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Sex Differences in the Antinociceptive Effects of the Enkephalinase Inhibitor, SCH 34826

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KAVALIERS, M. AND D. G. L. INNES. *Sex differences in the antinociceptive effects of the enkephalinase inhibitor, SCH 34826.* PHARMACOL BIOCHEM BEHAV **46**(4) 777–780, 1993.—The effects of endogenous opioid peptides are limited by proteolytic enzymes such as endopeptidase 24.11 ("enkephalinase"), which cleaves the Gly-Phe bonds in Met- and Leu-enkephalin. SCH 34826 {(*S*)-*N*-[*n*-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]carbonyl]-2-phenylethyl]-L-phenylalanine-B-alanine} is a potent, highly specific, enkephalinase inhibitor that has marked analgesic effects in laboratory rodents. The present study compared the effects of SCH 34826 on nociception and restraint stress-induced opioid analgesia in reproductive adult male and female deer mice, *Peromyscus maniculatus*. SCH 34826 had significantly greater antinociceptive actions and facilitatory effects on stress-induced analgesia in male than female mice. These antinociceptive effects of SCH 34826 were reduced by the general opioid antagonist naloxone and completely blocked by the specific delta opioid receptor antagonist, ICI 174,864, and nonsignificantly affected by the mu and kappa opioid receptor antagonists, β-funaltrexamine and nor-binaltorphimine, respectively. These results show that there are sex differences in the effects of the enkephalinase inhibitor, SCH 34826, on opioid-mediated antinociception and that these sex differences are associated with delta opioid mechanisms.

Sex differences Opioid analgesia Stress-induced analgesia Enkephalinase Endopeptidase 24.11

SEVERAL peptidases are involved in the metabolism of opioid peptides (2,3,13,18,19). Endopeptidase 24.11 (trivial name "enkephalinase"), which cleaves the Gly-Phe bonds in both Met- and Leu-enkephalin, is considered to have a critical role in the functioning of enkephalinergic systems (18,19). Recently, SCH 34826 {(*S*)-*N*-[*n*-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]carbonyl]-2-phenylethyl]-L-phenylanyl-B-alanine}, along with its active in vivo de-esterified constituent, SCH 32615 {*N*-[*l*-(-1-carboxy-2-phenyl)ethyl]-L-phenylalanyl-B-alanine}, were shown to potently, and selectively, inhibit enkephalinase activity in mammals (3,4). SCH 34826 was demonstrated to have potent and long-lasting, naloxone-reversible, antinociceptive effects in male laboratory mice and rats (4,14), consistent with the involvement of enkephalinase in the modulation of opioid-mediated analgesia.

There is also substantial evidence for sex differences in central opioid systems and the expression of opioid-mediated nociception and analgesia (1,11,12,16). Male rodents, including reproductive adult deer mice, *Peromyscus maniculatus*, display significantly greater levels of exogenous and endogenous opioid-mediated analgesia than females (7,8,11,12). As well, sex differences have been shown in the numbers and distribution of central opioid receptors in rodents (6,20). Recently, sex differences have also been reported in the effects of two purported peptidal endogenous antagonists or modulators of opioid activity (9,10). This raises the possibility of sex differences in a variety of components associated with the regulation of opioid activity. Whether or not these sex differences extend to enkephalinase and its modulation of opioid activity is, however, not known. Accordingly, in the present study, we examined the effects of the enkephalinase inhibitor, SCH 34826, on nociception and opioid-mediated restraint stress-induced analgesia in adult deer mice. In addition, we evaluated the effects of the prototypic opioid receptor antagonist, naloxone, the delta opioid receptor antagonist, ICI 174,864 (5), the mu opioid receptor antagonist, β-funaltrexamine (21), and the kappa opioid receptor antagonist, norbinaltorphimine (15,22), on the antinociceptive effects of SCH 34826.

METHOD

Experimental Animals

Sexually mature male and female deer mice, *Peromyscus maniculatus artemisiae* (20–30 g, 12–24 months of age), were housed as mixed sex pairs in polyethylene cages provided with Beta-chip bedding and cotton nesting material at 20 ± 2°C under a 14L : 10D cycle, with food (Purina Rat Chow 5012)

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and water freely available. Females were of mixed estrous phase with no pregnant animals being used.

Experimental Procedures

At midphotophase, separate groups of male and female mice (n = 5, in all cases) received intraperitoneal (IP) injections of either the enkephalinase inhibitor SCH 34826 (SCH; 3, 10, and 30 mg/kg) or isotonic (0.9%) saline vehicle (10 ml/kg). Additional groups of male mice (n = 5, in all cases) received IP injections of either the prototypic opioid antagonists, naloxone (1.0 mg/kg; Sigma, St. Louis, MO), the mu opioid receptor antagonist, β-funaltrexamine (20 mg/kg; Research Biochemical Inc., Natick, MA), the delta opioid receptor antagonist, ICI 174,864 (1.0 mg/kg; Research Biochemical Inc.), the kappa opioid receptor antagonist, nor-binaltorphimine (5.0 mg/kg; Research Biochemicals Inc.), or the saline vehicle (10 ml/kg; saline + DMSO for the delta and kappa antagonists) prior to receiving either SCH (10 mg/kg) or saline vehicle (10 ml/kg). Naloxone, ICI 174,864, and saline were injected 30 min before, nor-binaltorphimine 2 h before, and β-funaltrexamine 24 h before the SCH or saline treatments.

Other groups of mice were restrained individually for 30 min in sealed translucent 50-ml polypropylene centrifuge tubes (10 × 2.5 cm; Corning, NY). The tubes, which were provided with air vents, restricted the activity of the animals such that the mice were effectively immobilized. Additional male and female mice (n = 5, in all cases) were handled but not immobilized. The immobilization and handling procedures were preceded by IP injections of either SCH (3 and 10 mg/kg) or saline vehicle (10 ml/kg).

Nociceptive responses of the mice were determined prior to the antagonist, SCH, and saline injections, as well as 15, 30, and 60 min after the treatments. Nociception was also measured immediately before and 30 min after immobilization, injections, or handling. Nociception was measured as the latency of a front foot-lifting response to an aversive thermal stimulus (50 ± 0.5°C, Omnitech Analgesia Meter, Columbus, OH). The doses and time courses of the opioid receptor antagonists were established in prior (7,8) and pilot studies. Results of previous studies had also shown that maximum analgesia was evident 30 min after restraint (7).

Data were analyzed with separate (basal, drug, restraint) mixed design (pre-/posttest × male/female × treatment) analysis of variance. The Student-Newman-Keuls multiple range test was used for post hoc comparison of means. The significance level for hypothesis testing was set at 0.05.

RESULTS

Basal Nociception

There were no significant sex differences in the basal response latencies of the individual groups of mice, though in all cases males displayed greater basal thermal response latencies than females (Figs. 1, 2).

Male and female mice treated with SCH were analgesic, displaying significantly ($p < 0.001$, in both cases) greater thermal response latencies than before treatment or those administered saline vehicle (Figs. 1, 2). SCH induced a dose-dependent analgesic effect, with the doses of 10 and 30 mg/kg of SCH inducing significantly greater effects than 3.0 mg/kg (Fig. 1). In all cases, maximum analgesia was evident 30 min after treatment and declined to basal levels by 90 min postinjection (Fig. 2 for 10 mg/kg). There were also sex differences in these responses, with male mice displaying significantly ($p < 0.01$, at 30 and 60 min) greater levels of SCH-induced analgesia than females. The magnitude of the SCH-induced analgesia in males, relative to their basal response latencies, was significantly ($p < 0.01$, at 30 min) greater than that of the females relative to their baselines.

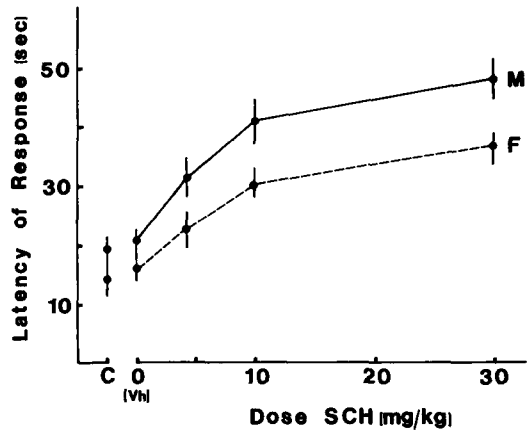

FIG. 1. Effects of IP injections of either the enkephalinase inhibitor, SCH 34826 (SCH; 3, 10, and 30 mg/kg) or saline vehicle (0 SCH, Vh; 10 ml/kg) on the thermal (50 ± 0.5°C) response latencies of adult male (M) and female (F) deer mice. Nociceptive responses were determined 30 min after injection as well as prior to injection. Representative preinjection response latencies (C) of the vehicle-injected mice are shown. N = 5, in all cases. Vertical lines denote the SEM.

Pretreatment with ICI 174,864 completely blocked ($p < 0.01$, for male mice) the antinociceptive effects of SCH (10 mg/kg) (Fig. 3). At 30 min postinjection, there were no significant differences between the response latencies of SCH + ICI 174,864- and control vehicle-injected mice. Naloxone (1.0 mg/kg) pretreatment also significantly reduced the analgesic effects of SCH, though the nociceptive responses of the SCH + naloxone were greater than those of just the vehicle-treated mice (Fig. 3). Neither β-funaltrexamine nor nor-binatorphimine had any significant effects on SCH-induced analgesia at 30 min postinjection. None of the opioid receptor antagonists

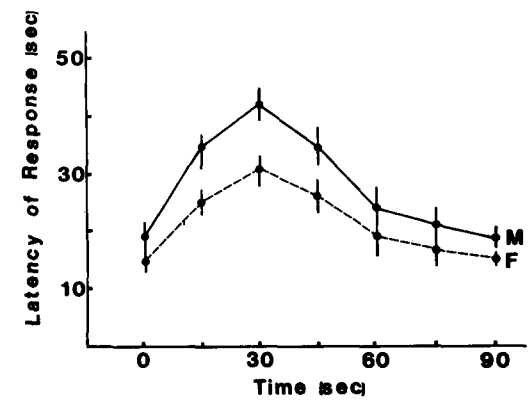

FIG. 2. Time course of the effects of IP injections of the enkephalinase inhibitor, SCH 34826, on the thermal (50 ± 0.5°C) responses of adult male (M) and female (F) deer mice. Response latencies prior to injection (time 0) are also shown. N = 5, in all cases. Vertical lines denote the SEM.

had any significant effects on basal nociceptive sensitivity of the male and female deer mice (not shown).

Restraint Stress-Induced Analgesia

Mice that had been restrained were analgesic, displaying significantly ($p < 0.01$) greater thermal response latencies than before restraint (Fig. 4). There were sex differences in the levels of restraint-induced analgesia, with males displaying significantly ($p < 0.05$) greater analgesia. The time courses of the analgesic responses were, however, equivalent, with both sexes displaying maximum analgesia immediately after the 30 min of restraint.

Administration of SCH significantly ($p < 0.01$, for 10 mg/kg SCH)), and in a dose-dependent manner, potentiated the levels of restraint-induced analgesia, with 10 mg/kg of SCH having a significantly ($p < 0.05$) greater effect than 3.0 mg/kg. There were also sex differences in these effects of SCH, with male mice displaying a significantly ($p < 0.05$) greater increase in, and final level of, restraint stress-induced analgesia, relative to their baseline, than the female mice.

DISCUSSION

The results of the present study with deer mice show that there are significant sex differences in both opioid-induced analgesia and the effects of the enkephalinase inhibitor, SCH 34826, on opioid analgesia. Consistent with the results of previous studies (8,9), it was found that reproductive male deer mice displayed significantly greater levels of opioid-mediated, restraint stress-induced analgesia than female mice. It was further observed that the enkephalinase inhibitor, SCH 34826, both induced and potentiated endogenous opioid-mediated analgesia in a sexually dimorphic manner, having significantly greater antinociceptive effects in male than female mice.

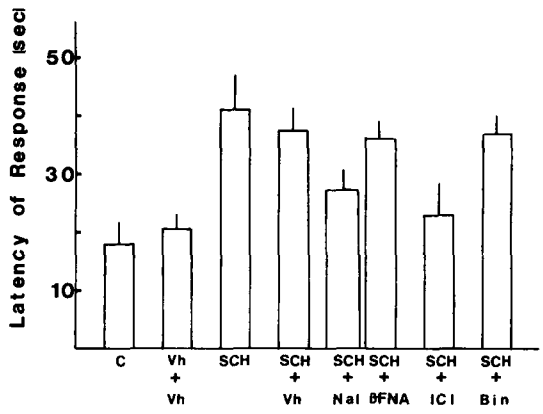

FIG. 3. Effects of IP injections of the enkephalinase inhibitor, SCH 34826 (SCH; 10 mg/kg) on the thermal (50 ± 0.5°C) response latencies of adult male deer mice. The effects of IP pretreatment with either the prototypic opioid antagonist, naloxone (Nal; 1.0 mg/kg; SCH + Nal), the mu opioid receptor antagonist, β-funaltrexamine (B-FNA; 20 mg/kg; SCH + B-FNA), the delta opioid receptor antagonist, ICI 174,864 (ICI; 1.0 mg/kg; SCH + ICI), the kappa opioid receptor antagonist, nor-binaltorphimine (Bin; 5.0 mg/kg; SCH + Bin), or the saline vehicle (Vh; 10 ml/kg; SCH + Vh) on the nociceptive responses of SCH (10 mg/kg)-treated mice are also shown. In addition, the nociceptive responses of just Vh-treated mice (Vh + Vh) and control untreated (C) animals are shown. Response latencies were determined 30 min after SCH or vehicle injection. Naloxone, ICI-174,864, and saline vehicle were injected 30 min before, nor-binaltorphimine 2 h before, and β-funaltrexamine 24 h before the SCH injection. $N = 5$, in all cases. Vertical lines denote the SEM.

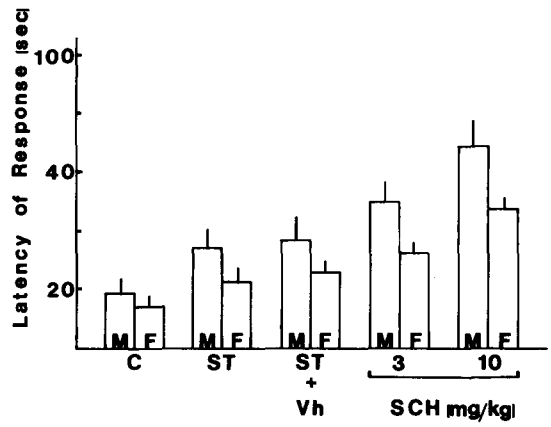

FIG. 4. Effects of IP injections of the enkephalinase inhibitor, SCH 34826 (SCH; 3 and 10 mg/kg) or saline vehicle (Vh; 10 ml/kg) on stress-induced analgesia in male (M) and female (F) deer mice. The nociceptive (50 ± 0.5°C thermal response latencies) of stressed mice that received no injections (ST) are also shown. Injections were given immediately prior to the 30-min immobilization period, with nociceptive sensitivity recorded at the termination of restraint. The response latencies of control (C) mice that received no restraint or injections are also shown. $N = 5$, in all cases. Vertical lines denote the SEM.

Treatment of deer mice with SCH 34826 resulted in significant, dose-dependent antinociceptive effects, similar to those previously reported from adult laboratory rodents (3,4,14). In addition, SCH 34826 potentiated naloxone-sensitive, restraint stress-induced analgesia. In both cases, reproductive male mice displayed significantly greater SCH-induced responses than females. These findings with SCH 34826 agree with and extend the sex differences that are evident in exogenous and endogenous opioid-mediated analgesia, as well opioid modulatory systems (1,7-13). The present findings do, however, also raise the possibility of sex differences in enkephalinase activity and its role in opioid metabolism.

The results of the present study with males, and in pilot studies with female mice, further show that the analgesic effects of SCH 34826 were reduced by the general opioid antagonist, naloxone, and completely blocked by the delta opioid receptor antagonist, ICI 174,864, with the kappa opioid receptor antagonist, nor-binaltorphimine, and the mu opioid receptor antagonist, β-funaltrexamine, having only weak, nonsignificant, antagonistic effects. This suggests that the antinociceptive effects of SCH 34826 are specific for delta opioid receptors and involve Met- and/or Leu-enkephalin. This is consistent with the specificity of endopeptidase 24.11 (enkephalinase) for the metabolism of Met- and Leu-enkephalin (2,18,19).

Sex differences in opiate effects, whereby adult male rodents have been shown to display greater analgesic effects than females (1,7,11,12), have been related to sex differences in the number and distribution of opioid receptors as well as levels of opioid peptides (1,6,20). The results of the present study with SCH are supportive of sex differences in the levels of and/or activity of delta opioid receptors and their ligands. These findings do not, however, preclude the possibility of there also being sex differences in the activity of SCH 34826-sensitive "enkephalinase" and/or of other endopeptidases involved in enkephalin metabolism. In this regard, the possibil-

ity of sex differences in the metabolism of SCH 34826, including its de-esterification to the active constituent SCH 32615, also cannot be excluded.

Sex differences in opiate effects, whereby males display greater antinociceptive responses than females, have been suggested to be mediated by gonadal steroids (11,12,16). There is evidence suggesting that opiate receptor activity and density can vary across the stages of the estrous cycle (17), though no such variation was evident in deer mice (7,8). Whether or not comparable sex differences and sensitivity to gonadal steroids exist in the levels and or activity of enkephalinase needs to be determined. As well, whether there are sex differences in the effects of, or responses to, other endopeptidases remains to be examined.

ACKNOWLEDGEMENTS

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