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Antinociceptive Effects of the Enkephalinase Inhibitor, SCH 34826, in the Snail, *Cepaea nemoralis*

LISA M. SAKSIDA,* LIISA A. M. GALEA† AND MARTIN KAVALIERS†[1]

*Department of Psychology, University of British Columbia, Vancouver, British Columbia, Canada V6TY 1Y7
and †Neuroscience Program, Department of Psychology and Division of Oral Biology,
University of Western Ontario, London, Ontario, Canada N6A 5C1*

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SAKSIDA, L. M., L. A. M. GALEA AND M. KAVALIERS. *Antinociceptive effects of the enkephalinase inhibitor, SCH 34826, in the snail, Cepaea nemoralis.* PEPTIDES **14**(4) 763–765, 1993.—In vertebrates the effects of endogenous opioid peptides are limited by proteolytic enzymes such as endopeptidase 24.11 (enkephalinase), which cleaves the Gly-Phe bonds in both methionine- and leucine-enkephalin. SCH 34826 {(*S*)-*N*-[*n*-[1-[(2,2-dimethyl-1,3-dioxolan-4yl) methoxy]carbonyl]-2-phenylethyl]-L-phenylalanine-B-alanine} is a potent, highly specific, enkephalinase inhibitor that has marked analgesic effects in mammals. The present study examined the effects of SCH 34826 on opioid-mediated aversive thermal (nociceptive) responses of an invertebrate, the land snail, *Cepaea nemoralis*. SCH 34828 had significant, dose-related antinociceptive effects in *Cepaea* that were reduced by naloxone and completely blocked by the specific delta opiate antagonist, ICI-174,864, and only weakly affected by the specific kappa opiate antagonist nor-binaltrophimine. These findings with SCH 34826 suggest that an enkephalinase similar to that in vertebrates is present and involved in the mediation of opioid (enkephalin) activity in the snail, *Cepaea*.

Mollusc Nociception Antinociception Enkephalinase Enkephalin degradation Opioids Snail

ALTHOUGH several peptidases are involved in the metabolism of opioid peptides, endopeptidase 24.11 (trivial name enkephalinase), which cleaves the Gly-Phe bonds in both methionine- and leucine-enkephalin, is considered to have a critical role in the functioning of enkephalinergic systems in vertebrates (1,18,19). 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 in vivo de-esterified constituent, SCH 32615 {*N*-[*l*-(-1-carboxy-2-phenyl)ethyl]-*l*-phenylalanyl-B-alanine}, were demonstrated to potently, and selectively, inhibit enkephalinase activity in mammals (2,3,16). SCH 34826 was shown to have potent and long-lasting, naloxone-reversible, antinociceptive effects in both rodents and primates (3,16).

Opioid peptides, such as methionine-enkephalin, and their receptors are also present in invertebrates (9,13,14). Endogenous opioid systems have been indicated to be similarly involved in the mediation of a number of biological processes in both vertebrates and invertebrates (6–12,14,21). Opioid peptides have been implicated in the modulation of the thermal avoidance behaviors (nociceptive responses) of gastropod molluscs. Morphine, as well as more specific opiate agonists, can enhance the latency of responses of the land snail, *Cepaea nemoralis*, to a thermal stimulus in a manner analogous to the analgesic or antinociceptive responses reported in mammals, while exposure of *Cepaea* to aversive or potentially aversive environmental stimuli leads to the increased activity of endogenous opioids and the display of naloxone-sensitive analgesia similar to that evident in vertebrates (8,9,12).

There is also evidence for enkephalin-degrading activity and enzymes in mollusc and arthropod hemolymph (4,15). Whether or not these substances and their effects are equivalent to that of vertebrate enkephalinase is, however, not known. Accordingly, in the present study we examined the effects of the enkephalinase inhibitor, SCH 34826 (SCH), on the nociceptive responses of the snail *Cepaea*. In addition, we describe the effects of the prototypic opiate antagonist, naloxone, the specific delta opiate antagonist, ICI-174,864 (5), and the specific kappa opiate antagonist, nor-binaltorphimine (17), on the SCH-induced responses of *Cepaea*.

METHOD

Experimental Animals

Snails were collected locally and maintained under a 12-h light:12-h dark cycle at 22 ± 2°C. The snails were fed ad lib on

[1] Requests for reprints should be addressed to M. Kavaliers, Division of Oral Biology, Faculty of Dentistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.

natural vegetation and lettuce. Water was freely available at all times.

Assessment of Nociception

As the activity of gastropods is affected by their state of hydration (20), all snails were allowed to fully hydrate under a saturated atmosphere at 22 ± 2°C for 15 min before being tested. Following hydration, individual snails were placed on a warmed surface (hot plate, Omnitech Electronics, Columbus, OH) at 40 ± 0.5°C and the latency of their avoidance, or more appropriately withdrawal, behavior to the thermal stimulus was determined. The withdrawal behavior was a characteristic elevation of the anterior portion of the fully extended foot (11). The behavioral end point was the time at which the foot reached its readily discernible maximum elevation. After displaying this nociceptive response, individual snails were quickly removed from the thermal surface. The appropriateness of the use of the term nociception for describing this behavioral response of *Cepaea* is discussed in Kavaliers (9).

This foot-lifting behavior is not observed in snails exposed to temperatures normally present in their natural habitat, but becomes evident as the experimental temperature is raised to 40°C (12). The test temperature of 40°C was chosen because it exceeds the temperatures present in the habitats from which the snails were collected (10).

Experimental Procedures

At midphotophase, seperate groups of hydrated snails (n = 25, in all cases) were injected with either the enkephalinase inhibitor SCH 34826 (SCH; 0.01 and 0.10 μg/2.0 μl saline) or 0.9% saline vehicle (2.0 μl). Thermal response latencies of the snails were determined prior to and 15, 30, 60, and 90 min after injection.

Other groups of hydrated snails (n = 10, in all cases) were injected with either the prototypic opiate antagonist, naloxone (1.0 μg/1.0 μl; Sigma, St. Louis, MO), the specific delta opiate antagonist, ICI-174,864 (1.0 μg/1.0 μl; Research Biochemical Inc, Natich, MA), the specific kappa opiate antagonist, nor-binaltorphimine (1.0 μg/1.0 μl; Research Biochemicals Inc.), or saline vehicle (1.0 μl) prior to receiving either SCH 34826 (0.10 μg/1.0 μl) or saline vehicle (1.0 μl). Naloxone, ICI-174,864, and saline were injected 15 min prior to, and nor-binaltrophinmine 2 h before, the SCH or saline treatments. Thermal response latencies of hydrated snails were determined prior to the antagonist, SCH, and saline injections, as well as 15, 30, and 60 min after the latter treatments. The doses and time courses of the opiate antagonists were established in prior (11) and pilot studies.

Solutions were injected with a 5.0-μl microsyringe (Hamilton, NV) into the side of the foot, in either the vicinity of, or directly in, the mantle cavity into the hemocoel. Injections were made on the basis of 1.0 g body mass. Body masses of snails without shells ranged from 0.7 to 1.3 g. Control and prior determinations had shown that the injections had no evident effects on the activity levels of the snails. Ongoing motor fuctions, including vertical and horizontal movements, climbing, and related muscular activity, as well as feeding, were also not adversely affected by the drug treatments. This indicates that SCH 34826 at the doses used did not have any apaprent confounding motor debilitating effects in *Cepaea*.

Data were analyzed by repeated measures analysis of variance and Newman-Keuls test with the significance level for hypothesis testing set at 0.05.

RESULTS

A stereotyped elevation of the anterior portion of the fully extended foot was observed in all fully hydrated snails that were exposed to the 40°C surface temperature. Adminstration of SCH 34826 caused a significant, $F(1, 91) = 16.55$, $p < 0.001$, increase in the latency of the foot-lifting response, indicative of the induction of analgesia (Fig. 1). Maximum antinociceptive effects of SCH 34826 were evident 15–30 min after administration, $F(4, 68) = 4.76$, $p < 0.01$, with a decline to basal levels by 60 min after treatment. SCH 34826 had dose-related actions, with at 15 min postinjection 0.10 μg having a significantly ($p < 0.05$) greater antinociceptive effect than 0.01 μg (Fig. 1). There were no significant differences between the basal preinjection response latencies or values recorded 60 min after treatment. Saline injections has no significant effects on the thermal response latencies.

Pretreatment with ICI-174,864 completely blocked ($p < 0.01$) the antinociceptive effects of SCH (Fig. 2). There were no significant differences between the response latencies of ICI (SCH + ICI)- and saline control (saline + saline)-treated snails at 15 and 30 min postinjection. Naloxone pretreatment also reduced the antinociceptive effects of SCH, though the response latencies of the naloxone (SCH + naloxone)-treated snails were still significantly ($p < 0.05$) greater at 15, though not at 30, min postinjection than those of saline-treated (saline + saline) snails. After treatment with nor-binaltorphimine, a significant ($p < 0.05$) reduction in thermal response latencies was evident only at 30 min after injection. Saline pretreatments had no significant effects on SCH-induced antinociception. Treatment with either ICI-174,864, naloxone, nor-binaltorphimine, or saline had no significant effect on basal thermal response latencies.

DISCUSSION

The results of the present study show that the enkephalinase inhibitor, SCH 34826, has antinociceptive or analgesic effects in the snail *Cepaea*. Treatment with SCH resulted in significant, dose-related, and relatively prolonged increases in the latency of the aversive thermal responses of *Cepaea*. The effects of SCH were reduced by naloxone and completely blocked by the highly specific opiate antagonist, ICI 174,864, and only weakly affected by the specific kappa opiate antagonist, nor-binaltorphimine.

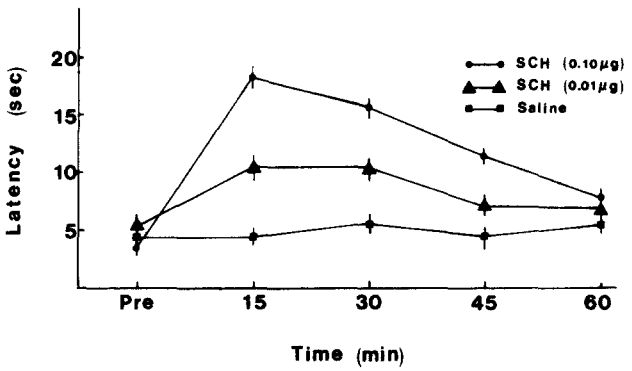

FIG. 1. Effects of the enkephalinase inhibitor, SCH 34826 (SCH; 0.01 and 0.10 μg) or the saline vehicle (2.0 μl) on the thermal (40°C) thermal response latencies (nociceptive responses) of individual hydrated snails. Response latencies were determined prior to (pre) and after injection. n = 25 in all cases. Vertical lines denote SEM.

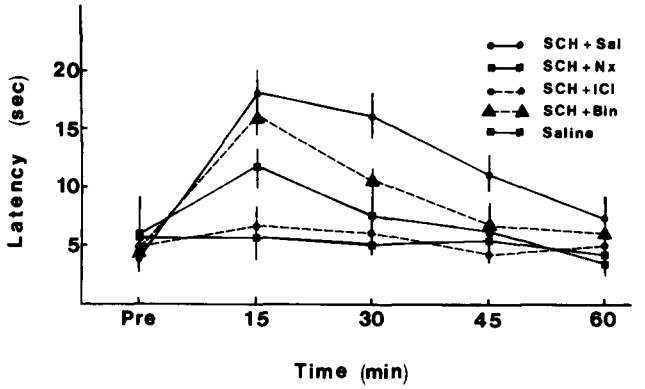

FIG. 2. Effects of pretreatment with either naloxone (Nx; 1.0 μg), the delta opiate antagonist ICI-174,864 (ICI; 1.0 μg), the kappa opiate antagonist nor-binaltorphimine (Bin; 1.0 μg), or saline vehicle (Sal; 1.0 μl) on the thermal (40°C) response latencies (nociceptive responses) of individual hydrated snails injected with the enkephalinase inhibitor SCH 34826 (SCH; 0.10 μg). Response latencies of snails receiving just saline treatments (saline; 2.0 μl) are also shown. Naloxone, ICI-174,864, and saline were injected 15 min prior to, and nor-binaltorphimine 2 h before, SCH treatment. Response latencies were determined prior to (pre) and after SCH or saline treatment. n = 10 in all cases. Vertical lines denote SEM.

This indicates that the antinociceptive effects of SCH 34826 in *Cepaea* involve delta opioid receptors and likely the delta opioid ligands Met- and/or Leu-enkephalin. This is consistent with the isolation of enkephalins and their receptors in molluscan tissue, and the report of Met-enkephalin peptidases in molluscan hemolymph (13–15). The relatively small and delayed effect of nor-binaltorphimine may reflect either weak, nonspecific effects of SCH and/or interactions between kappa and delta opiate mechanisms in *Cepaea*. Whether or not these opiate antagonists have comparable effects on SCH-induced analgesia in vertebrates remains to be determined.

The present findings with *Cepaea* are, however, consistent with the analgesic effects of SCH 34826 and SCH 32615 in mammals (2,3,16). These analgesic effects in *Cepaea*, along with their blockade by a specific delta opiate antagonist, agree with and extend the data indicating that SCH 34826 produces its analgesic effects in mammals through a blockade of enkephalinase and the augmentation of endogenous enkephalin activity.

There is mounting evidence for the presence of multiple receptor types and endogenous opioid peptides similar to those in vertebrates in molluscs and other invertebrates (6–12,21). The present results suggest that an enkephalinase similar to that in vertebrates is present and involved in the regulation of opioid metabolism and actions in *Cepaea*. These findings provide further support for a phylogenetic continuity in underlying mechanisms and components associated with the regulation of opioid activity.

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