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Capsaicin Pretreatment Interferes with the Thermoregulatory Effect of Morphine[1]

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SZIKSZAY, M , G BENEDEK AND F OBAL, JR *Capsaicin pretreatment interferes with the thermoregulatory effect of morphine* PHARMACOL BIOCHEM BEHAV **18**(3) 373–378, 1983 —Body temperature changes after the administration of 8 mg/kg morphine sulphate were studied in freely-moving and restrained rats, which were pretreated with capsaicin (300 mg/kg) Morphine caused a hyperthermic response irrespective of the previous capsaicin treatment in freely-moving rats the lag time from the application of morphine to the hyperthermic maximum was, however, significantly delayed in capsaicin pretreated rats A careful habituation to the experimental procedure (including injections, taking temperature, etc) facilitated the hyperthermic response both in the pretreated and the control groups Restrained rats typically showed a hypothermic response to the same dose of morphine The drop of body temperature in habituated animals was significantly larger in the case of capsaicin pretreated rats This potentiation of the hypothermic effect of morphine can be regarded as the primary site of interaction between capsaicin and morphine Since capsaicin pretreated rats also showed an exaggerated thermoregulatory response to experimental stress, we conclude that the thermoregulatory effects of morphine and endogenous opiates are facilitated after capsaicin pretreatment

Morphine Capsaicin Thermoregulation Stress Habituation

JANCSÓ and Jancsó-Gábor reported that the analgesic effect of morphine in tail-withdrawal test could be depressed by previous capsaicin treatment [9] This finding lent support to speculations concerning to the possible interaction between capsaicin-sensitive and opiate-sensitive elements in the central nervous system

As it is known both morphine and capsaicin exert a powerful action on the nociception and the thermoregulation as well [11] As to the thermoregulatory reactions, morphine administration causes either hypothermia or hyperthermia in rats, depending on the dose, degree of restraint, ambient temperature, etc [6, 16, 18, 19, 28, 31] A small dose of capsaicin (1–10 mg/kg) results in a fall of body temperature by exciting warm receptors in the preoptic region and at the periphery [13,14] Hyperthermic reactions after capsaicin administration have also been reported [13, 14, 24] Large doses of capsaicin (50–300 mg/kg) induce ultrastructural damages in the preoptic region [25] and severe irreversible thermoregulatory disturbances in warm environment [20,21] Besides the impaired adaptation to heat, capsaicin pretreated rats show an enhanced sensitivity towards emotional stress [13] Since morphine tolerant rats also show a similar enhanced sensitivity towards emotional stress [2] and a lessened survival capacity to heat stress [32], the question arises whether there are certain common features in the behavioural effects of capsaicin and morphine Consequently, capsaicin pretreatment can be supposed to affect the thermoregulatory reactions to morphine

The aim of our study was to test the thermoregulatory effects of morphine in the case of capsaicin pretreated rats Special care was given to determine the role of emotional stress induced by the experimental procedures including handling, administering injections and taking temperature [1] Our results show that both previous capsaicin treatment and one week habituation to the experimental circumstances can markedly changed the thermoregulatory reactions to morphine or physiological saline injections

METHOD

CFY male rats of the Sprague-Dawley strain were used (body weight 359±11 3 g) Capsaicin (Merck) was injected subcutaneously in increasing doses (20, 30, 50, 100 and 100 mg/kg) on five consecutive days The drug was dissolved as described earlier by Jancsó *et al* [12] The effect of morphine sulphate (8 mg/kg SC) on the body temperature was tested at least one month after the capsaicin treatment

The thermoregulatory reaction was tested with 16 groups of rats (Table 1) which were divided according to the following four aspects (1) Primarily all the animals were divided into two main groups The first group had been habituated to the experimental procedure for six days before testing the thermoregulatory reactions The habituation included housing in individual cages, daily injections with physiological saline solution and taking the temperature 10–18 times a day according to the same protocol as used on the experimental

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TABLE 1
DESCRIPTION OF THE EXPERIMENTAL GROUPS

Group	Experience in experimental stress	Pretreatment	Treatment	n	Baseline temperature T_c (°C)
Experiments with freely-moving animals					
1	naive	—	saline	22	37 13 ± 0 08
2	naive	capsaicin 300 mg/kg	saline	24	36 89 ± 0 08
3	naive	—	morphine 8 mg/kg	23	36 57 ± 0 07
4	naive	capsaicin 300 mg/kg	morphine 8 mg/kg	21	36 70 ± 0 11
5	habituated	—	saline	5	37 08 ± 0 03
6	habituated	capsaicin 300 mg/kg	saline	5	36 70 ± 0 02
7	habituated	—	morphine 8 mg/kg	5	37 42 ± 0 01
8	habituated	capsaicin 300 mg/kg	morphine 8 mg/kg	5	36 56 ± 0 06
Experiments with restrained animals					
9	naive	—	saline	9	37 48 ± 0 16
10	naive	capsaicin 300 mg/kg	saline	9	37 03 ± 0 13
11	naive	—	morphine 8 mg/kg	11	37 46 ± 0 09
12	naive	capsaicin 300 mg/kg	morphine 8 mg/kg	11	37 16 ± 0 09
13	habituated	—	saline	9	37 21 ± 0 21
14	habituated	capsaicin 300 mg/kg	saline	9	37 07 ± 0 12
15	habituated	—	morphine 8 mg/kg	20	37 51 ± 0 14
16	habituated	capsaicin 300 mg/kg	morphine 8 mg/kg	19	37 09 ± 0 11

day The second group (naive animals) was not handled except a repeated temperature probing on the day before morphine/saline administration (2) The second aspect was the degree of restraint during the thermoregulatory tests The experiments were performed both with freely-moving animals and with restrained ones (3) The third aspect was whether the animals were pretreated with capsaicin or not (4) Finally, the fourth aspect was the injection of morphine or physiological saline applied on the experimental day Each rat was tested only once

For the test period the animals were housed individually at an ambient temperature of 22±1 0°C A light-dark schedule of 12–12 hr was maintained Food and water were not available during six hours of temperature measurement Body temperature (T_c) was taken by a thermometer probe inserted 6 5 cm deep into the colon On the experimental day, T_c was taken three times at 15 min intervals, the mean of these records served as a baseline value for each rat The restrained animals were kept in special wire-mesh cages which prevented them turning around They had been restrained for an hour before the injection Morphine sulphate was administered at 9 00 a m and T_c was taken then repeatedly as indicated in the figures Control rats were injected with physiological saline in the same way The results are presented as the mean±standard error of means The mean latency of the hypo- and hyperthermic maxima was also calculated by taking into account the individual recordings Statistical significance was calculated by Student's *t*-test

RESULTS

Experiments with Freely-Moving Rats

Subcutaneous treatment with 8 mg/kg morphine sulphate resulted in an elevation of the body temperature both in the case of capsaicin pretreated and of control rats The extent of the hyperthermia depended on the previous habituation to the experimental stress situation In the case of naive animals without any previous habituation procedure morphine treatment produced about a 2°C rise in the body temperature irrespective of capsaicin pretreatment (Fig 1) The maximum temperature response of the capsaicin pretreated rats was, however, considerably delayed in time after the application of morphine the temperature rise reached its maximum in 142±9 minutes in the case of control rats, whereas the capsaicin pretreated animals showed their maximal response after 201±6 minutes (Table 2) There was a significant difference between the two groups in the body temperature reactions to saline injection, too Both groups showed rising body temperature after the injection, but the capsaicin pretreated rats responded to the injection and the temperature taking procedure with a significantly higher body temperature ($p<0$ 01) (Fig 1)

The development of hyperthermia after morphine injection was more pronounced in the case of those rats which were previously habituated to the handling procedure In these cases a rise of about 3°C was observed in the body temperature, irrespective of the capsaicin pretreatment (Fig 1) The hyperthermic maximum was, however, again significantly delayed in the case of the capsaicin-pretreated rats after habituation (Table 2) Habituation eventually abolished the differences previously found in the reactions of the two naive groups to saline injection (Fig 1) Neither group of habituated rats showed any significant thermoregulatory response to saline injection and temperature taking procedures

Experiments with Restrained Rats

Restraining the animals into small wire-mesh cages reversed the temperature responses of the rats to the same dose of morphine, i e , the application of morphine caused hypothermia in these rats (Fig 2)

TABLE 2

TIME LAG BETWEEN THE ADMINISTRATION OF 8 mg/kg MORPHINE SC AND ITS MAXIMUM EFFECT ON BODY TEMPERATURE

Group		Time of hyperthermic maximum in freely-moving rats mean±SEM (min)	Time of hypothermic maximum in restrained rats mean±SEM(min)
Naive rats	control	142 ± 9	68 ± 12
	capsaicin pretreated	201 ± 6*	93 ± 16
Habituated rats	control	111 ± 9	93 ± 7
	capsaicin pretreated	186 ± 6*	93 ± 5

*Significantly different from the control group at a level of $p<0.001$

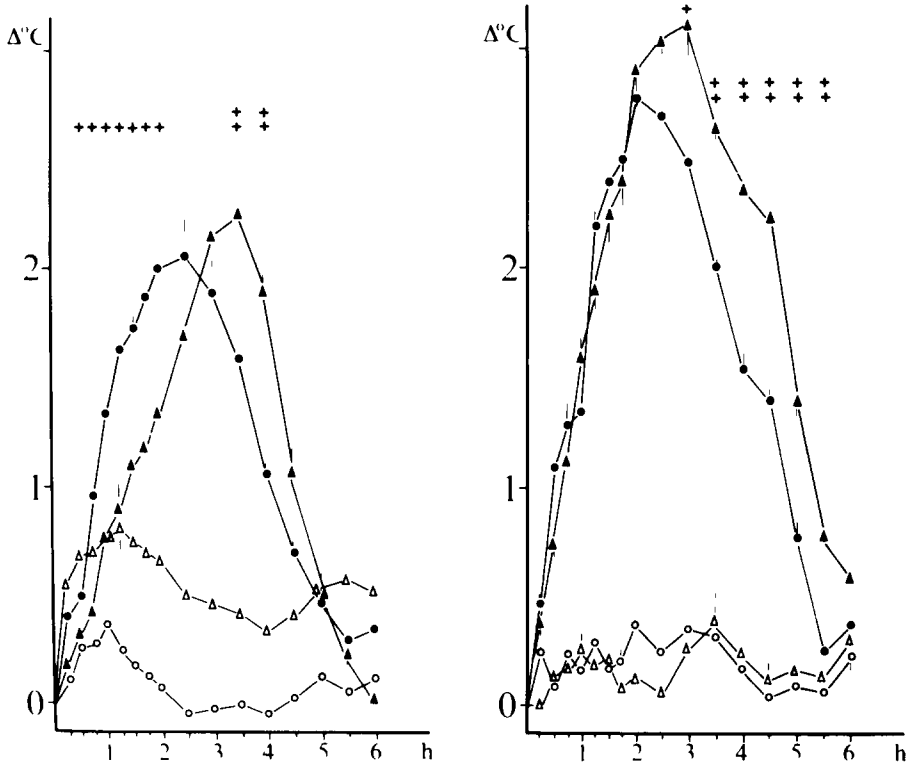

FIG 1 Effect of 8 mg/kg morphine sulphate on the body temperature of freely-moving rats Each value represents the temperature difference measured from the baseline temperature (see text), given as the mean±S E M Left side morphine effects on naive rats without previous habituation Right side morphine effects in habituated rats Symbols ▲–▲ morphine effects on capsaicin pretreated rats, ●–● morphine effects on control rats, △–△ physiological saline injection to capsaicin pretreated rats, ○–○ physiological saline injections to control rats The crosses denote significance levels between the morphine administered groups at a level of $^{+}p<0.02$, and $^{++}p<0.001$

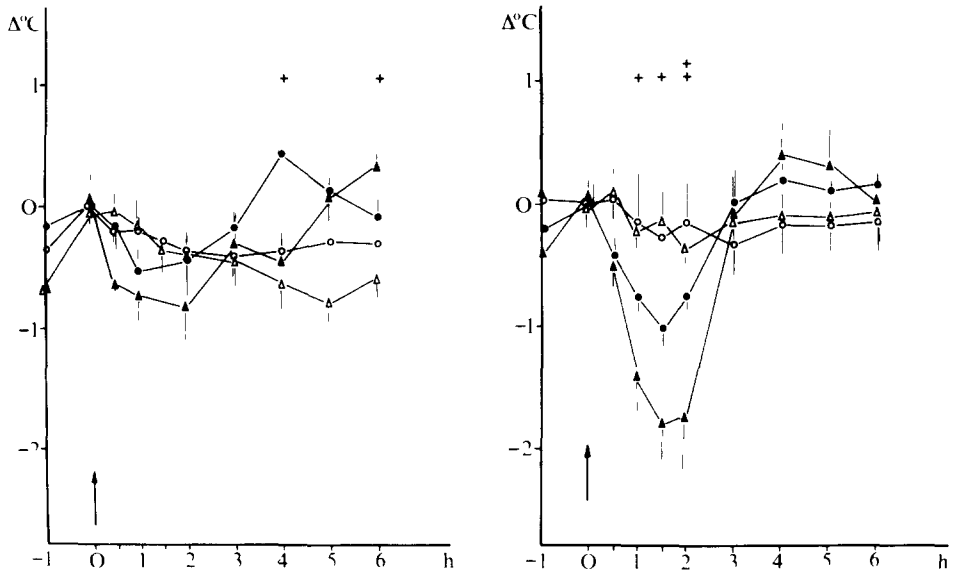

FIG 2 Effect of 8 mg/kg morphine sulphate on the body temperature of restrained rats The animals had been restrained for 1 hour before morphine was administered Left side morphine effects on naive rats, without previous habituation Right side morphine effects on rats after one week habituation to the experimental procedure For symbols see Fig 1

If naive animals were restrained, the intervention itself produced a rise of about 0 5°C in body temperature Morphine administration thereafter caused a temperature drop of about 0 9°C In these animals no significant differences were obtained between the reactions of capsaicin pretreated and control rats to morphine The differences were highly accentuated, however, if the animals were previously habituated to the experimental procedure The most striking consequence of the habituation was that the temperature responses of capsaicin pretreated and control rats to morphine were dissociated Morphine application caused a considerable decrease in the body temperature of capsaicin pretreated rats, the peak decrease was about 2 0°C The temperature drop observed in the case of control rats was significantly smaller than that seen in the case of capsaicin pretreated ones The administration of saline solution failed to induce any marked effect in habituated rats under these restrained conditions either Consequently, no differences were found between the reactions of the capsaicin pretreated and control rats to the saline injections

DISCUSSION

The principal finding of the present study is that capsaicin pretreatment strongly affects the thermoregulatory reactions of rats to morphine Although it is known that the thermoregulatory effect of morphine is highly dependent on ambient temperature [22], still no interaction between morphine and any agent which excites warm-sensitive elements has been described yet Capsaicin pretreatment changes the animals' reactions to morphine both in freely-moving and restrained conditions In the case of freely-moving animals a characteristic lag time was observed before the onset of the peak hyperthermic effect whereas under restrained conditions the capsaicin pretreatment greatly facilitated the hypothermic response to morphine, at least in the case of animals habituated to the experimental stress

It is interesting to compare this delayed hyperthermic effect of morphine with the thermoregulatory changes which accompany the establishment of morphine tolerance Gunne found a decreased time lag from morphine administration to the hyperthermic maximum in the case of morphine tolerant rats and an increasing time lag during the cessation of morphine tolerance [6] The decreasing and increasing time lag during the development and the cessation of morphine tolerance, respectively was attributed to a diminution and potentiation of an early though concealed hypothermic effect [6] The existence of this early hypothermic effect has been substantiated by several authors [19,31] Hence we suggest that the increased lag time of the hyperthermic effect observed in freely-moving capsaicin pretreated rats is the result of a potentiation of this early hypothermic effect Our experiments with restrained animals have revealed that this potentiated hypothermic effect does exist, although previous habituation to the experimental procedure is inevitably necessary for its manifestation On the basis of the present data we can conclude that capsaicin pretreatment acts primarily via the facilitation of the hypothermic effect of morphine whereas the changes observed in the hyperthermic response should be regarded as a secondary phenomenon This conclusion is in agreement with the generally accepted notion [16,19] that hypothermia is the primary thermoregulatory effect of morphine

The differences found between the response of the naive and habituated animals both to morphine and saline injections clearly show the involvement of stress-induced factors in the thermoregulatory responses, in agreement with the results of Stewart and Eikelboom [23] Endorphins also were reported to reduce the intensity of changes in body temperature due to warm or cold exposure [8,27] Hence endorphins which are liberated by the experimental stress, probably reduce the hyper- and hypothermic reactions to morphine of freely-moving and restrained rats respectively The more

pronounced reactions what we observed in the case of habituated rats should be due to a declining endorphin effect on repeating the stress procedure [3, 5, 17]

The rats which were pretreated with capsaicin showed an exaggerated sensitivity towards stress-induced thermoregulatory influences This is reflected in the case of capsaicin pretreated rats by the strongly facilitated emotional hyperthermia following saline injection and in the case of naive, restrained rats by the concealment of the potentiated hypothermic response Since the thermoregulatory consequences of an emotional stress were proven to be naloxone-sensitive [1], this observation can be regarded as an indirect evidence for a potentiated thermoregulatory effect of endogenous opiates in capsaicin pretreated rats

As far as the biochemical bases of the altered reaction of capsaicin pretreated rats are concerned, only fragmentary data are available Apart from the severe alterations in the substance P and somatostatin content of the spinal cord [7,33], no change in the peptide content of the forebrain has been substantiated [4,33] There is some evidence concerning the possible involvement of the 5-HT system in the thermoregulatory effects of capsaicin treatment [26,30] It is interesting to note that an increased adenylate cyclase activity was found in the preoptic region of capsaicin treated rats [10] Since both the adenylate cyclase activity [15] and the 5-HT system [22,29] are closely affected by morphine treatment, this suggests a possible interaction site between the thermoregulatory effect of morphine and capsaicin

Taking into account the present results and the finding that capsaicin pretreatment decreases the analgetic effect of morphine [4,9], we conclude that the thermoregulatory and analgesic effects of opiates are accomplished via different mechanisms which are oppositely affected by capsaicin Further studies on the interaction between opiates and capsaicin-sensitive mechanisms may therefore give an insight into the central opiate sensitive mechanisms

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