Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7203–7207, August 1992
Pharmacology

Dermorphin-related peptides from the skin of *Phyllomedusa bicolor* and their amidated analogs activate two μ opioid receptor subtypes that modulate antinociception and catalepsy in the rat

([Lys^7]dermorphin/[Trp^4,Asn^7]dermorphin/[Trp^4,Asn^5]dermorphin-(1–5)/guinea pig ileum/mouse vas deferens)

LUCIA NEGRI*†, GIULIANA FALCONIERI ERSPAMER*, CINZIA SEVERINI‡, ROSA LUISA POTENZA*, PIETRO MELCHIORRI*, AND VITTORIO ERSPAMER*

*Institute of Pharmacology, University La Sapienza, 00185 Rome, Italy; and ‡Institute of Neurobiology, National Research Council, 00185 Rome, Italy

Contributed by Vittorio Erspamer, April 20, 1992

ABSTRACT **Three naturally occurring dermorphin-like peptides from the skin of the frog *Phyllomedusa bicolor*, the related carboxyl-terminal amides, and some substituted analogs were synthesized, their binding profiles to opioid receptors were determined, and their biological activities were studied in isolated organ preparations and intact animals. The opioid binding profile revealed a very high selectivity of these peptides for μ sites and suggested the existence of two receptor subtypes, of high and low affinity. The peptides tested acted as potent μ opioid agonists on isolated organ preparations. They were several times more active in inhibiting electrically evoked contractions in guinea pig ileum than in mouse vas deferens. When injected into the lateral brain ventricle or peritoneum of rats, the high-affinity-site-preferring ligand, [Lys^7-NH$_2$]dermorphin, behaved as a potent analgesic agent. By contrast, the low-affinity-site-preferring ligand, [Trp^4,Asn^7-NH$_2$]dermorphin, produced a weak antinociception but an intense catalepsy.**

Dermorphin is an opioid peptide (see peptide 1 in Table 1) isolated from the skin of South American frogs belonging to the subfamily Phyllomedusinae (1). It has high affinity and selectivity for μ opioid receptors and produces analgesia in laboratory animals and humans (2–4). By means of a cDNA library prepared from the skin of *Phyllomedusa bicolor*, the amino acid sequences of three dermorphin-like peptides were recently predicted (5). These dermorphin analogs have now been isolated from methanol extracts of skin from this frog, and their amino acid sequences have been confirmed (15). Like dermorphin, these peptides contain a D-alanine as the second amino acid and share the common amino-terminal sequence Tyr-DAla-Phe. The D-alanine present in the final products is encoded in the skin mRNA by a normal codon for L-alanine. While other opioid sequences in the frog skin precursors are all followed by a glycine residue required for the formation of terminal amides, this is not the case for these three dermorphin-like peptides. Accordingly, the end products extracted from the skin were found to contain a free α-carboxyl group at the carboxyl terminus. In the previous paper (5) the binding affinity and selectivity of these peptides for μ opioid receptors were briefly reported. In this paper, we present the binding profile to μ, δ, and κ opioid receptors and the biological activities of the *Phyllomedusa bicolor* dermorphin-like peptides, their related carboxyl-terminal amides, and a few substituted analogs.

MATERIALS AND METHODS

Synthesis of Peptides. The peptides were synthesized by using fluorenylmethoxycarbonyl-polyamide active-ester chemistry on a Biolynx automated peptide synthesizer (Pharmacia Biochrom, Cambridge, U.K.). The products were purified by preparative HPLC on a Vydac reverse-phase column (22 × 250 mm, 10-μm particle size, 300-Å pore diameter, C_{18} packing; The Separations Group) with a linear gradient from 10% to 90% of solvent B (5 mM trifluoroacetic acid in acetonitrile) in solvent A (5 mM trifluoroacetic acid in water) over 60 min. After hydrolysis in 6 M HCl (24 hr, 110°C, *in vacuo*), amino acid analyses gave ratios consistent with the desired structures. The expected amino acid sequences were confirmed by automated Edman degradation performed with an Applied Biosystems 470A protein sequencer.

Binding Assays. Binding of the peptides to μ, δ, and κ opioid sites was assayed in crude membrane preparations (6) from rat (μ, δ) and guinea pig (κ) brain at pH 7.4 in 50 mM Tris·HCl buffer. Each assay mixture contained, in a final volume of 2 ml, the membrane preparation (0.8–1.0 mg of membrane proteins), protease inhibitors (100 μM phenylmethanesulfonyl fluoride, soybean trypsin inhibitor at 20 μg/ml, and bestatin at 10 μg/ml) (Sigma), and the tritiated ligand at the desired concentration with or without unlabeled ligand. The μ binding site was selectively labeled with [*tyrosyl*-3,5-^{3}H][DAla^2,Me-Phe^4,Gly-ol^5]enkephalin ([^{3}H]DAGO, Amersham, UK) (0.5 nM); the δ binding site with [3,5-^{3}H]Tyr-DAla-Phe-Asp-Val-Val-Gly-NH$_2$, ([^{3}H][DAla^2]deltorphin I) (7) (0.3 nM); and the κ binding site with (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl][3,4-^{3}H]benzeneacetamide ([^{3}H]U-69,593; DuPont/NEN) (1 nM). Radioligands were checked for purity by reverse-phase HPLC (Vydac C_{18} column, 5-μm particle size, 300-Å pore size, 2.1 × 250 mm) and eluted according to the linear gradient described above. Nonspecific binding was determined in the presence of 50 μM naloxone (S.A.L.A.R.S., Como, Italy) for the μ and κ systems or 5 μM naltrindole (Research Biochemicals, Natick, MA) for the δ system. After a 90-min incubation at 35°C, the free ligand was separated from membrane-bound ligand by filtration over Millipore AP40 glass fiber filters (soaked in 0.1% bovine serum albumin incubation buffer for 1 hr) under reduced pressure; the filters were then washed three times with 5 ml of ice-cold buffer. The radioactivity was extracted in 10 ml of Kontrogel (Kontron, Milan) and measured in a liquid scintillation counter (Betamatic V, Kontron). The percent of depletion of free radioligand by binding in the absence of a competing ligand (8) was 4% for [^{3}H]DAGO and [^{3}H]U-69,593 and 6% for [^{3}H][DAla^2]deltorphin I. Competition curves were determined in triplicate and were usually obtained over the concentration range of 1 pM to 120 μM. The inhibition

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DAGO, [DAla^2,Me-Phe^4,Gly-ol^5]enkephalin; U-69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide; [DAla^2]deltorphin I, Tyr-DAla-Phe-Asp-Val-Val-Gly-NH$_2$; i.c.v., intracerebroventricular.

†To whom reprint requests should be addressed.

constants of the various nonradioactive peptides were calculated by fitting the displacement curves with the nonlinear regression program LIGAND (9), using one-site or two-site models. The results are given as mean ± SEM of at least six separate determinations. IC_{50} is the concentration of the competing ligand at which specific binding of labeled ligand is reduced by half. K_d and K_i are the equilibrium dissociation constants for the radioligand and inhibitor respectively, η_H is the slope of the log-logit plot of competition data, and B_{max} is the maximum binding capacity of the radioligand. Because it is impractical to measure concentrations of unbound high-affinity unlabeled competing ligands, whenever an observed IC_{50} is less than the radioligand K_d, the computed value of K_i may be too large by an indeterminate factor (8).

Pharmacological Assays in Isolated Tissues. Preparations of the myenteric plexus–longitudinal muscle obtained from the small intestine of male guinea pigs (400–500 g) and preparations of mouse vas deferens were used for field stimulation with bipolar rectangular pulses of supramaximal voltage as described by Gyang and Kosterlitz (2). Synthetic reference peptides were dermorphin (Farmitalia) for guinea pig ileum, and [DAla^2]deltorphin I (7) for mouse vas deferens preparations. Assays were performed in the presence or absence of naloxone and the δ opioid receptor antagonist naltrindole (10). The results were expressed as the IC_{50} values obtained from concentration–response curves. When peptides were assayed, [DAla^2]deltorphin I and dermorphin were used as internal standards with mouse vas deferens and guinea pig ileum preparations, respectively. Because preparations varied considerably in sensitivity, the results obtained for the various peptides were normalized as described by Kosterlitz *et al.* (11). The IC_{50} value obtained in an individual assay was multiplied by the following fraction: mean IC_{50} value of the standard determined for all the assays ÷ IC_{50} value of the standard found in the individual assay.

Test of Peptide Degradation. The integrity of the radioligands after 90-min contact with the membranes was examined by HPLC as described above; no degradation was observed. Degradation of the peptides was also determined by incubating them with rat brain homogenates in 50 mM Tris·HCl, pH 7.4, at a concentration of 100 μg of peptide per g of tissue, in the presence or absence of the protease inhibitor cocktail used in the binding studies. After incubation, peptides were extracted with methanol and the recovery was determined by pharmacological assay on mouse vas deferens preparation, as described above.

Intracerebroventricular (i.c.v.) Injections. Male Wistar rats weighing 220–230 g when they were received from the supplier (Charles River Breeding Laboratories) and 250–270 g at the time of surgery were housed singly in 25 × 35 cm cages placed in a thermostatically controlled cabinet at an environmental temperature of 21°C. Under light diethyl ether anesthesia, each rat was implanted surgically with a plastic guide cannula (Linca, Tel Aviv) screwed into a skull hole drilled over the left lateral cerebral ventricle (AP, −0.5 mm and L, +1.8 mm relative to bregma; V, −1.0 mm relative to the skull surface). The cannula was secured to the bone with dental cement and the rat was returned to its cage and allowed to recover from surgery for at least a week. Peptides dissolved in saline were delivered to the lateral ventricle through the plastic cannula, with a Hamilton microliter syringe. All i.c.v. injections were made in a volume of 5 μl.

Test of Antinociception. Antinociceptive responses to peptide administration were determined in rats by the tail-flick test (12). The latency to the first sign of a rapid tail-flick was taken as a measure of the nociceptive response. Before peptide administration, each rat was tested and the latency to flick was recorded (control latency, CL). Animals not flicking their tails within 3 sec were discarded. The test was repeated every 15 min during the first hour after i.c.v. administration of peptides and thereafter every 30 min for a total period of 4 hr. The latency to flick of peptide-injected rats was defined as the test latency (TL). To avoid tissue damage, rats with a test latency of more than 10 sec (cutoff time), were removed from the nociceptive stimulus and assigned a maximal antinociceptive score of 100%. For drawing dose- and time-response curves, antinociception (A) was expressed as $A = 100 \times (TL - CL)/(10 - CL)$. To determine if antinociceptive effects were the result of activity at opioid receptors, naloxone (0.1 mg/kg, s.c.) was administered 15 min before i.c.v. injection of the peptides and antinociception was measured as described above. Each peptide dose was tested in a 10-rat group. The AD_{50} of each peptide was defined as the dose that produced an antinociceptive response in 50% of the animals tested. An antinociceptive response was considered to occur when an individual animal displayed a TL value greater than its CL value plus three SDs of the control mean CL of all animals in the group.

Test of Catalepsy. Catalepsy was evaluated by placing both front limbs of the rat over a 10-cm-high horizontal bar and measuring the time the animal maintained that posture. Rats remaining more than 1 min on the bar were defined as cataleptic (positive bar test). The cataleptic effect of each peptide dose was expressed as the percentage of animals with a positive bar test. A group of eight rats was used for each peptide dose. The CD_{50} of each peptide was defined as the dose that produced a positive bar test in 50% of the animals tested. To confirm the opioid origin of the catalepsy, nalox-

Table 1. Affinities for opioid receptors and biological activities on guinea pig ileum (GPI) and mouse vas deferens (MVD) of dermorphin-like peptides

Peptide	μ system		δ system		κ system		GPI	MVD
	K_i, nM	η_H	K_i, nM	η_H	K_i, nM	η_H	IC_{50}, nM	IC_{50}, nM
1. Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH_2	0.540 ± 0.021	0.96	929 ± 41	0.93	8162 ± 979	1.00	1.29 ± 0.11	16.5 ± 1.3
2. Tyr-DAla-Phe-Gly-Tyr-Pro-Lys-NH_2	0.090 ± 0.008	0.62	1105 ± 185		617 ± 66	0.87	1.15 ± 0.13	13.6 ± 1.5
High-affinity site	0.007 ± 0.001	—	—	—	—	—		
Low-affinity site	0.250 ± 0.013	—	—	—	—	—		
3. Tyr-DAla-Phe-Gly-Tyr-Pro-Lys-OH	5.700 ± 0.510	0.98	1150 ± 172	0.81	—	—	3.82 ± 0.45	56.3 ± 7.8
4. Tyr-DAla-Phe-Gly-Tyr-Pro-Arg-NH_2	0.200 ± 0.012	0.90	391 ± 25	0.98	192 ± 19	0.85	0.90 ± 0.17	10.2 ± 1.7
5. Tyr-DAla-Phe-Gly-Tyr-Pro-Asn-NH_2	0.444 ± 0.031	0.89	421 ± 42	0.82	5348 ± 1175	0.86	1.57 ± 0.23	17.0 ± 2.6
6. Tyr-DAla-Phe-Trp-Asn-NH_2	0.900 ± 0.052	0.85	480 ± 45	0.98	177 ± 12	1.01	5.00 ± 0.52	73.7 ± 9.1
7. Tyr-DAla-Phe-Trp-Asn-OH	4.440 ± 0.395	0.97	715 ± 73	1.01	2396 ± 351	0.89	13.10 ± 1.20	205.2 ± 25.0
8. Tyr-DAla-Phe-Trp-Tyr-Pro-Asn-NH_2	0.320 ± 0.026	0.95	690 ± 57	1.00	427 ± 47	1.03	0.58 ± 0.06	6.6 ± 0.9
9. Tyr-DAla-Phe-Trp-Tyr-Pro-Asn-OH	2.900 ± 0.208	0.84	865 ± 69	0.91	905 ± 61	0.97	1.30 ± 0.20	10.4 ± 1.3
10. Tyr-DAla-Phe-Trp-Tyr-Pro-Ser-NH_2	0.390 ± 0.013	0.93	—	—	—	—	1.00 ± 0.15	8.7 ± 1.2

K_i, equilibrium dissociation constant of the competing ligand; η_H, slope of log–logit plot; IC_{50}, agonist concentration that produced 50% inhibition of the electrically evoked twitch.

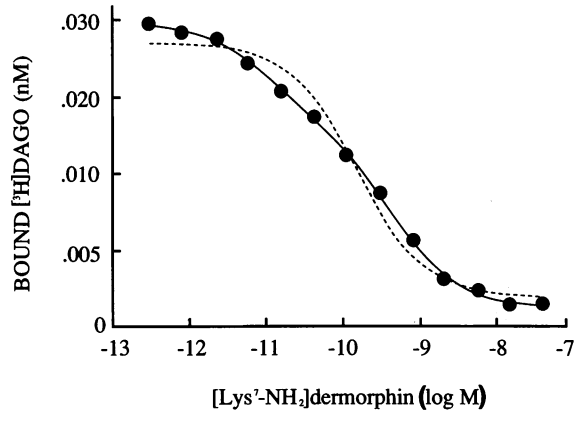

FIG. 1. [Lys⁷]Dermorphin competition curve in the μ opioid system of the rat brain. Data were fitted by the nonlinear regression program LIGAND. The solid line was the best fit for the two-site model, the broken line that for the one-site model. The apparent improvement in fit for the two-site model was significant ($P < 0.01$ versus one-site).

one (1 mg/kg of body weight) was administered to five-rat groups 15 min before i.c.v. injection of the peptides.

RESULTS

Binding Assay. Table 1 shows the results of the inhibition of binding of [³H]DAGO, [³H][DAla²]deltorphin I, and [³H]U-69,593 to brain membrane preparations by the peptides. With the exception of [Lys⁷-NH_2]dermorphin (peptide 2 in Table 1), the peptides produced smooth competition curves that were fitted best by a one-site model. Log-logit transformation of [Lys⁷-NH_2]dermorphin inhibition of the specific [³H]DAGO binding gave a slope significantly lower than 1 (0.65) and an apparent K_i of 0.09 ± 0.008 nM. Because the Hofstee plot (13) was curvilinear and the line of best fit assuming a one-site model was inadequate, a nonlinear regression analysis with a two-site model was used. This resulted in a shallow competition curve (Fig. 1), that fitted data significantly better than the monophasic curve ($P < 0.01$ versus one-site model). The computed K_i values were 0.007 ± 0.001 nM for the high-affinity site and 0.250 ± 0.013 nM for the low-affinity site. However, for this analysis we assumed that the K_d values of [³H]DAGO for the two sites were identical (0.78 nM). If this was not the case, the computed K_i values of the competing ligand were offset by an indeterminate amount. To provide further evidence for two μ binding sites of different affinity, [³H]DAGO binding was displaced by increasing concentrations of [Lys⁷-NH_2]dermorphin (peptide 5 in Table 1) in the presence of [Trp⁴,Asn⁷-NH_2]dermorphin at three different concentrations (0.1, 0.3, and 0.5 nM). For each concentration of [Trp⁴,Asn⁷-NH_2]dermorphin, Fig. 2 depicts the displacement curve fitted by the nonlinear regression program with a two-site model. The three competition curves show that the density of the low-affinity site was progressively reduced as the concentration of [Trp⁴,Asn⁷-NH_2]dermorphin increased from 0.1 nM to 0.5 nM. The peptides with a free carboxyl-terminal carboxyl group had a two orders of magnitude lower affinity for μ sites than their amidated analogs. Because all peptides tested had very low affinity for δ and κ sites, they behaved as highly selective ligands for μ sites (Table 1). Nevertheless, the new peptides showed higher affinity for κ opioid receptors of guinea pig brain than did dermorphin.

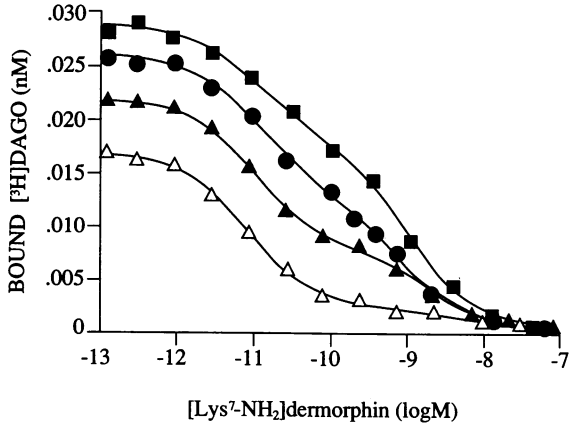

FIG. 2. Displacement of [³H]DAGO binding to μ opioid receptors by increasing concentrations of [Lys⁷-NH_2]dermorphin in the absence (■) and in the presence of [Trp⁴,Asn⁷-NH_2]dermorphin (●, 0.1 nM; ▲, 0.3 nM; and △, 0.5 nM).

Peptide Degradation. The peptides were degraded by crude brain homogenates (Fig. 3). An unexpected finding was that within few minutes of incubation with brain homogenates the biological activity of [Trp⁴,Asn⁷-NH_2]dermorphin and to a lesser extent that of [Trp⁴]dermorphin was completely destroyed. Since both dermorphin and Tyr-DAla-Phe-Trp-Asn-NH_2 were degraded slowly, the rapid cleavage of [Trp⁴,Asn⁷-NH_2]dermorphin and [Trp⁴]dermorphin probably occurred between Trp⁴ and Tyr⁵. The mixture of inhibitors we used did not afford protection against this type of enzyme degradation.

Biological Activity on Isolated Preparations. The peptides tested all acted as potent μ opioid agonists on isolated organ preparations (Table 1). They were several times more active in inhibiting electrically evoked contractions in guinea pig ileum than in mouse vas deferens. The rank order of their biological potencies roughly paralleled that of the respective μ binding affinities. However, there were notable exceptions. [Trp⁴,Asn⁷-NH_2]dermorphin had twice the potency of [Lys⁷-NH_2]dermorphin on guinea pig ileum, but one-third the affinity for μ opioid receptors. [Trp⁴,Asn⁷]dermorphin had the same biological potency as [Lys⁷-NH_2]dermorphin but a 1/20th the affinity for μ opioid receptors.

Antinociception and Catalepsy. Peptides with a carboxyl-terminal amide group produced dose-related antinociceptive effects in rats (Table 2), whereas their analogs with a free α-carboxyl group were about two orders of magnitude less active (results not shown). By i.c.v. administration, [Lys⁷-NH_2]dermorphin, [Arg⁷-NH_2]dermorphin, and [Asn⁷-NH_2]dermorphin behaved as potent analgesic agents in the tail-flick test. The antinociception of [Lys⁷-NH_2]dermorphin was long lasting (3–4 hr), exceeding that of dermorphin itself. In contrast, [Trp⁴,Asn⁷-NH_2]dermorphin, and [Trp⁴]dermorphin were about 1/80th and 1/20th as active as [Lys⁷-NH_2]-dermorphin, respectively. Their antinociceptive effects

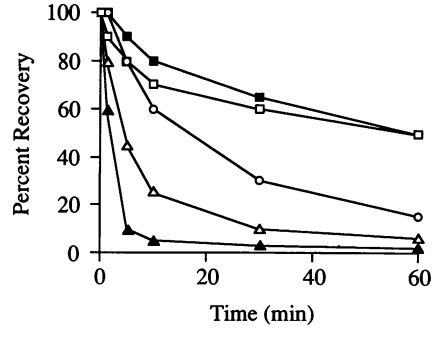

FIG. 3. Time course of peptide degradation by rat brain homogenates. □, [Lys⁷]Dermorphin (peptide 3 in Table 1); ■, [Trp⁴,Asn⁵]dermorphin-(1–5) (peptide 7); ○, dermorphin (peptide 1); △, [Trp⁴]dermorphin (peptide 10); and ▲, [Trp⁴,Asn⁷]dermorphin (peptide 9).

Table 2. Antinociceptive and cataleptic effects of dermorphin-like peptides

Peptide	No. in Table 1	M_r	AD_{50}	CD_{50}	AD_{50}/CD_{50}
[Lys^7-NH_2]Dermorphin	2	845	0.026 ± 0.009	0.364 ± 0.126	0.07
[Arg^7-NH_2]Dermorphin	4	872	0.034 ± 0.008	0.149 ± 0.016	0.23
Dermorphin	1	804	0.035 ± 0.010	0.112 ± 0.037	0.31
[Asn^7-NH_2]Dermorphin	8	830	0.034 ± 0.009	0.082 ± 0.024	0.41
[Trp^4,Asn^5-NH_2]Dermorphin-(1–5)	6	700	0.430 ± 0.044	0.210 ± 0.033	2.05
[Trp^4]Dermorphin	10	932	0.791 ± 0.048	0.130 ± 0.021	6.12
[Trp^4,Asn^7-NH_2]Dermorphin	8	959	2.086 ± 0.311	0.052 ± 0.013	40.11

AD_{50}, ED_{50} (nmol per rat, i.c.v.) for antinociception in the tail-flick test; CD_{50}, ED_{50} (nmol per rat, i.c.v.) for catalepsy in the bar test.

lasted less than 1 hr. In addition to antinociception, i.c.v. injection of these peptides produced catalepsy (Table 2). With the exception of the tryptophan-containing analogs, the peptides tested showed CD_{50} values higher than the respective AD_{50} values. However, the ratio AD_{50}/CD_{50} varied widely among the different agonists. [Lys^7-NH_2]Dermorphin showed the lowest ratio (0.073): at i.c.v. doses lower than 100 pmol per rat the peptide produced antinociception alone. In contrast, when given at i.c.v. doses that did not modify the reaction time in the tail-flick test, [Trp^4,Asn^7-NH_2]dermorphin induced catalepsy. For the latter peptide we calculated AD_{50}/CD_{50} = 40, a value 500 times higher than that calculated for [Lys^7-NH_2]dermorphin. Both antinociception and catalepsy were reversed by naloxone (data not shown). When injected intraperitoneally (i.p.) in rats, [Lys^7-NH_2]-dermorphin produced a long-lasting antinociception that was dose-related (Fig. 4). In the range of doses tested, it did not induce catalepsy. By i.p. administration, the AD_{50} of [Lys^7-NH_2]dermorphin (36 ± 9 nmol/kg of body weight) was about 1/40th and 1/6th that of morphine (1500 ± 260 nmol/kg) and dermorphin (225 ± 38 nmol/kg), respectively.

DISCUSSION

The results obtained clearly demonstrate that the dermorphin-related peptides from the skin of *Phyllomedusa bicolor* and their amidated analogs are highly potent and selective μ opioid agonists. In binding to opioid receptors, [Lys^7-NH_2]dermorphin showed an affinity for the preferred μ site at least 3 or 4 orders of magnitude greater than its affinity for the κ and δ sites. Moreover, its competition curve for [^{3}H]DAGO binding was fitted best by a two-site model. At very low concentrations, in the picomolar range, this peptide reduced binding by some small amount, to produce a distinct change in the curve slope, followed at higher concentrations (nanomolar) by a steeper reduction in the remaining bound [^{3}H]DAGO. Previous data in the literature (14) show that DAGO binding to μ opioid receptors sometimes reveals high- and low-affinity sites. The current explanation of this phenomenon is that the high-affinity site represents a high-affinity state of the receptor rather than a distinct μ receptor subtype. Our data are difficult to reconcile with this interpretation. The self-competition curves of DAGO were smooth and symmetrical, whereas the heterologous competition curves with [Lys^7-NH_2]dermorphin clearly showed two binding sites. In addition, displacement of [^{3}H]DAGO binding by [Lys^7-NH_2]dermorphin in the presence of [Trp^4,Asn^7-NH_2]dermorphin at three different concentrations (0.1, 0.3, and 0.5 nM) produced three biphasic competition curves in which the density of [^{3}H]DAGO binding to the low-affinity site was progressively shifted toward lower B_{max} values. A simple explanation for all these results might be that the two binding sites represent two distinct receptor subtypes of the μ system, one being the site preferred by [Lys^7-NH_2]-dermorphin and the other the site preferred by [Trp^4,Asn^7-NH_2]dermorphin. Evidence also exists for distinct functional roles of the two μ receptor subtypes. When [Lys^7-NH_2]-dermorphin was injected i.c.v. in the rats, its AD_{50} in the test of antinociception was 1/10th of its CD_{50} in the test of catalepsy, whereas in the case of [Trp^4,Asn^7-NH_2]dermorphin the AD_{50} was 40 times higher than the CD_{50}. However, until cloning and sequence analysis provide conclusive proof, the evidence provided here for the existence of two subtypes of the μ receptor must be regarded with circumspection. Our experiments with brain homogenates demonstrated a large difference in the degradation rates of the tested peptides and no suitable protective cocktails could be devised. The possibility that *in vivo* enzyme degradation may generate cleavage products that can account in part for the difference observed in the ED_{50} values between the test of antinociception and that of catalepsy is not suggested by our data, but it cannot be rigorously excluded. However, both antinociception and catalepsy showed classical opioid behavior as evidenced by naloxone antagonism. Of the peptides tested, [Lys^7-NH_2]dermorphin was the most potent μ opioid agonist even when injected peripherally. When given i.p., its AD_{50} in the tail-flick test was 36 nmol/kg of body weight, which is a value 1/40th of the AD_{50} of morphine (1500 nmol/kg). The duration of the antinociceptive effect also exceeded that of morphine. Thus its higher affinity and selectivity for μ sites and its greater antinociceptive potency, as compared with morphine, might well make [Lys^7-NH_2]dermorphin a superior analgesic agent.

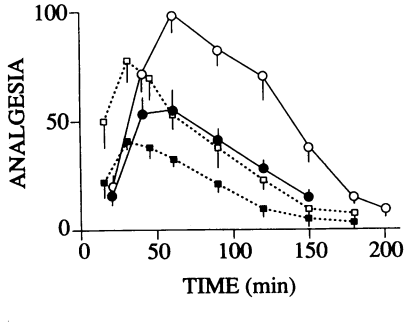

FIG. 4. Time course of the antinociception (analgesia) produced by i.p. injections of [Lys^7-NH_2]dermorphin (●, 0.2 μmol/kg of body weight; ○, 0.5 μmol/kg) and morphine·hydrochloride (■, 5 μmol/kg; □, 13 μmol/kg). Bars indicate SEM.

This work was supported by grants from the Italian National Research Council (Progetto finalizzato Cimica Fine II) and the Italian Ministry of University and of Scientific and Technological Research.

1. Montecucchi, P. C., de Castiglione, R., Piani, S., Gozzini, L. & Erspamer, V. (1981) *Int. J. Pept. Protein Res.* **17**, 275–279.
2. Gyang, E. A. & Kosterlitz, H. W. (1966) *Br. J. Pharmacol. Chemother.* **27**, 514–527.
3. Basso, N., Marcelli, M., Ginaldi, A. & De Marco, N. (1985) *Peptides* **6**, 177–179.
4. Sandrini, G., Degli Uberti, E., Salvadori, S., Margutti, A., Trasforini, G., Tomatis, R., Nappi, G. & Pansini, R. (1986) *Brain Res.* **371**, 364–367.

5. Richter, K., Egger, R., Negri, L., Corsi, R., Severini, C. & Kreil, G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4836–4839.
6. Gillan, M. G. C., Kosterlitz, H. W. & Paterson, S. J. (1980) *Br. J. Pharmacol.* **70,** 481–490.
7. Erspamer, V., Melchiorri, P., Falconieri Erspamer, G., Negri, L., Corsi, R., Severini, C., Barra, D., Simmaco, M. & Kreil, G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5188–5192.
8. Goldstein, A. & Barrett, R. W. (1987) *Mol. Pharmacol.* **31,** 603–609.
9. Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107,** 220–239.
10. Portoghese, P. S., Sultana, M. & Takemori, A. E. (1988) *Eur. J. Pharmacol.* **146,** 185–186.
11. Kosterlitz, H. W., Lord, J. A. H., Paterson, S. J. & Waterfield, A. A. (1980) *Br. J. Pharmacol.* **68,** 333–338.
12. D'Amour, F. E. & Smith, D. L. (1941) *J. Pharmacol. Exp. Ther.* **72,** 74–79.
13. Molinoff, P. B., Wolfe, B. B. & Weiland, G. A. (1981) *Life Sci.* **29,** 427–438.
14. Goldstain, A. & Naidu, A. (1989) *Mol. Pharmacol.* **36,** 265–272.
15. Mignogna, G., Severini, C., Simmaco, M., Negri, L., Falconieri Erspamer, G., Kreil, G. & Barra, D. (1992) *FEBS Lett.* **302,** 151–154.