Pharmacology Biochemistry & Behavior Vol 24, pp 561–566 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Mu- and Kappa-Opiate Agonists Modulate Ingestive Behaviors in the Slug, *Limax maximus*

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Received 11 March 1985

KAVALIERS, M , R W RANGELEY, M HIRST AND G C TESKEY *Mu- and kappa-opiate agonists modulate ingestive behaviors in the slug*, Limax maximus PHARMACOL BIOCHEM BEHAV **24**(3) 561–566, 1986 — Administration of the prototypical mu opiate agonist, morphine sulphate, 1–10 mg/kg, produced over three hours a significant dose-dependent increase in the ingestive responses of free-feeding slugs, *Limax maximus*, although lower doses, 0 10 mg/kg, attenuated feeding The mixed mu and kappa opiate agonist, ketocyclazocine hydrochloride, in the dose range 1 0–10 mg/kg, also induced significant increases in food consumption With both of these opiates there was a latency of about 0 5 hr before initiation of feeding The more specific kappa opioid agonist, U-50,488H, given over the dose range 0 10–1 0 mg/kg, produced a more potent increase in three hour food consumption by *Limax*, whereas a dose of 10 mg/kg produced a significant increase in ingestive responses for 3–4 hr after a 1–2 hr period of inactivity The prototypic mu opiate antagonist, naloxone hydrochloride (1 0 mg/kg) blocked the feeding effects of morphine and ketocyclazocine and reduced the effects of U-50,488H The delta antagonist, ICI 154,129, in a dose of 10 mg/kg, reduced the effects of morphine as well as decreasing food intake of free-feeding slugs These results indicate that activation of differential opiate receptors in invertebrates has similar effects on feeding behavior as occur in mammals, suggesting early evolutionary development of opioid involvement in the control of feeding

Feeding Mu opioid Delta opioid Morphine Naloxone Kappa opioid ICI 154,129 U-50,488H Slug *Limax maximus* Evolution

A substantial body of evidence supports the hypothesis that endogenous opioid peptides participate in the control of appetite Several opiate agonists are known to induce feeding and it is probable that opioid peptides are involved in the physiological regulation of mammalian ingestive behaviors and food intake [8, 16–20, 24, 30] Recent studies have suggested that opiate systems may also participate in the regulation of ingestive behaviors of invertebrates Peripheral administration of the prototypic mu opiate agonist, morphine sulfate [15] stimulates food intake in the slug, *Limax maximus* [9], in a manner similar to that found in mammals [20] In both *Limax* and mammals the morphine-induced feeding effects could be blocked by low doses of the prototypic mu opiate antagonist, naloxone hydrochloride [9,20] Naloxone by itself caused a significant decrease in feeding of food deprived individuals [1, 2, 9] These similarities in feeding responses of mammals and slugs suggest an early evolution of opiate involvement in the mediation of ingestive behaviors

As multiple opioid peptides and differing opioid receptors have been recognized [15], interest has focused on determining which of the opioid receptors are involved in the control of feeding behaviors There is data to indicate that opiates affecting delta, kappa, mu and sigma opiate receptors can influence mammalian feeding [20] The results from a number of investigations have suggested that the endogenous kappa opioid peptide, dynorphin, and the kappa opiate receptor may have specific roles in the control of mammalian ingestive responses of food intake [7, 17–20] Evidence has also been presented to suggest that under certain circumstances the mu and/or other opioid receptor(s) affected by morphine may inhibit food [20] intake rather than stimulating ingestion It has become evident that morphine can act as a relatively non-specific opioid agonist affecting both mu and delta receptors [15, 21, 22] The relative roles of delta opioids in the determination of feeding are largely unknown [20], though there is some evidence to suggest that they stimulate food intake in rats [29] The present study was undertaken to determine whether kappa opiates influence food intake in *Limax*, as well as to examine in more detail the effects of morphine, mu and delta opioid receptors on ingestion in these animals As the temporal profiles of the effects of var-

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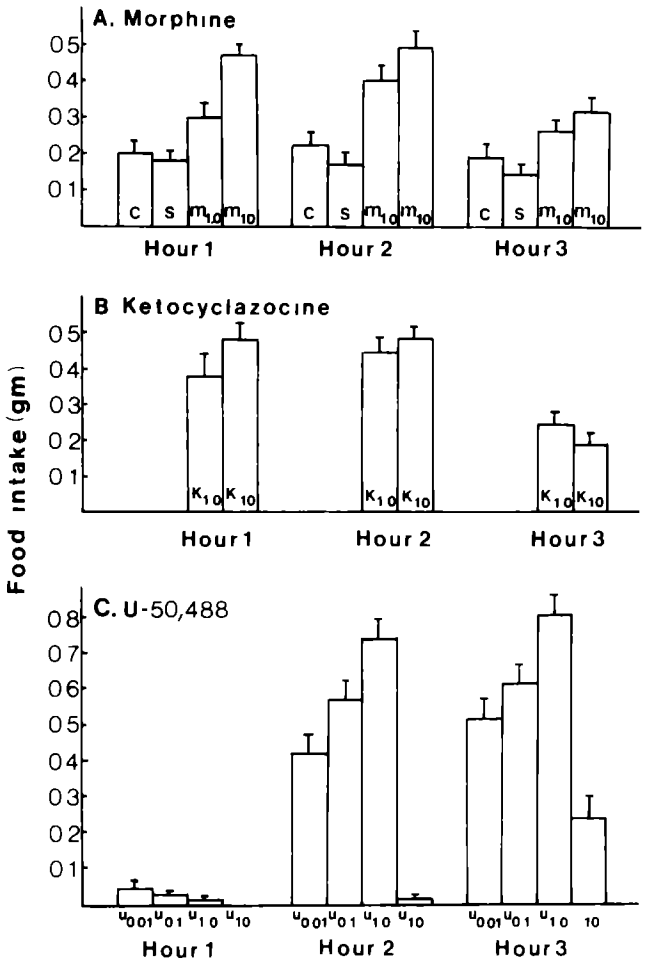

FIG 1 A–C Food intakes over three hours of free-feeding slugs injected intramuscularly with A The mu opiate agonist, morphine sulfate (m_1 and m_{10}, 1 0 and 10 mg/kg/10 ml, respectively), B The mixed kappa-mu opiate agonist, ketocyclazocine hydrochloride (k_1 and k_{10}, 1 0 and 10 0 mg/kg/10 ml, respectively), and C The kappa opiate agonist, U-50,488H ($u_{0\,01}$, $u_{0\,10}$, u_{10}, 0 01, 0 10, 1 0 and 10 0 mg/kg/10 ml, respectively) S represents saline injected (10 ml/kg) and C represents control untreated slugs The latter two are the same for panels A–C N = 10 in all cases Vertical lines denote two standard errors of the mean

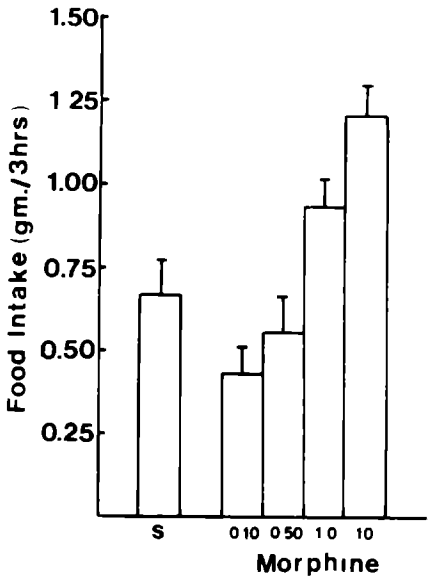

FIG 2 Dose dependent relations of the effects of intramuscular injections of morphine sulfate (0 10–10 mg/kg/10 ml) on the total three hour food intake of free-feeding slugs N = 10 in all cases Vertical lines denote two standard errors of the mean

ious opiates on ingestive behaviors in mammals vary with the type of opiate agonist employed [8, 9, 17, 20, 29, 31, 32], the time courses of mu and kappa opioid effects on feeding in *Limax* were also examined In this study we describe the effects of various doses of the preferential kappa-mu opiate agonist, ketocyclazocine [14,35], the highly selective kappa opiate agonist, U-50,488H (trans-3,4-dichloro-N-methyl-N[2-pyrolidinyl)cyclohexyl]-benzenecetamide methane-sulfonate) [33], the prototypic mu opiate agonist, morphine, the prototypic mu opiate antagonist, naloxone, the specific delta opioid antagonist, ICI 154,129 (N,N-Bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH) [5,25] on the food intakes of *Limax* In addition, the latencies and time courses of the ingestive effects of these opioid agonists and antagonists are presented

METHOD

Slugs (20–25 g) were field collected (Victoria, British Columbia) and maintained in the laboratory at 22±1°C under a 12 hr light 12 hr dark cycle (light, 25 μW cm^{-2}) They were communally housed in a glass aquarium (95×51×46 cm) that was provided with a soil substrate Animals were kept moist and had continous access to water, lettuce and other leafy vegetation Forty-eight hours prior to testing slugs were placed individually in 10 cm diameter petri dishes to which they were confined by 5 cm high plastic mesh sides and a plastic cover A substrate of lettuce was placed on the bottom of the petri dishes To ensure full hydration of the slugs a thin film of water was also maintained in each dish The transparent dishes were placed on an elevated platform, thus allowing direct visual determinations of the onset (latency) of feeding by the animals

Determinations were made of the effects of intramuscular (IM) injections of the mu opiate agonist, morphine sulfate (0 10, 0 50, 1 0 and 10 mg/10 ml/kg, B D H , Canada), the mixed kappa-mu opiate agonist, ketocyclazocine hydrochloride (1 0 and 10 mg/10 ml/kg, Sterling-Winthrop, NY) and the specific kappa opiate agonist, U-50,488H (0 01, 0 10, 1 0 and 10 mg ml/kg, Upjohn, MI), dissolved in saline, on the amounts of pre-weighed lettuce (2 0–3 0 g) ingested by the slugs One group of control animals received injections of saline (10 ml/kg) Uninjected slugs served as an additional control for the experimental procedures Measurements were made of the amounts of food consumed by individual slugs (n = 10, in all cases) each hour for three hours during the light period (0900–1200 hr) Measurements of the food intakes of the animals treated with U-50,488H were made over six hours Determinations were also made of the latencies of the initiation of concerted (greater than 5 min) feeding behavior and food intake in the injected animals At the end of each hour slugs were removed from the dishes and the remaining lettuce was blotted dry and weighed Any food adhering to

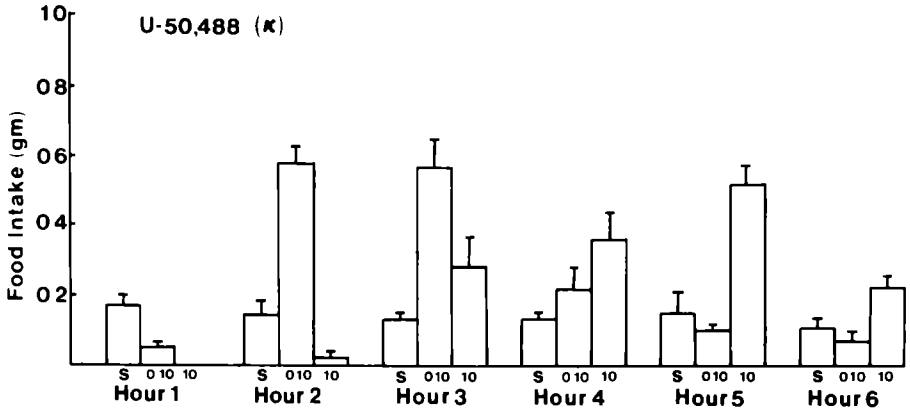

FIG 3 Effects of intramuscular injections of U-50,488H (0 10 and 10 mg/kg/10 ml) and saline (S, 10 ml/kg) on the hourly food intakes of free-feeding slugs over six hours N=5 in all cases Vertical lines denote two standard errors of the mean

the slugs was removed and corrected for in the hourly determinations of food intake

Determinations were also made of the effects of the mu opiate antagonist, naloxone hydrochloride (Endo Laboratories, NJ) and the delta opioid antagonist, ICI 154,129 (Imperial Chemical Industries, England) pre-treatments on the ingestive effects of the agonists Individual slugs (n=10, in all cases) received IM injections of either naloxone hydrochloride (1 0 mg/10 ml/kg), ICI 154,129 (10 mg/10 ml/kg) in saline, or saline control (10 ml/kg) before receiving morphine sulfate (10 mg/10 ml/kg, ketocyclazocine hydrochloride (10 mg/10 ml/kg), or U-50,488H (0 10 and 10 mg/ml/kg) An additional 5 animals received either saline and naloxone, saline and ICI 154,129, or either saline solutions of naloxone hydrochloride (0 10, 1 0 and 10 mg/ 10 ml/kg), or ICI 154,129 (10, 20 and 30 mg/10 ml/kg) alone Determinations were made of the latencies to initiation of feeding All data were analyzed by two-way analysis of variance

RESULTS

Opiate Agonist, Antagonist and Food Intake

Mu opiate agonists Administration of morphine sulfate resulted in a significant ($p<0$ 01, for 1 0 mg/kg), dose-dependent increase in the ingestive responses of slugs (Figs 1A, 2) The lowest dose, 0 10 mg/kg of morphine sulfate, caused a significant ($p<0$ 05) decrease in food consumption, as well as significantly ($p<0$ 05) decreasing the latency to the onset of feeding when compared to saline treated animals (Figs 2, 5) Maximal ingestive effects of morphine (1 0 and 10 mg/kg) occurred within 1–2 hr of administration, with there being a decline in food consumption in the third hour to basal levels

Mixed kappa-mu opiate agonists Administration of ketocyclazocine also resulted in a significant ($p<0$ 01, for 1 0 mg/kg) dose-dependent increase in the food consumption of slugs (Fig 1B) The ketocyclazocine treated slugs showed maximal ingestive responses within 1–2 hr after injection Food intake in slugs treated with 1 0 mg/kg of ketocyclazocine hydrochloride was significantly ($p<0$ 05) greater than that in slugs injected with an equivalent dose of morphine There were no differences in effect at the 10 0 mg/kg dosage Control determinations showed that the use of different portions and sources of lettuce had no apparent effects

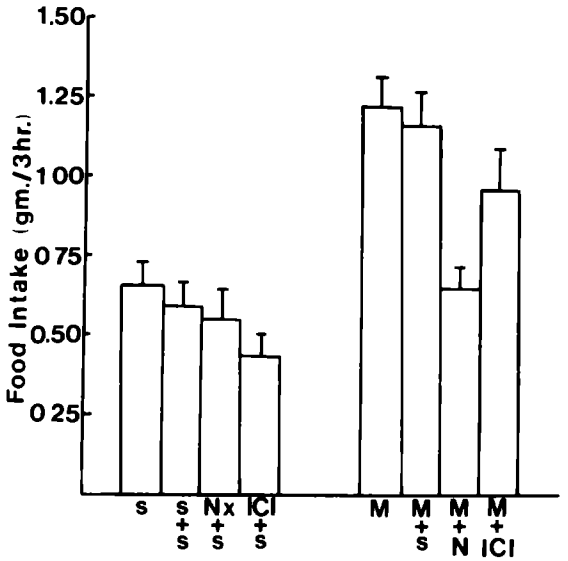

FIG 4 Effects of intramuscular injections of naloxone hydrochloride (Nx, 1 0 mg/kg/10 ml), ICI 154,129 (ICI, 10 mg/kg/10 ml) on the food intakes of saline (S, 10 ml/kg) and morphine sulfate (M, 10 mg/kg/10 ml) treated slugs N=10 in all cases Vertical lines denote two standard errors of the mean

on ingestive responses Responses were similar to that obtained with a homogenous carrot puree [9]

Kappa opiate agonists Low doses of U-50,488H, 0 01–1 0 mg/kg, caused a significant ($p<0.01$, for 0 10 mg/kg) dose-dependent enhancement of three hour food intake (Fig 1C) The total food consumption after treatment with 0 10 mg/kg of U-50,488H was not significantly different from that obtained with 10 mg/kg morphine sulphate, while that obtained with 1 0 mg/kg of U-50,488H was significantly ($p<0$ 01) greater Feeding was minimal during the first 1–1 5 hr after injection of U-50,488H, 0 01–10 mg/kg, but significant ($p<0$ 001, for 0 10 mg/kg) augmentations of food intake occurred during the later period (Figs 1C, 3, 5) for all but the highest dose In an extended study, slugs treated with 10 mg/kg U-50,488H displayed significantly ($p<0$ 01) enhanced food intake 4–6 hr after treatments, with food intake returning to basal levels by

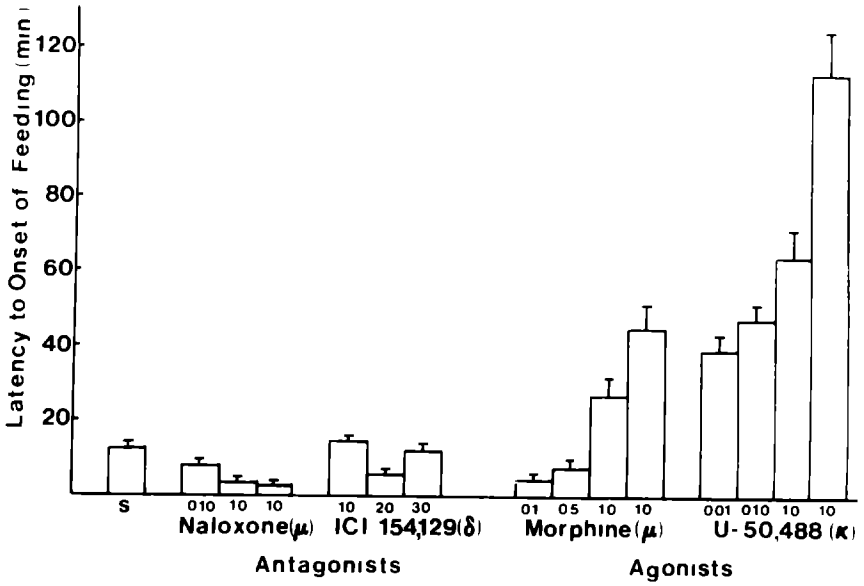

FIG 5 Latency to the onset of feeding of slugs receiving intramuscular injections of either saline (10 ml/kg), naloxone hydrochloride (0 10–10 mg/kg/10 ml), ICI 154,129 (10–30 mg/kg/10 ml), morphine sulfate (0 10–10 mg/kg/10 ml) or U-50,448H (0 01–10 mg/kg/10 ml) N=10 in all cases Vertical lines denote two standard errors of the mean

hour 7 (Fig 3) The effects of 0 10 mg/kg of U-50,488H on ingestive responses were non-significant by hour 4

Mu opiate antagonists Naloxone hydrochloride, 1 0 mg/kg, suppressed morphine (Fig 4) and to a lesser extent ketocyclazocine stimulated feeding The saline control treatment had no significant effects on either morphine- or ketocyclazocine-induced food intake Naloxone also significantly ($p<0$ 05) reduced, but did not block, U-50,488H (0 10 (mg/kg) induced food intake (not shown) This dose of naloxone, by itself, had no significant effects on the total or hourly food consumption of free-feeding animals (Fig 4)

Delta opiate antagonists ICI 154,129 significantly ($p<0$ 05, for 10 mg/kg) reduced the food intakes of free-feeding slugs and significantly ($p<0$ 05) reduced morphine-induced food intake (Fig 4) This effect of ICI 154,129 was significantly ($p<0$ 05) less than that obtained with naloxone

Opiate Agonist, Antagonists and Latency to Feeding

The latencies to initiation of concerted feeding after administration of different dosages of various opioid agonists and antagonists are shown in Fig 5 Naloxone hydrochloride, 0 10–10 mg/kg, caused a significant ($p<0$ 05, for 1 0 mg/kg) dose-dependent decrease in the time to the onset of feeding when compared to saline treatments ICI 154,129 had variable effects on latency, with only the dose of 20 mg/kg causing a significant ($p<0$ 05) decrease in feeding latency Morphine also had a significant effect on the initiation of feeding Low doses of morphine significantly ($p<0$ 05, for 0 10 mg/kg) increased the latency to feed After injection with morphine (10 mg/kg) there was a lag of 30–45 min during which the majority of animals did not feed However, all animals once they commenced feeding, injested large quantities of food such that their food intakes during the first hour of treatment were markedly greater than that of the saline controls. This effect is accentuated by the morphine-treated animals that showed a relatively short lag to the initiation of feeding Ketocyclazocine hydrochloride, at 1 0 and 10 mg/kg (not shown), caused increases in the latency of feeding response that were comparable to those observed after treatment with morphine The kappa agonist, U-50,488H, caused a significant ($p<0$ 01, for 0 01 mg/kg) dose-dependent increase in the latency to the initiation of feeding The latency to feeding observed after U-50,488 was significantly ($p<0$ 01, for 1 0 mg/kg) greater than that obtained with equivalent doses of morphine During this latency period the general locomotor behavior of the slug was considered to be reduced This type of marked behavioral inhibition was not observed after morphine or ketocyclazocine treatments

DISCUSSION

Evidence obtained from behavioral biochemical, electrophysiological and pharmacological investigations of the effects of opiates, their agonists and antagonists, in molluscs, suggests the existence of regulatory opioid systems that resemble those found in mammals [7, 12, 13, 14, 26–28] The present results provide further evidence of this similarity, demonstrating that mu and kappa opiate agonists known to stimulate feeding behavior in mammals can stimulate food intake in free-feeding *Limax* As shown previously using a carrot food source [9], administration of the preferential mu opiate agonist, morphine, resulted in significant dose-dependent (1 0–10 mg/kg) increases in the food consumption of free-feeding slugs Furthermore, the time courses of response, with 0 5 to 1 hr latencies to initiation of feeding by the slugs were analogous to latencies observed after peripheral administration of equivalent doses of morphine to rats and mice [8, 20, 30, 32] Surprisingly, however, a lower dose of morphine (0 10 mg/kg) had an anorexic effect in slugs, decreasing their food intake during the first hour after administration Although it is generally considered that morphine, given peripherally at doses of 1 0–10 mg/kg, stimulates feeding in mammals [20], there is evidence to indicate

that under certain situations mu opioids might in fact inhibit food consumption [20] The low dose of morphine also caused a significant decrease in the latency of the initiation of feeding by the slugs that was similar to that obtained after administration of the mu opiate antagonist, naloxone Since morphine has been shown to act at both mu and delta opiod receptors [15,22], it is possible that in slugs, the high and low doses of morphine are exerting their effects through opioid binding sites of different affinities Both high and low affinity opioid binding sites have been shown to be present in molluscan neural tissue [12,28] Results from mammalian studies have also suggested the existence of high and low affinity mu opioid receptors that may be involved in the mediation of different behavioral and physiological responses [21,22] The ability of both naloxone and the delta antagonist, ICI, 154, 129, to reduce morphine-induced feedings in slugs further supports the proposal that morphine may be exerting its effects at different opiate receptors Studies with rats have indicated that central administration of enkephalin analogues, which act at delta receptors, results in significant increases in food intake [29] Whether or not this occurs in *Limax* remains to be determined Nonetheless, the significant reduction in the food intake of free-feeding slugs obtained after administration of the specific delta opioid antagonist ICI 154,129 [5], in conjunction with the demonstration of the presence of leucine- and methionine-enkephalin in molluscan neural tissue [13], raises the possibility that delta opioid receptor activation is involved in the modulation of feeding in invertebrates A comparable inhibition of opioid-induced feeding following peripheral administration of ICI 154,129 has also been observed in mice [31] As the animals in the present experiment were well hydrated, it is not known whether water uptake accompanied feeding, analogous to the situation in mammals [10] There is some evidence, however, to suggest that endogenous opiates may also have independent stimulatory effects on mammalian drinking [19] It remains to be determined whether or not opioid systems have a role in the control of the fluid balance and water intake by *Limax* and other molluscs

The mixed kappa-mu agonist, ketocyclazocine [20,35], also had a stimulatory effect on the food intake of *Limax* over three hours similar to that reported in mammal [8, 19, 20] Ketocyclazocine hydrochloride at 1 0 mg/kg was significantly more potent than the same dose of morphine sulphate, while there were no differences in effect at the 10 0 mg/kg doses The duration of feeding response and latency in the initiation of feeding were also similar to those observed in mice [8] These dose-response relationships may arise from the mixed mu and kappa agonist actions of ketocyclazocine [15,35] In addition, low doses (0 01–1 0 mg/kg) of the more specific kappa agonist, U-50,488H [33], had a marked dose-dependent stimulatory effect on the feeding of *Limax* over three hours Over this dose range there was also a longer 1–1 5 hr lag before the initiation of feeding This potent effect on feeding is similar to that observed in mice and rats [16,31] Higher doses of U-50,488H (5–10 mg/kg) significantly attenuated feeding during the initial three hour measurement periods, while significantly increasing food intake during the next 2–3 hours This attenuation of food intake, which incorporates a prolonged time lag in the intiation of feeding, was characterized by immobility in treated animals In rats and mice there are similar 2–4 hr latencies of reduced activity before peripherally administered doses of 5 and 10 mg/kg of U-50,488H cause significant augmentations of food intake [16,31] These feeding effects of U-50,488H suggest that activation of the kappa opioid receptor is involved in the control of feeding in *Limax*

As in mammals [8, 17, 20], the preferential mu opiate antagonist, naloxone, blocked the increased ingestive effects of morphine and reduced the actions of ketocyclazocine and U-50,488H These responses further indicate effects at specific opiate receptors In addition, naloxone by itself decreased the food consumption of both 24 hr food deprived rodents [2] and slugs [9] Furthermore, administration of naloxone also resulted in a significant dose-dependent decrease in the latency of the initiation of feeding, while not affecting the total food intake of free-feeding slugs This raises the possibility that different components of the opioid system may be involved in the determination of appetitive food-seeking behaviors as compared to the mediation of food intake Similar decreases in the time to the onset of feeding have also been suggested to occur in rats treated with naloxone [11] In contrast, the delta antagonist, ICI 159,124, reduced food intake by free-feeding animals while over a higher dose range consistent effects on the latency to initiation of feeding were not obtained The lack of consistent effect may in part arise from partial agonistic actions that higher doses of delta antagonists are reported to show [3] The delta antagonist also reduced food intake in morphine-treated animals, while not having any effects on the actions of U-50,488H These results may be interpreted as reflecting differential effects of various opioid receptors on the initiation, duration and temporal patterning of feeding Taken together, the present results suggest that a complex mu, kappa and delta opioid mediation of ingestive responses similar to that proposed for mammals [20] may also exist in *Limax* Moreover, these findings also raise the possibility that mu and kappa opioid involvement in the modulation of feeding and possibly other crucial body functions may have a broad phylogenetic continuity

These findings provide evidence to suggest that activation of kappa, mu and delta opioid receptors is involved in the control of the ingestive responses and feeding behavior of *Limax* There is direct immunohistochemical evidence for the presence of enkephalins and β-endorphin in various ganglia in *Limax* [14] Dynorphin-like immunoreactive material has been reported in molluscs and other invertebrates [12], indicating the existence of a possible endogenous ligand for the kappa receptor As mentioned earlier, enkephalins could serve as ligands for other receptors It has been suggested that the control of ingestive behaviors in mammals involves an interaction between neuropeptides and monoamines [20] Both dynorphin and catecholamines have been shown to be involved in the control of feeding in rats [20] There is also substantial evidence for opioid-dopamine interactions [26] in molluscs Additionally, there is evidence for dopamine having a role in the initiation and modulation of feeding behavior in *Limax* [34] While the present study has not addressed the issue, there is the additional possibility of opioid-monoamine connections in feeding related activities across many species from invertebrates to mammals

ACKNOWLEDGEMENTS

We thank Dr P Von Voigtlander and the Upjohn Company for the gift of the U-50,488H, Dr P Cotton and Imperial Chemical Industries for the ICI 154,129 and Endo Laboratories for naloxone This research was supported by NSERC grant S222A1 to M K and MRC grant MA-7278 to M H

REFERENCES

1 Brands, B , J A Thornhill, M Hirst and C W Gowdey Suppression of food intake and body weight by naloxone in rats *Life Sci* **24.** 1772–778, 1979

2 Brown D R and S G Holtzman Suppression of deprivation-induced food intake and water intake in rats by naloxone *Pharmacol Biochem Behav* **11.** 5567–573, 1979

3 Dray, A and L Nunan Selective δ-opioid receptor antagonism by ICI 174,864 in the central nervous system *Peptides* **5** 1015–1106, 1984

4 Fitzsimons, T J and J Le Magnen Eating as a regulatory control of drinking in the rat *J Comp Physiol Psychol* **67.** 273–283, 1969

5 Gormley, J J , J S Morley, T Priestly, J S Shaw, M J Turnbull and H Wheeler In vivo evaluation of the opiate delta receptor antagonist ICI 154,129 *Life Sci* **31** 1263–1266, 1983

6 Kavaliers, M and M Hirst Evolutionary aspects of opiate involvement in thermoregulation In *Handbook of Comparative Aspects of Opioid and Related Mechanisms* edited by G R Stefano Florida CRC Press, 1986, in press

7 Kavaliers, M , G C Teskey and M Hirst The effects of aging on day-night rhythms of kappa opiate-mediated feeding in the mouse *Psychopharmacology (Berlin)* **87:** 286–291, 1985

8 Kavaliers, M and M Hirst The influence of opiate agonists on day-night feeding rhythms in young and old mice *Brain Res* **326** 160–167, 1985

9 Kavaliers, M , M Hirst, and G C Teskey Opioid-induced feeding in the slug, *Limax maximus* *Physiol Behav* **33** 765–767, 1984

10 Kissileef, H R Food-associated drinking in the rat *J Comp Physiol Psychol* **67·** 284–300, 1969

11 Kirkam, T C and J E Blundell Dual action of naloxone on feeding revealed by behavioral analysis separate effects on initiation and termination of eating, *Appetite* **5** 42–52, 1984

12 Kream, R M , R S Zukin and G B Stefano Demonstration of two classes of opiate binding sites in the nervous tissue of the marine mollusc *Mytilus edulis* *J Biol Chem* **225:** 9218–9224, 1980

13 Leung, M K and G B Stefano Isolation and identification of enkephalins in pedal ganglia of *Mytilus edulis (Mollusca)* *Proc Natl Acad Sci USA* **81** 955–958, 1984

14 Marchand, C R , P G Sokolove and M P Dubois Immunocytological localization of a somatostatin-like substance in the brain of the giant slug, *Limax maximus* L *Cell Tissue Res* **238** 349–358, 1984

15 Martin, W R Pharmacology of opioids *Pharmacol Rev* **35** 283–323, 1984

16 McLean, S and B G Hobel Feeding induced by opiates injected into the paraventricular hypothalamus *Peptides* **4·** 321–326, 1983

17 Morley, J E and A S Levine Involvement of dynorphin and the kappa opioid receptor in feeding *Peptides* **4** 797–800 1983

18 Morley, J E , A S Levine, M Grace and J Kniep An investigation of the role of kappa opiate receptor agonists in the initiation of feeding *Life Sci* **31** 2617–2626, 1983

19 Morley J E , A S Levine, M Grace and J Kneip Dynorphin-(1–13), dopamine and feeding in rats *Pharmacol Biochem Behav* **16** 701–705, 1982

20 Morley, J E , A S Levine, B A Gosnell and C I Billington Which opioid receptor mechanism modulates feeding? *Appetite* **5.** 61–68, 1984

21 Pasternak, G W B R Childers and S H Syder Opiate analgesia evidence for mediation by a subpopulation of opiate receptors *Science* **208.** 514–516, 1980

22 Pasternak, G W , A R Gintzler R A Houghten G S F Ling, R R Goodman, K Spiegel, S Nishimura, N Johnson and L D Recht Biochemical and pharmacological evidence for opioid receptor multiplicity in the central nervous system *Life Sci* **33** 167–173, 1983

23 Sanger, D J and P S McCarthy Differential effects of morphine on food and water intake in food deprived and freely-feeding rats *Psychopharmacology (Berlin)* **72** 103–108, 1980

24 Sanger, D J and P S McCarthy Increased food and water intake produced in rats by opiate receptor agonists *Psychopharmacology (Berlin)* **74.** 217–220, 1981

25 Shaw, J S L Miller, M J Turnbull and J S Morley Selective angatonists at the opiate delta-receptor *Life Sci* **31** 1263–1266, 1983

26 Stefano, G B Comparative aspects of opioid-dopamine interaction *Cell Mol Neurobiol* **2** 167–178, 1982

27 Stefano, G B , B Hall, M H Markham and B Dvorkin Opioid inhibition of dopamine release from nervous tissue of *Mytilus edulis* and *Octopus bimaculatus* *Science* **213** 928–930, 1980

28 Stefano, G B , R M Kream and R S Zukin Demonstration of stereospecific opiate binding in the nervous tissue of the marine mollusc *Mytilus edulis* *Brain Res* **181.** 445–450 1980

29 Tepperman F S and M Hirst Effect of interhypothalamic injection of (D-Ala^2,D-Leu^5) enkephalin on feeding and temperature in the rat *Eur J Pharmacol* **96** 243–249, 1983

30 Teskey, G C , M Kavaliers and M Hirst Social conflict activates opioid analgesic and ingestive behaviors in male mice *Life Sci* **35** 303–315, 1984

31 Teskey, G C and M Kavaliers Opioids and aggression influence of opioid agonists and antagonists on aggressive behaviour in mice *Soc Neurosci Abstr* **10** 1105, 1984

32 Thornhill, J A , M Hirst, and C W Gowdey Changes in diurnal temperature and feeding patterns of rats during repeated injections of heroin and withdrawl *Arch Int Pharmacol Ther* **223** 120–131, 1976

33 Von Voigtlander, P F R A Lahti, and J H Ludens U-50,488 A selective and structurally novel non-mu (kappa) opioid agonist *J Pharmacol Exp Ther* **224** 7–12, 1983

34 Wieland, S J and A Gelperin Dopamine elicits feeding motor program in *Limax maximus* *J Neurosci* **2·** 1735–1745 1983

35 Wood P L D Sanschagrin J W Richard and M Thakur Multiple opiate receptor affinities of kappa agonist/antagonist analgesics In vivo assessment *J Pharmacol Exp Ther* **226** 545–550 1981