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Captopril and Capsaicin Modify Opioid Withdrawal in the Morphine-Dependent Rat

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SHARPE, L. G. AND J. H. JAFFE. *Captopril and capsaicin modify opioid withdrawal in the morphine-dependent rat.* PHARMACOL BIOCHEM BEHAV **33**(4) 899–902, 1989.—The involvement of neurokinins, especially substance P, in the opiate withdrawal syndrome was studied by treating rats with drugs that have been reported to increase (captopril) or decrease (capsaicin) tissue levels of substance P. Preliminary experiments with captopril (0.1, 0.3, 1 or 3 mg/kg, SC) showed that the 0.3 mg/kg dose enhanced some of the naloxone-precipitated withdrawal signs. Captopril alone had no effect in the morphine-dependent rat. On experimental days, either saline or captopril (0.3 mg/kg) was injected (SC) immediately before naloxone in morphine-dependent rats that were pretreated (4 to 10 days before the morphine pellet implantation) with either capsaicin (125 mg/kg, SC) or the capsaicin vehicle (N = 8 for each of 4 groups). Capsaicin treatment inhibited the following withdrawal signs: rhinorrhea, lacrimation and salivation. Captopril increased the occurrence of these secretory responses in vehicle-treated but not in capsaicin-treated animals. Other withdrawal signs were not altered by either captopril or capsaicin treatment. The results support the conclusion that substance P and related neurokinins may be involved in the expression of some signs of opioid withdrawal.

Captopril Capsaicin Naloxone-precipitated withdrawal Substance P Morphine

CAPTOPRIL is a potent and selective inhibitor of angiotensin-converting enzyme (ACE) (6). Substance P may be a substrate for ACE, as captopril potentiates substance P-induced salivation (4) and increases the level of substance P-like immunoreactivity in the CNS (10). Thus, aside from converting angiotensin I to angiotensin II (A-II), ACE may also degrade substance P in the brain and peripheral tissues (17,19).

We previously reported that substance P or related neurokinins may be released during naloxone-precipitated withdrawal because morphine-dependent adult rats which were pretreated as neonates with capsaicin showed less salivation, lacrimation and rhinorrhea during withdrawal than vehicle-pretreated controls (16). The purpose of the present study was to determine the effects of captopril and capsaicin (given alone or combined) on naloxone-precipitated withdrawal in the morphine-dependent rat. We hypothesized that if substance P is involved in any opioid withdrawal signs, captopril would aggravate them, whereas capsaicin would ameliorate the withdrawal and prevent the enhancing effects of captopril.

METHOD

Animals

Thirty-two male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN), weighing from 300–450 g, were caged in groups of 3 until the first day of treatment. Thereafter, they were housed individually in clear plastic cages (17 × 8 × 8 cm high). The animals had free access to food and water in a temperature- and humidity-controlled environment under a 12-hr light-dark cycle.

Naloxone-Precipitated Withdrawal Procedure

A 75 mg morphine pellet [NIDA, (20)] was implanted SC in animals anesthetized with halothane as previously described (5). Three days later, the rats were tested for naloxone-precipitated withdrawal. This regimen produces tolerance and a physical dependence peak after the third day of morphine-pellet implantation (5). The dose of naloxone used (0.5 mg/kg, SC) produces a moderate withdrawal such that increases or decreases of these signs by other treatments were readily detected (16). The following twelve withdrawal signs were scored: wet-dog shakes, penile licking, grooming, forepaw shakes, stretching, rhinorrhea, lacrimation, salivation, hyperactivity, teeth chattering, mouth movement and weight loss. The first 5 behaviors were scored each time they occurred. The remaining signs, except for weight loss, received a score of "1" if it occurred within a 15-min time period. Thus, in the 1-hr period, a maximum score of 4 was possible for each of these signs. The 3 secretory responses (rhinorrhea, lacrimation, salivation) were combined to yield a maximum score of "3" for a 15-min epoch (or a score of 12 for the 1-hr period).

Weight loss, used as an index of diarrhea, was expressed as percent body weight loss during a 75-min period after the naloxone injection.

Capsaicin Treatment

Capsaicin (125 mg/kg) was injected SC into 16 animals over 3 days. This dose of capsaicin produces a long-term depletion of substance P in several peripheral and central tissues (8). On the first day, the rats' responses on the hot-plate and tail-flick apparatus were determined. Before being anesthetized in a halothane-contained chamber, the rats were then injected with freshly prepared theophylline (5 mg/kg, IP) to reduce respiratory impairment caused by capsaicin (9) and then anesthetized with halothane. The rats were injected with capsaicin (25 mg/kg) while anesthetized and allowed to recover. On the second and third day, the animals were tested for capsaicin desensitization [see (3)] using the hot-plate, tail-flick, and the corneal chemical-irritation tests (see below). If antinociception was evident (compared with baseline and vehicle controls), theophylline and capsaicin (50 mg/kg) were administered again without anesthetizing the animal because the animals would display no signs of distress to the capsaicin treatment. The remaining 16 rats formed the control group and underwent experimental procedures identical to the capsaicin-treated rats except that they received the capsaicin vehicle (1 ml/kg of 10% ethanol–10% Tween 80 in 0.9% NaCl).

Capsaicin-Desensitization Tests

The hot-plate, tail-flick, and corneal chemical-irritation (CCI) tests were used to determine whether capsacin treatment reduced the response to heat and chemical irritants. In the hot-plate test, the animal was placed on the surface (55°C) of a commercially supplied hot-plate apparatus until it licked its hind paw. The animal was immediately removed, and the lick latency was recorded. If paw licking did not occur within 20 sec the animal was removed. The same animal was then tested on a commercially supplied tail-flick apparatus. The mid portion of the tail (2 to 3 mm) rested under a heat lamp activated by a foot pedal. The tail-flick latency was automatically recorded when the animal removed its tail from the heat. A cut-off limit of 8 sec was used. Only animals showing analgesia in the hot-plate and tail-flick tests participated in the CCI test, which consisted of touching the cornea with a cotton-tipped stick soaked in a solution of capsaicin (1.2%). A positive score was assigned if the animal wiped its eye within 10 sec. All vehicle-control animals received the CCI test on the first day of testing, but, thereafter, only one of the control animals was tested on any given day. The control animals that received the CCI test were alternated from day to day to minimize the number of animals that experienced distress from the test. Capsaicin-desensitization tests were repeated every day just prior to morphine pellet implantation (4 to 10 days after vehicle or capsaicin treatment).

Experimental Testing Procedure

Preliminary experiments were conducted on another group of 32 rats in which 4 doses (0.1, 0.3, 1 and 3 mg/kg) of captopril (8 animals per dose) were used to test its effect on naloxone-precipitated withdrawal. Only the 0.3 mg/kg dose significantly increased naloxone-precipitated withdrawal and was therefore the dose of captopril used in the next series of experiments. Three days after morphine pellet implantation, rats were injected with saline or captopril (0.3 mg/kg, SC) 15 min before naloxone such that four separate groups of 8 animals each were formed: capsaicin vehicle + saline; capsaicin vehicle + captopril; capsaicin + saline; and capsaicin + captopril. The animals were then tested for naloxone-precipitated withdrawal as described above. The 15-min interval between captopril and naloxone was used to test the effects of captopril alone. Four animals, one from each of the four groups, were scored for withdrawal signs simultaneously. The animals were tested only once and no more than eight animals were tested in one day. One of the two observers was blind as to the group to which the animals belonged. Placebo-pelleted controls were omitted because in previous experiments naloxone did not affect animals implanted SC with placebo pellets for 3 or 6 days (16).

Drugs

Naloxone (NIDA) and captopril (SQ14,225, generously supplied by The Squibb Inst.) were dissolved in saline (0.9%). Capsaicin (Fluka AG) was suspended in a solution of 10% ethanol–10% Tween 80 in 0.9% NaCl.

Statistical Analysis

A 2×2 factorial design ANOVA was used to analyze the effects of the pretreatments (vehicle or capsaicin), treatments (saline or captopril) and their interactions on naloxone-precipitated withdrawal. Student's t-test was used for post hoc analysis among the four groups (capsaicin vehicle + saline, capsaicin vehicle + captopril, capsaicin +saline and capsaicin + captopril).

RESULTS

In both the preliminary and capsaicin experiments, captopril had no effect on behavior during the 15-min observation period before the naloxone injection. Only the 0.3 mg/kg dose of captopril produced a statistically significant increase from saline control in secretory responses of naloxone-precipitated withdrawal ($p<0.05$).

All capsaicin-treated animals showed evidence of capsaicin desensitization (Table 1). Their hot-plate and tail-flick latencies were significantly increased over baseline values at the time of morphine pellet implantation ($p<0.05$) and each responded negatively to the CCI test. None of the vehicle-treated animals showed signs of analgesia or desensitization in the 3 tests. During naloxone-precipitated withdrawal, only the secretory responses showed statistically significant pretreatment, treatment and interaction effects: $F(1,28)=28.4$, ($p<0.01$), 6.6 ($p<0.05$) and 9.1 ($p<0.01$), respectively (Fig. 1).

DISCUSSION

The results of this study support the suggestion that the release of substance P or related neurokinins may occur in some tissues during morphine withdrawal. Only the secretory responses of the opioid abstinence syndrome (salivation, rhinorrhea and lacrimation) appear to be involved, as indicated by previous work in our laboratory (16). Substance P is a potent sialogogue (13) and captopril (IV) potentiates substance P-induced salivation in the rat (4). Capsaicin, on the other hand, acts on small afferent sensory neurons in a way that leads to a long-term depletion of substance P from neural and nonneural elements [see (7,15)]. Substance P is present in tissues of the eye, the salivary glands and the nasal mucosa (11,14) and is depleted by capsaicin in the nasal mucosa of several species (13). As reported previously (16), we found that capsaicin-treated rats had significantly fewer abstinence signs of salivation, lacrimation and rhinorrhea than the vehicle controls. Depletion of substance P in these tissues may account for the reduced secretions and for the absence of enhancement by captopril in the capsaicin-treated animals. Additional support for this

TABLE 1

CAPSAICIN-INDUCED DESENSITIZATION AS MEASURED BY THE HOT-PLATE (HP), TAIL-FLICK (TF) AND CORNEAL CHEMICAL-IRRITATION (CCI) TESTS

		Baseline (day of capsaicin treatment)			Day of Morphine Pellet Implant (4–10 days after capsaicin or vehicle)		
		Latency (sec)			Latency (sec)		
Treatment	N	HP	TF	CCI no.	HP	TF	CCI no.
Vehicle	16	7.7 ± 0.4	3.7 ± 0.2	8/8	7.8 ± 0.7	3.6 ± 0.2	8/8
Capsaicin	16	8.1 ± 0.5	3.6 ± 0.3	8/8	17.5 ± 1.1*	7.1 ± 0.6*	0/16†

*$p<0.01$ compared with capsaicin vehicle, Student's t-test.
†$p<0.001$ compared with capsaicin vehicle, chi-square test.

conclusion is that all capsaicin-treated animals displayed capsaicin desensitization, a condition which, to some extent, is related to substance P depletion [see (3)].

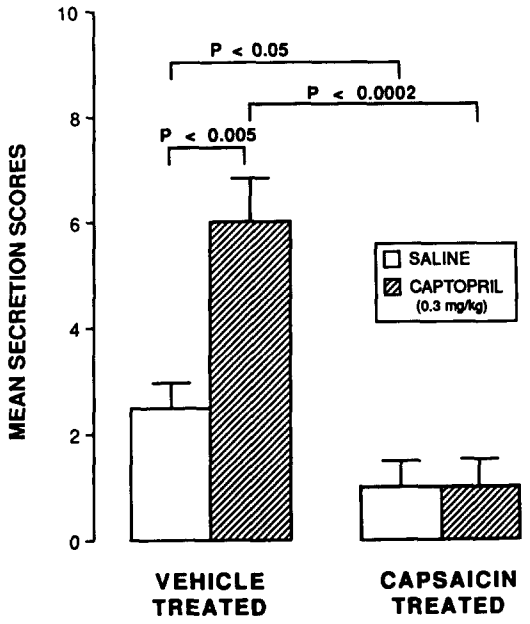

FIG. 1. Capsaicin treatment (125 mg/kg, IP) reduced naloxone-precipitated withdrawal of secretory responses (rhinorrhea, lacrimation and salivation) and prevented the captopril (0.3 mg/kg) enhancement of secretory withdrawal in morphine-dependent rats. N = 8 for each group.

Previous studies have shown that, in rats treated with capsaicin (either as neonates, 50 mg/kg, or as adults, 300 mg/kg), the number of naloxone-precipitated wet-dog shakes was increased over that in rats treated with the vehicle (16,18). However, we did not observe such an increase in the present study, perhaps because a lower dose of capsaicin (125 mg/kg) was used.

There appear to be limitations for using captopril and capsaicin as pharmacologic probes to study the role of substance P and other neurokinins in the opioid withdrawal syndrome. The effects of both drugs on naloxone-precipitated withdrawal are mediated by a peripheral site of action located only in fibers and tissues related to the secretory elements of salivation, rhinorrhea and lacrimation. Although capsaicin penetrates the blood-brain barrier, it does not appear to influence substance P levels in several brain areas such as the midbrain, hypothalamus, striatum, cortex and cerebellum nor in the gut [see (3,7)]. The latter finding could explain why weight loss (an index of diarrhea) after naloxone did not differ between the groups treated with capsaicin and capsaicin vehicle. Apparently, captopril does not penetrate the blood-brain barrier adequately. Injected in the cerebral ventricles, but not systemically, captopril increases substance P-like immunoreactivity in several brain sites (10). In addition, [^{3}H]captopril binds to several brain sites with high ACE activity but where endogenous A-II and A-II receptors are lacking (17). Therefore, the principal substrate for ACE in these sites may be substance P. In this regard, there are indications to suggest that the release of substance P in the CNS may play an important role in the expression of several opioid withdrawal signs (1,2). However, other techniques and/or pharmacologic tools that would alter simultaneously both the central and peripheral levels of substance P may be required to better understand the role of substance P in the opiate withdrawal syndrome.

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