 to 5 min intervals. In 6 cats, morphine hydrochloride 
(2 mg/kg) and naloxone hydrochloride (0.4 mg/kg) were administered intravenously into 
the cephalic vein through a polyethylene cannula.

Histology: At the end of each experiment, a current of 20 μA was passed through the 
electrode for 15 sec to mark the position of the electrode tip. The animals were then sac-
crificed by an i.v. administration of pentobarbital sodium and the brains were fixed by trans-
carotid perfusion 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.
RESULTS

General findings: Fifty-six neurons in the medial thalamus were examined. The majority were responsive to various types of somatic stimulation, e.g. hair, tapping and pinching. Twenty-eight of 56 neurons were activated by both noxious (pinching) and non-noxious (hair and/or tapping) stimulation and 16 were activated only by non-noxious stimulation. The remaining 12 neurons did not respond to any natural stimulation. The somatic receptive fields of the nociceptive and non-nociceptive neurons were widely distributed over the whole body.

The effects of capsaicin, dihydrocapsaicin and bradykinin were examined in these 56 neurons described above. Twenty-six of 28 nociceptive neurons revealed an excitation with the intra-arterial administration of capsaicin, dihydrocapsaicin and bradykinin. On the other hand, only two of 16 non-nociceptive neurons were activated by these agents. Two nociceptive neurons, 14 non-nociceptive neurons and 12 neurons which were not activated by any natural stimulation showed no appreciable changes in their firing frequency with these agents. An intra-arterial administration of the solvent did not produce any changes in the firing rate of any neuron. These results are summarized in Table 1.

Response patterns of the medial thalamic neuron to the application of capsaicin, dihydrocapsaicin and bradykinin: All neurons sensitive to bradykinin and capsaicin were also activated by dihydrocapsaicin, although the latency and duration of excitatory effects of capsaicin and dihydrocapsaicin differed from those of bradykinin.

Figure 1 shows a typical increase in firing rate of the same medial thalamic neuron following intra-arterial administration of capsaicin, dihydrocapsaicin and bradykinin in a dose of 3 μg. Increase in the number of spikes appeared with a latency of 7-8 sec after the application of bradykinin, whereas the latency of capsaicin and dihydrocapsaicin was about 1 sec. Although the duration of the action of bradykinin was longer than that of capsaicin and dihydrocapsaicin, there was no apparent difference between capsaicinoids and bradykinin regarding the peak frequency of this neuron. Similar observations were obtained in the other neurons which responded to these agents. The mean latency and duration of bradykinin were 8.7 and 12.5 sec, those of capsaicin were 1.2 and 6.1 sec and those of dihydrocapsaicin were 1.3 and 5.0 sec, respectively (Table 2).

| Stimuli                          | Number of Neurons | Percentage |
|---------------------------------|-------------------|------------|
| Noxious + non-noxious + cap + dihydrocap + brady | 26 | 46.4 |
| Noxious + non-noxious           | 2                 | 3.6        |
| Non-noxious + cap + dihydrocap + brady | 2 | 3.6 |
| Non-noxious                     | 14                | 25.0       |
| Not Driven                      | 12                | 21.4       |
| Totals                          | 56                | 100.0      |

Noxious: pinching skin with toothed forceps.
Non-noxious: Bending hairs with a blower and/or tapping superficial and deep tissues.
cap: capsaicin, dihydrocap: dihydrocapsaicin, brady: bradykinin.
FIG. 1. Effects of capsaicin, dihydrocapsaicin and bradykinin on spontaneous unit discharges in the medial thalamic single neuron. "Spikes" in Figs. 1, 3 and 4 are reshaped pulses produced by the spike height discriminator.

TABLE 2. The mean latency and duration for capsaicin, dihydrocapsaicin and bradykinin on medial thalamic single neurons

| Drug (3 μg i.a.) | Latency Mean ± S.E. (sec) | Duration Mean ± S.E. (sec) |
|------------------|--------------------------|---------------------------|
| Capsaicin        | 1.17 ± 0.12*             | 6.11 ± 1.11*              |
| Dihydrocapsaicin | 1.31 ± 0.15*             | 5.04 ± 0.94*              |
| Bradykinin       | 8.73 ± 0.76              | 12.50 ± 1.06              |

*: Significantly different from bradykinin, Student's t-test, p<0.01.

Results are the mean and standard error of 22 neurons.

We previously reported that activation of the neurons in the medial thalamus with bradykinin and capsaicin was inhibited by the intravenous administration of morphine (3). In the present work, the intravenous administration of morphine (2 mg/kg) also decreased the number of dihydrocapsaicin-induced discharges of the medial thalamic single neurons and this inhibition was antagonized by the intravenous administration of naloxone (0.4 mg/kg) (Fig. 2). In contrast, the activity of the medial thalamic single neuron with non-noxious stimuli (hair and/or tapping) was not affected by morphine or naloxone (Fig. 2). Similar results were obtained in the other 5 nociceptive neurons in the 6 trials. These results indicate that the pain-conducting fibers were selectively activated by dihydrocapsaicin as well as by bradykinin and capsaicin.

Tachyphylaxis: It has been reported that repeated intra-arterial application of capsaicin induced a tachyphylaxis on muscle fibrillation in the rat (1). Similar results were obtained in the medial thalamic single neurons in cats.

When capsaicin was injected into the femoral artery 3 times at intervals of about 20 sec, tachyphylaxis was apparent (Fig. 3). When each administration was given at intervals greater than 3 min, tachyphylaxis did not occur, whereas it was apparent at 1 min intervals. In general, about one half of these neurons responded to hair blowing and tapping but not
pinching during the tachyphylaxis induced by capsaicin. Similar results were obtained by repeated administration of dihydrocapsaicin (Fig. 4).

**DISCUSSION**

We (3) previously showed that the intra-arterial administration of capsaicin excites the neuronal activity of medial thalamic neurons, as these particular neurons play an important role in pain perception (3, 8–10). In the present study, we found that dihydrocapsaicin, one variety of the pungent substance contained in Capsicum annuum L., also has selective action on the nociceptive neurons in the medial thalamus and its response modalities.
are much the same as those of capsaicin. In addition, it was noted that administration of morphine markedly inhibited the dihydrocapsaicin-induced activity of the medial thalamic single neurons. This finding substantiates the data of our preliminary experiments (in which the neuronal activity of the medial thalamus induced by painful stimuli such as intra-arterial administration of bradykinin and pinching was selectively blocked by the administration of morphine) (3, 8). These results indicate that capsaicin and dihydrocapsaicin may exert their effects on the pain-conducting fibers, in a similar manner.

A long latency (about 5-20 sec) is required to activate the pain sensitive neurons in the spinal cord, the brain stem or the medial thalamus by bradykinin (3, 8, 11, 12). In the present work, the mean latency of bradykinin was significantly longer than those of capsaicinoids (Table 2). This phenomenon demonstrates that the mechanism of action in the excitation of pain-conducting fibers by bradykinin differs from that of capsaicinoids.

Jancsó et al. (13) reported that the subcutaneous administration of capsaicin to newborn rats produces a selective degeneration of chemosensitive primary afferents of the spinal cord. Other recent studies have demonstrated that capsaicin induces a release of substance P from sensory afferents (14, 15). In our study, we found that when capsaicin and dihydrocapsaicin were repeatedly administered within a short time (about 20 sec), the activity of the medial thalamic neurons was significantly reduced and returned to control responses within 5-10 min after the last administration. It is probable that the mechanism of tachyphylaxis can be explained by the reversible desensitization of pain-receptors and the transient depletion of substance P from pain-conducting fibers.

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