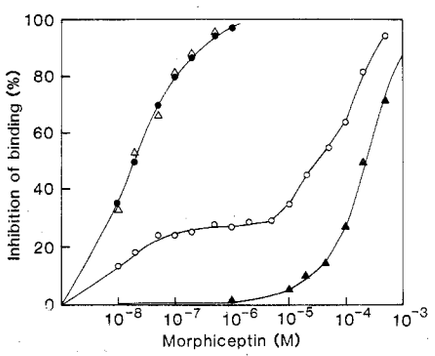
an affinity for μ receptors that is at least 1000 times greater than that for δ receptors; for these reasons it has been named morphiceptin. Such a highly selective morphine receptor ligand may prove useful for understanding the mode of action of opiates and the physiological function of opiate receptors.

Henschen *et al.* (*13*) first observed that a heptapeptide fragment of β-casein had some opiate-like effect in guinea pig ileum preparations. This heptapeptide was named β-casomorphin. It inhibits electrically stimulated muscle contractions of longitudinal muscle preparations of myenteric plexus from guinea pig ileum. This inhibition can be reversed and blocked by the specific opiate antagonist naloxone. β-Casomorphin is 250 times less potent than normorphine in the ileum assay and shows no effect in the vas deferens assay. The pentapeptide, NH_2-terminal fragment of β-casomorphin is 22 times less potent than normorphine. The NH_2-terminal tripeptide is completely inactive. Because of its relatively low activity, the possible biological significance of β-casomorphin has been held in doubt.



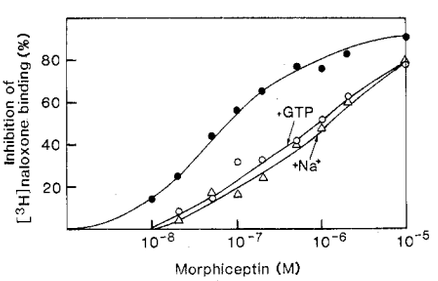


Fig. 1 (left). Differential inhibition of morphiceptin on the binding of [^{3}H]dihydromorphine (●), ^{125}I-labeled [D-Ala^2,N^{α}Me-Phe^4,Met(*O*)^5-ol]enkephalin (△), and ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin (○) to rat brain membranes and ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin to mouse neuroblastoma cell membranes (▲). Rat (Sprague-Dawley) brain membranes and mouse neuroblastoma cell (N4TG1) membranes were prepared as described previously (*2, 3*). Binding assays were performed at 24°C for 60 minutes essentially as described previously, with a filtration (GF/C) method. Nonspecific binding was determined in the presence of 1 μ*M* of the respective unlabeled ligand. ^{125}I-labeled enkephalin analogs were prepared as described previously (*2, 3*). The concentrations of labeled ligands were 0.06 n*M*, 0.1 n*M*, and 0.08 n*M* for ^{125}I-labeled [D-Ala^2,N^{α}Me-Phe^4,Met(*O*)^{5}ol]enkephalin (specific activity, 2 Ci/μmole), ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin (2 Ci/μmole), and [^{3}H]dihydromorphine (70 Ci/mmole), respectively. The total incubation volumes were 0.25 ml and 2 ml for ^{125}I-labeled and ^{3}H-labeled ligands, respectively. Fig. 2 (right). Effects of Na^+ and GTP on the morphiceptin inhibition of [^{3}H]naloxone binding to rat brain membranes. The inhibition of morphiceptin on the binding of [^{3}H]naloxone (0.4 n*M*; 23 Ci/mmole) were carried out in the absence (●) and presence of Na^+ (100 m*M*) (△) and GTP (0.1 m*M*) (○).

Table 1. Relation between structure and activity of morphiceptin in binding assays of ^{125}I-labeled enkephalin analogs and guinea pig ileum and mouse vas deferens assays. Myenteric plexus–longitudinal muscle strips were removed from lengths (3 to 4 cm) of ileum and suspended with 1 g of tension in a bath chamber containing Tyrodes solution. The strips were stimulated with electrical square-wave pulses of 0.1 Hz, 0.5-msec pulse duration at supramaximal voltage. Vas deferentia were suspended with 0.5 g of tension in a Mg^{2+}-free Krebs buffer and stimulated at supramaximal voltage with 10-Hz pulse trains for 400 msec, train interval 10 seconds, and 0.5-msec pulse duration. The percentage inhibition of the electrically induced muscle contractions was determined for the compounds at varying concentrations. The ED_{50} values for the peptides were extrapolated from those curves showing the dose concentration plotted against the response. The potencies of the β-casomorphin analogs in inhibiting the binding of ^{125}I-labeled enkephalin analogs were determined by the concentration which reduced the binding of labeled compounds by 50 percent. The conditions for binding assays were the same as for Fig. 1. The values are expressed as mean ± standard error.

β-Casomorphin fragment or analog	^{125}I-labeled FK33824 IC_{50} (μ*M*)*	Guinea pig ileum ED_{50} (μ*M*)†	^{125}I-labeled [D-Ala^2, D-Leu^5]-enkephalin IC_{50} (μ*M*)*	Mouse vas deferens ED_{50} (μ*M*)†
Tyr-Pro-Phe-Pro-Gly-Pro-Ile	1.8 ± 0.6	>10	15	No effect at 10
Pro-Phe-Pro-Gly-Pro-Ile	15 ± 2	No effect	250	No effect at 10
Tyr-Pro-Phe-Pro-Gly	0.5 ± 0.1	0.71	25	2.0‡
Tyr-Pro-Phe-Pro	1.2 ± 0.1	2.45	70	No effect at 10
Tyr-Pro-Phe	8.3 ± 0.9	No effect	70	No effect at 10
Tyr-Pro-Phe-Pro-NH_2	0.019 ± 0.004	0.13	30	15
Tyr-D-Pro-Phe-Pro-NH_2	10 ± 1	No effect	50	No effect at 10
Try-D-Ala-Gly-Phe-D-Leu	0.004 ± 0.0002	0.024	0.0016 ± 0.00012	0.0014
Morphine	0.0004 ± 0.0002	0.1	0.035 ± 0.005	0.9

*IC_{50} is the concentration which inhibits the binding of the labeled ligand by 50 percent. †ED_{50} is the concentration which supresses the electrically stimulated muscle contraction by 50 percent. ‡This effect is not blocked by naloxone.

Morphiceptin potently inhibits the binding of [^{3}H]dihydromorphine, ^{125}I-labeled FK33824, and [^{3}H]naloxone to rat brain membrane preparations with a 50 percent inhibition concentration (IC_{50}) of about 20 n*M* (Fig. 1). It is at least 1000 times less active in competing with the binding of ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin to rat brain membrane preparations and mouse neuroblastoma cell (N4TG1) membranes (Fig. 1) which bear only δ receptors (*2*). A small portion (about 30 percent) of the binding of ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin to rat brain membrane preparations can be inhibited by low concentrations of morphiceptin with a middle point of about 20 n*M*. This suggests that 30 percent of ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin in rat brain membranes binds to μ receptor sites, consistent with our previous data using morphine as an inhibitor (*2*).

Sodium ion and GTP reduce the binding of opiate agonists and enkephalins to both receptors (*4, 10, 11*) but have little effect on the binding of antagonists. The effects of Na^+ and GTP may therefore be used as an indication of the agonist or antagonist properties of a ligand. The potency of morphiceptin in competing with binding of [^{3}H]naloxone is greatly reduced by Na^+ (100 m*M*) and GTP (0.1 m*M*) (Fig. 2), thus suggesting agonist properties for this peptide. This is consistent with the observation that morphiceptin administered into brain has analgesic activity (*14*), and applied to guinea pig ileum preparations has agonist properties.

The binding affinities of various β-casomorphin fragments and analogs to μ receptors are measured by their potencies in competing with the binding of ^{125}I-labeled FK33824 (Table 1). β-Casomorphin is at least 100 times less potent than morphiceptin. The pentapeptide fragment Tyr-Pro-Phe-Pro-Gly is 25 times less potent than morphiceptin. The stereospecificity of the binding of morphiceptin is indicated from the data that shows virtually no activity of the 2-D-proline analog. The importance of an amide group at the carboxyl terminal is suggested by the data showing that the tetrapeptide NH_2-Tyr-Pro-Phe-Pro-COOH is 60 times less potent. Des-Tyr-β-casomorphin shows no affinity for the μ receptor, whereas the tripeptide Tyr-Pro-Phe retains some affinity.

All the above peptides show very little affinity for δ receptors (Table 1). None of them has an IC_{50} value below 10 μ*M* in inhibiting the binding of ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin to rat brain membrane preparations.

Kosterlitz and colleagues have provided strong evidence that the myenteric plexus of the guinea pig ileum contains mainly μ receptors and the mouse vas deferens mainly δ receptors (*1, 15*). Opiates and enkephalins suppress the electrically stimulated smooth muscle contractions of these two isolated organ preparations by inhibiting the release of excitatory neurotransmitters. Morphine is more potent than enkephalin in inhibiting the muscle contractions of the guinea pig ileum, whereas the reverse is found in the mouse vas deferens. Morphiceptin has a potency comparable to morphine in inhibiting electrically stimulated contractions of myenteric plexus–longitudinal muscle preparations (median effective dose, $ED_{50} = 1.3 \times 10^{-7}M$), whereas concentrations 100 times higher are required for depression of the mouse vas deferens ($ED_{50} = 1.5 \times 10^{-5}$, which is about 15 times higher than that of morphine). Naloxone reversed or blocked the depressant effects of morphiceptin in both preparations. Morphiceptin is without effect on electrically stimulated contractions of the rat vas deferens at concentrations as high as $10^{-5}M$. Other casomorphin analogs and fragments show considerably less activity (see Table 1). The ranking of potencies of the casomorphin analogs and fragments in the ileum bioassay are in excellent agreement with their binding affinities to μ receptors in brain membrane preparations. These data substantiate the highly selective affinity of morphiceptin for μ receptors.

Although opioid alkaloids and enkephalins show selectivity for μ and δ receptors, the significant cross-reactivity between receptor subtypes has make it difficult to understand the mode of action of opiates and the selective physiological role of the opiate receptor subtypes. The availability of a relatively specific ligand for the μ receptor subtype should be especially useful in this respect.

The discovery of this specific μ receptor ligand supports the hypothesis of multiple opiate receptors, and the analgesic property of morphiceptin (*14*) clearly establishes the role of μ receptors in modulating pain perception. The possibility of designing a morphine receptor-selective drug with less unwanted side effects may now be feasible. The highly peptidase-resistant property of morphiceptin (*13, 16*) is also attractive. The physiological significance of this and related peptides in milk remains to be determined.

A peptide isolated from enzymatic digests of β-casein is very similar or identical to morphiceptin (*17*). It behaves similarly on high-pressure liquid chromatography and gel filtration, and it cross-reacts with antibodies selective for morphiceptin that react only poorly with the carboxyl-free tetrapeptide. Perhaps this or related peptides derived from dietary constituents are able to interact physiologically with μ receptors under certain circumstances.

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References and Notes

1. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **267**, 495 (1977).
2. K.-J. Chang and P. Cuatrecasas, *J. Biol. Chem.* **254**, 2610 (1979).
3. K.-J. Chang, B. R. Cooper, E. Hazum, P. Cuatrecasas, *Mol. Pharmacol.* **16**, 91 (1979).
4. K.-J. Chang, E. Hazum, A. Killian, P. Cuatrecasas, *ibid.*, in press.
5. S. R. Childers, I. Creese, A. M. Snowman, S. H. Snyder, *Eur. J. Pharmacol.* **55**, 11 (1979).
6. L. Terenius, *Psychoneuroendocrinology* **2**, 53 (1977).
7. G. W. Pasternak, S. R. Childers, S. H. Snyder, *Science* **208**, 514 (1980).
8. H. L. Fields, P. C. Emson, B. K. Leigh, R. F. T. Gilbert, L. L. Iverson, *Nature (London)* **284**, 351 (1980).
9. K.-J. Chang, E. Hazum, P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4469 (1980).
10. C. B. Pert and S. H. Snyder, *Mol. Pharmacol.* **10**, 868 (1974); E. J. Simon and J. Groth, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2404 (1975).
11. S. R. Childers and S. H. Snyder, *Life. Sci.* **23**, 759 (1978); A. J. Blume, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1713 (1978).
12. β-Casomorphin and its fragments were synthesized by a solid-phase method. After hydrogen fluoride cleavage of protected peptide from the resin, the amide peptide was purified by counter-current distribution with the use of *n*-butyl alcohol, acetic acid, and water [*n*-BuOH: HOAC:H_2O (4:1:5 by volume)] as partition solvents. Peptides were characterized by thin-layer chromatography, electrophoresis, amino acid analyses, and high-performance chromatography. The purity of the peptide was 98 percent or better.
13. A. Henschen, V. Brantl, H. Teschemacher, F. Lottspeich, in *Endogenous and Exogenous Opiate Agonists and Antagonists*, E. E. Way, Ed. (Pergamon, New York, 1980), pp. 233–236.
14. E. T. Wei, A. Lee, J.-K. Chang, *Life Sci.* **26**, 1517 (1980).
15. A. A. Waterfield, F. M. Leslie, J. A. H. Lord, N. Ling, H. W. Kosterlitz, *Eur. J. Pharmacol.* **58**, 11 (1979); R. Miller and P. Cuatrecasas, *Vitam. Horm.* **36**, 297 (1978).
16. V. Brantl and H. Teschemacher, *Arch. Pharmacol.* **306**, 301 (1979).
17. K.-J. Chang, P. Cuatrecasas, J.-K. Chang, unpublished observations.
18. We thank M. A. Collins for technical assistance.

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Genetic Basis of Migratory Behavior in European Warblers

Abstract. *The seasonal course and magnitude of migratory restlessness recorded in four populations of the blackcap* Sylvia atricapilla *differ in a population-specific fashion that is related to the distance of travel. Experimentally produced hybrids of an exclusively migratory European population and a partially migratory African population showed intermediate migratory restlessness and an intermediate percentage of birds displaying restlessness compared to the two parental stocks. These results demonstrate the genetic basis of migratory behavior in this avian species and support the hypothesis that partial migration of populations is due to polymorphism.*

In Old World warblers of the genera *Phylloscopus* and *Sylvia* there are close relations between migratory restlessness (nocturnal hopping by captive birds at the time of migration) and several aspects of actual migration. In particular, the amount of migratory restlessness measured in the laboratory is closely correlated with the length of the migratory route. Thus it is possible that young, inexperienced birds migrate to their species- or population-specific winter quarters as a result of an endogenous program for migration that is controlled by or linked with an endogenous annual rhythm (*1*).

These observations can be explained by assuming that the patterns of migratory behavior are genetically fixed (*2*). Alternatively, environmental conditions (such as differences in photoperiod, temperature, or food supply) under which individuals grow up may be responsible for the differences in migratory performance (*3*). The genetic hypothesis would be supported if F_1 hybrids of birds from two populations that migrate over different distances showed intermediate patterns of migratory behavior (*4*).

The blackcap *Sylvia atricapilla*, which has a wide distribution from the Cape Verde Islands off the West African coast to approximately 65°N in Europe, is a suitable species for the necessary cross-breeding experiment. Blackcaps of different populations differ sharply in their migratory performance and in the percentage of individuals that display migratory restlessness when caged. Moreover, migratory activity can be easily measured as nocturnal activity in this nocturnally migrating species (*5*).

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