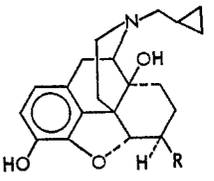
Activities of Morphinone and *N*-(Cyclopropylmethyl)normorphinone at Opioid Receptors

Sunan Fang,† A. E. Takemori,‡ and P. S. Portoghese*†

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received February 29, 1984*

Morphinone (3) and *N*-(cyclopropylmethyl)normorphinone (4) were synthesized and tested on electrically stimulated smooth muscle preparations (guinea pig ileum and mouse vas deferens) and in mice. Compound 3 behaved as an agonist and 4 as an antagonist in vitro and in vivo. No pronounced nonequilibrium agonist or antagonist activity was observed with either compound.

Affinity labels are finding increased use as tools for investigating opioid receptors. The ligands that have been employed most widely for this purpose are β-chlornaltrexamine[1] (1, β-CNA) and β-funaltrexamine[2] (2, β-FNA).



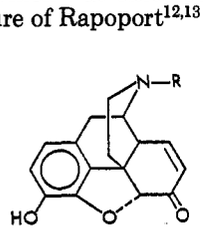
1, R = $N(CH_2CH_2Cl)_2$

2, R = $NHCO-\underset{H}{C}=\underset{H}{C}-COOMe$

β-CNA is capable of alkylating at least three types of opioid receptors, and β-FNA is known to block irreversibly only μ receptors.[3-9] These ligands are effective both in vitro and in vivo.

As the specificity of β-FNA for μ receptors is related to the narrow spectrum of reactivity and the orientation of its Michael acceptor group, we were intrigued by a report[10] that described the irreversible binding of morphinone (3) to mouse-brain opioid receptors. In this publication we have evaluated the in vitro and in vivo pharmacological properties of 3 and its *N*-cyclopropylmethyl analogue 4 in order to determine whether or not these ligands possess a nonequilibrium effect.

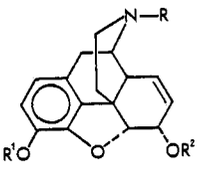
Chemistry. Morphinone (3) was synthesized from morphine (5) via the *tert*-butyldimethylsilyl (TBDMS)[11] intermediate 6, which was oxidized to the ketone 12 by using the procedure of Rapoport[12,13] and deprotected with HCl.



3, R=CH_3

4, R = $CH_2CH(CH_2)_2$

N-(Cyclopropylmethyl)normorphinone (4) was obtained from morphine (5). This involved N-demethylation of 5 with vinyl chloroformate[14-16] to give the desired diacylnormorphine 7 along with a small amount of the triacyl compound 8. Treatment of 7 with HBr afforded the vinyl carbonate 9. As alkylation of 9 with cyclopropylmethyl bromide and sodium carbonate consistently afforded the deprotected phenolic compound 10, this phenol was therefore converted to the TBDMS ether 11, which then was oxidized to the enone 13. Deprotection with aqueous HCl gave the desired compound 4.



	R	R^1	R^2
5,	Me	H	H
6,	Me	t-Bu-$SiMe_2$	H
7,	CH_2=CHOCO	CH_2=CHOCO	H
8,	CH_2=CHOCO	CH_2=CHOCO	CH_2=CHOCO
9,	H	CH_2=CHOCO	H
10,	$CH_2CH(CH_2)_2$	H	H
11,	$CH_2CH(CH_2)_2$	t-Bu-$SiMe_2$	H

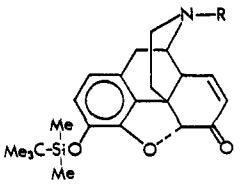
Pharmacological Results

In Vitro Studies. Compounds 3 and 4 were evaluated in vitro for opioid agonism and antagonism with use of the guinea pig ileal longitudinal muscle (GPI)[17] and the mouse vas deferens (MVD)[18] preparations. The ligands employed

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

(1) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Takemori, A. E.; Caruso, T. P. *J. Med. Chem.* **1978**, *21*, 598.
(2) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. *J. Med. Chem.* **1980**, *23*, 233.
(3) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Caruso, T. P.; Takemori, A. E. *J. Med. Chem.* **1979**, 22, 168.
(4) Caruso, T. P.; Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Science* **1979**, *204*, 316.
(5) Caruso, T. P.; Larson, D. L.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1980**, *213*, 539.
(6) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Eur. J. Pharmacol.* **1981**, *70*, 445.
(7) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* **1982**, *80*, 377.
(8) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* **1982**, *85*, 163.
(9) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1982**, *220*, 494.
(10) Nagamatu, K.; Kido, Y.; Terao, T.; Ishida, T.; Toki, S. *Life Sci.* **1982**, *31*, 1451.
(11) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(12) Rapoport, H.; Baker, D. R.; Reist, H. N. *J. Org. Chem.* **1957**, *22*, 1489.
(13) Rapoport, H.; Reist, H. N. *J. Am. Chem. Soc.* **1955**, *77*, 490.
(14) Olofson, R. A.; Schnur, R. C.; Bunes, L.; Pepe, J. P. *Tetrahedron Lett.* **1977**, *18*, 1567.
(15) Olofson, R. A.; Schnur, R. C. *Tetrahedron Lett.* **1977**, *18*, 1571.
(16) Olofson, R. A.; Pepe, J. P. *Tetrahedron Lett.* **1977**, *18*, 1575.
(17) Rang, H. P. *Br. J. Pharmacol.* **1964**, *22*, 356.

0022-2623/84/1827-1361$01.50/0



12, R = Me

13, R = $CH_2CH(CH_2)_2$

as standard agonists were morphine (μ selective), ethylketazocine (κ selective), and [D-Ala^2,D-Leu^5]enkephalin (DADLE) (δ selective). These pharmacological results are listed in Table I.

On the GPI and MVD, morphinone (3) possessed equivalent and 5 times greater potency than morphine, respectively. In both preparations 3 (200 nM) behaved as a reversible agonist and it exhibited no irreversible antagonism to morphine, ethylketazocine, or DADLE.

In the GPI that was depleted of functional μ receptors by preincubation with β-FNA (2),[8] the concentration–response curve of 3 was shifted to approximately 5-fold higher concentration with a concomitant >50% reduction of its maximum response. This suggested that 3 mediates its principal agonist effect through μ opioid receptors in the GPI.

N-(Cyclopropylmethyl)normorphinone (4) displayed partial agonist activity in both preparations. In the GPI, 4 (200 nM) reversibly antagonized morphine (IC_{50} ratio, 22.5) and ethylketazocine (IC_{50} ratio, 3.5). Irreversible antagonism of borderline significance (IC_{50} ratio, 3.2) was observed for morphine agonism in the MVD by exposure to 4 (200 nM for 30 min), but no irreversible blockage of morphine was observed in the GPI. Moreover, no sustained antagonism toward ethylketazocine or DADLE was exhibited in preparations that were preincubated with 4.

In Vivo Studies. The compounds 3 and 4 were tested after intracerebroventricular (icv) administration in mice by using the tail-flick procedure (Table II).[19] Morphinone (3) behaved as an analgesic with a potency one-fourth that of morphine. The cyclopropylmethyl analogue 4 possessed no agonist activity and it was effective in antagonizing morphine 0.5 h after administration. Neither 3 nor 4 (10 nmol/mouse) possessed sustained agonist or antagonist effects, and it appeared that the response of the mice to the morphine challenge fell within the control range 2 h after injection. The analgesic test was performed 30 min after sc administration of morphine.

Discussion

The fact that morphinone (3) behaved as a potent agonist on the GPI and MVD but possessed no sustained pharmacological effect on these preparations and in vivo suggests that 3 does not bind covalently to opioid receptors under the conditions employed in this study. The reported[10] irreversible binding was carried out with 2500-fold greater concentration of 3 than that employed in our study, which is 5000 times greater than the IC_{50} concentration in the GPI. Thus, it appears that impractically high concentrations may be required to block opioid receptors with this ligand (3).

Unlike the published in vivo study with 3, we detected no significant blockage of morphine antinociception 24 h after pretreatment. As the route of administration and test procedure differed from that reported, a direct comparison is difficult. However, if 3 possessed antagonist activity, it would have been more easily detected by the icv route of administration that was used in this study.

N-(Cyclopropylmethyl)normorphinone (4 had affinity for μ opioid receptors, as suggested by its ability to reversibly antagonize the agonist effect of morphine in vitro and in vivo. However, 4 afforded little or no irreversible blockage of the agonist effect of morphine in these tests.

The apparent inertness of 3 and 4 toward opioid receptors is not related to the intrinsic reactivity of its enone system, as the presumably less reactive α,β-unsaturated ester group in β-FNA (2) confers potent irreversible blockage of μ receptors in the GPI at one-tenth the concentration of ligands employed in the present study.[6] It therefore is likely that the inability of 3 and 4 to covalently bind to opioid receptors is due to a deficient secondary recognition step, i.e., inadequate alignment between the electrophilic moiety and an appropriate proximal nucleophile on the receptor.[20]

Table I. Activities of Morphinone (3) and *N*-(Cyclopropylmethyl)normorphinone (4) in the GPI and MVD

no.	agonism (IC_{50} ± SE)	irreversible antagonism (IC_{50} ratio ± SE)[a] morphine[b]	ethylketazocine[c]
	A. Guinea Pig Ileum Preparation (GPI)		
3	108 ± 17 nM (3)	0.67 ± 0.21 (3)	0.92 ± 0.17 (3)
4	40% (300 nM)[f] (3)	1.0 ± 0.2[d] (3)	0.82 ± 0.08[e] (3)

no.	agonism (IC_{50} ± SE)	irreversible antagonism (IC_{50} ratio ± SE)[a] morphine[g]	DADLE[h]
	B. Mouse Vas Deferens Preparation (MVD)		
3	47 ± 11 nM (4)	0.68, 0.59	0.74, 0.55
4	18% (300 nM)[i] (3)	3.2 ± 0.9 (3)	1.2 ± 0.2 (3)

[a] Agonist IC_{50} [after 30-min incubation of the preparation with 200 nM of 3 or 4 followed by washing (20×)] divided by the control agonist IC_{50} in the same preparation. Number of experiments indicated in parentheses. [b] Morphine IC_{50} = 79.8 nM (6). [c] Ethylketazocine IC_{50} = 1.6 nM (3). [d] Reversible IC_{50} ratio was 22.6 ± 6.3 (4) in the presence of 200 nM 4. [e] Reversible IC_{50} ratio was 3.5 ± 0.5 (3) in the presence of 200 nM 4. [f] Partial agonist with 40% of maximal response. [g] Morphine IC_{50} = 244 nM (5). [h] [D-Ala^2,D-Leu^5]enkephalin IC_{50} = 0.22 nM (3). [i] Partial agonist with 18% of maximal response at 300 nM.

Table II. Antagonism of Morphine Analgesia in Mice

no.	ED_{50},[a] nmol/mouse	% of animals exhibiting analgesia[b] pretreatment time, h 0.5	2	24
3	11.1 (7.2–17.1)	c	80	67
4	d	10	60	67

[a] Tested by the tail-flick procedure[19] 30 min after icv administration; morphine ED_{50}, 2.8 (1.8–4.1) nmol/mouse. [b] Mice (9–10/group) administered 3 or 4 (10 nmol) icv were challenged with a 10 mg/kg sc dose of morphine sulfate; the usual analgesic response to this dose is 70–90%. [c] Not tested due to the residual analgesic activity of 3. [d] Inactive at 10 nmol/mouse; morphine antagonist.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, Az, and were within ±0.4% of the theoretical values. IR spectra were determined on a Perkin-Elmer Model 281 infrared spectrophotometer. Mass spectra were obtained on an AEI-MS-30 instrument.

(18) Henderson, G.; Hughs, J. Kosterlitz, H. W. *Br. J. Pharmacol.* **1972**, *46*, 764.
(19) Ward, S. J.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1983**, *224*, 525.
(20) Sayre, L. M.; Larson, D. L.; Fries, D. S.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1983**, *26*, 1229.

3-(Butyldimethylsilyl)morphine (6). To a THF solution (100 mL) containing morphine sodium salt (3.07 g, 10 mmol) was added a solution of *tert*-butyldimethylsilyl chloride (2 g, 12.8 mmol) in THF (20 mL). The reaction mixture was stirred under nitrogen at 25 °C and monitored by TLC until the reaction had gone to completion (40 h). The mixture was filtered, the THF was evaporated, and the residue was heated in vacuo at 40–50 °C for 1 h to remove the excess *tert*-butyldimethylsilyl chloride. The crude product (2.5 g, 65%) was crystallized from EtOAc–MeOH to afford **6**: mp 122–123 °C; EIMS, *m/e* 399 (1, M^+), 284 ($M^+ - t$-BuSiMe$_2$); NMR (base in $CDCl_3$) δ 0.95 (9 H, *t*-Bu), 0.19 (6 H, $SiMe_2$), 5.25 and 5.67 (2 H, t, vinyl), 4.25 (1 H, br, H-6), 4.85 (1 H, d, H-5).

3-(*tert*-Butyldimethylsilyl)morphinone (12). Intermediate **6** (399 mg, 1 mmol) was dissolved in benzene (20 mL) and freshly prepared dry silver carbonate (1.52 g, 6 mmol) was added. Under a nitrogen atmosphere the mixture was refluxed with vigorous stirring for 2 h and filtered, and the insoluble residue was washed twice with benzene. The filtered solution and filtrate were combined and evaporated in vacuo to afford the crude oxidation product **12** (200 mg, 50%). The crude compound was subjected to dry column chromatography on silica gel (MeOH–EtOAc, 20:80), which afforded **12** contaminated with 20% of **6**: EIMS, *m/e* 397 (M^+); NMR (base in $CDCl_3$) δ 5.96, 6.06 (2 H, t, vinyl), 4.59 (1 H, s, H-5); IR (neat) 1680 cm^{-1} (conjugated C=O).

Morphinone (3). 3-(*tert*-Butyldimethylsilyl)morphinone (**12**; 100 mg, 0.25 mmol) was dissolved in 1 N HCl solution (5 mL) and the solution stirred at 25 °C for 20 h. The solution was cooled in an ice bath, adjusted to pH 8.7 with $NaHCO_3$, and extracted with several portions of chloroform. The chloroform extract was evaporated in vacuo to afford crude product **3** (24 mg, 33%). The amine **3** was converted to the perchlorate salt with 50% perchloric acid–ethanol solution and crystallized from ethanol: mp 156–159 °C (lit.[12] mp 151–155 °C); EIMS, *m/e* 283 (M^+); NMR (base in $CDCl_3$) δ 5.86, 6.02 (2 H, t, vinyl), 4.62 (1 H, s, H-5); IR (KBr) 1680 cm^{-1} (conjugated C=O). Anal. ($C_{17}H_{17}O_3N\cdot HClO_4$) C, H, N.

3,17-Bis[(vinyloxy)carbonyl]normorphine (7). A mixture of morphine (**5**; 285 mg, 1 mmol), vinyl chloroformate (530 mg, 5 mmol), and 1,8-diaminonaphthalene (514 mg, 2.4 mmol) was heated for 6 h in $ClCH_2CH_2Cl$ (50 mL) at 65 °C with vigorous stirring. The reaction mixture was filtered and evaporated in vacuo and the residue was subjected to dry column chromatography on silica gel (hexane–EtOAc, 70:30). The first fraction (50 mg, 12%), EIMS *m/e* 481 (M^+), consisted of compound **8**. The desired product **7** (200 mg, 48%), mp 70–71 °C, was obtained in the second fraction of eluate: EIMS, *m/e* 411 (M^+); NMR (Me_2SO) δ 8.25 (phenolic OH); IR (KBr) 1775 (carbonate C=O), 1720 cm^{-1} (carbamate C=O). Anal. ($C_{22}H_{21}O_7N$) C, H, N.

3-[(Vinyloxy)carbonyl]normorphine Hydrobromide (9·HBr). Intermediate **7** (1.27 g, 3 mmol) was dissolved in EtOH (2 mL) and Et_2O (20 mL). Anhydrous HBr (400 mg, 5 mmol) in EtOH was added and the reaction mixture was stirred for 8 h at 25 °C. The precipitate of **9**·HBr (640 mg, 63%) was recrystallized from EtOH: mp 266–267 °C; EIMS, *m/e* 341 (M^+); IR (KBr) 1775 cm^{-1} (carbonate C=O). Anal. ($C_{19}H_{19}O_5N\cdot HBr$) C, H, N.

17-(Cyclopropylmethyl)normorphine Hydrobromide (10·HBr). This was prepared by a modification of the procedure reported for the free base.[21] A mixture of **9**·HBr (375 mg, 208 mmol), cyclopropylmethyl bromide (450 mg, 3.3 mmol), and sodium bicarbonate (530 mg, 6.6 mmol) in EtOH (25 mL) was heated at 80 °C under N_2 with stirring for 10 h. The mixture was cooled, filtered, and evaporated in vacuo, and the residue was subjected to dry column chromatography on silica gel (EtOAc–MeOH, 80:20). The crude amine **10** (386 mg, 57%) was converted to the HBr salt and recrystallized from MeOH–EtOAc–petroleum ether: mp 214–216 °C; EIMS, *m/e* 325 (M^+). Anal. ($C_{20}H_{23}NO_3\cdot HBr\cdot H_2O$) C, H, N.

3-(*tert*-Butyldimethylsilyl)-17-(cyclopropylmethyl)normorphine (11). 17-(Cyclopropylmethyl)normophine (**10**; 109 mg, 0.3 mmol) was suspended in dimethoxyethane (DME) (15 mL), and 1.43 M *n*-butyllithium–hexane (0.23 mL, 0.33 mmol) was added under nitrogen. To this solution was added *tert*-butyldimethylsilyl chloride (57 mg, 0.38 mmol) in DME. The reaction mixture was stirred for 5 h at 25 °C, filtered, and evaporated in vacuo to afford crude product **11** (90 mg, 68%): EIMS, *m/e* 439 (M^+); NMR ($CDCl_3$) δ 1.1 (9 H, *t*-Bu), 0.2 (6 H, $SiMe_2$), 5.08 and 5.81 (2 H, t, vinyl), 4.86 (1 H, d, H-5), 4.04 (1 H, br, H-6).

3-(*tert*-Butyldimethylsilyl)-17-(cyclopropylmethyl)normorphinone (13). Compound **11** (90 mg, 0.2 mmol) was dissolved in dry benzene (10 mL), and traces of moisture were removed by azeotropic distillation of a small fraction of solvent. Silver carbonate (0.5 g, 2 mmol) then was added and the mixture was heated for 4 h. The mixture was cooled, filtered, and evaporated in vacuo to afford 20 mg (22%) of crude **13** as an oil: IR (neat) 1680 cm^{-1} (conjugated C=O); NMR ($CDCl_3$) δ 5.89 and 6.10 (2 H, d, vinyl), 4.78 (1 H, s, H-5).

17-(Cyclopropylmethyl)normorphinone Perchlorate (4·HClO$_4$). The crude enone **13** (20 mg) was dissolved in 1 N HCl (5 mL) and was stirred at 25 °C for 24 h. The chilled solution was adjusted to pH 8.7 with $NaHCO_3$ and extracted with several portions of $CHCl_3$. The chloroform extract was evaporated and the residue was subjected to dry column chromatography (EtOAc–MeOH, 90:10). The amine **4** was converted to perchlorate salt and crystallized from ethanol–ether to afford 5 mg (25%) of **4**: mp 170–173 °C; EIMS, *m/e* 323 (M^+). Anal. ($C_{20}H_{21}O_3N\cdot HClO_4\cdot 2H_2O$) C, H, N.

Acknowledgment. This research was supported by the National Institute on Drug Abuse (DA 01522). We thank Victoria Darrow and Joan Naeseth for the capable technical assistance in performing the biological testing, Suz Bartell for typing the manuscript, and Dr. D. Loncrini of Mallinckrodt for furnishing the morphine for this study.

Registry No. **3**, 467-02-7; **3**·HClO$_4$, 91265-67-7; **4**, 91265-68-8; **4**·HClO$_4$, 91265-69-9; **5**, 57-27-2; **6**, 91265-70-2; **7**, 91265-71-3; **8**, 64643-81-8; **9**·HBr, 91265-72-4; **10**, 1976-45-0; **10**·HBr, 91265-73-5; **11**, 91265-74-6; **12**, 91265-75-7; **13**, 91280-58-9; morphine sodium salt, 50291-32-2; *tert*-butyldimethylsilyl chloride, 18162-48-6; cyclopropylmethyl bromide, 7051-34-5.

(21) Gates, M.; Montzka, T. A. *J. Med. Chem.* **1964**, *7*, 127.