Communications to the Editor

Stereostructure–Activity Relationship of Opioid Agonist and Antagonist Bivalent Ligands. Evidence for Bridging between Vicinal Opioid Receptors[1]

Sir:

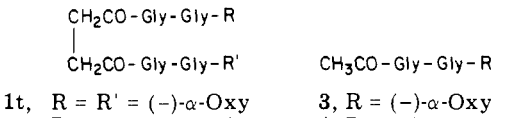
Ligands having two pharmacophores connected by a spacer (bivalent ligands) have the potential for bridging vicinal receptors. Such bridging should be manifested by a substantial increase in potency due to the high local concentration of the free pharmacophore in the vicinity of the proximal recognition site when the bivalent ligand is bound in a monovalent mode.[2,3] Indeed, bivalent ligands containing opiate or peptide pharmacophores have been reported to possess enhanced opioid agonist or antagonist potencies at a specific spacer length.[3-11] However, since such potency enhancement also is consistent with bridging between an opioid receptor and a vicinal nonopioid recognition site, we have attempted to distinguish between these possibilities by utilizing the high enantioselectivity[12,13] of opioid receptors for the opiate pharmacophore. Here we present data that suggest that the observed potency enhancements of bivalent opiates are a consequence of bridging between vicinal opioid recognition sites.

If a bivalent ligand containing two identical (−)-opiate pharmacophores (threo isomers **1t** and **2t**) bridges vicinal opioid receptors, then replacement of one of the (−)-opiate pharmacophores with the antipodal (+)-opiate (meso isomers **1m** and **2m**) should afford a potency decrease because of the lower affinity of the (+)-opiate component for the neighboring opioid receptor. This is suggested from reports that the affinities of (+)-morphine and (+)-naloxone are at least 1000 times less than those of the corresponding (−)-isomers.[14,15] In this regard, the potency of the meso isomer should be less than that of the threo isomer but more than that of the monovalent ligands **3** and **4** if the (+)-opiate component interacts nonspecifically with the vicinal opioid site or other membrane sites.

Table I. Activity of Bivalent and Monovalent Ligands on the Electrically Stimulated Guinea Pig Ileal Longitudinal Muscle (GPI)[a]

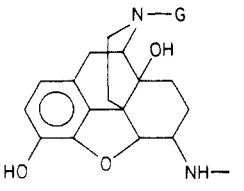
	agonist activity	
compd	potency ratio[b]	rel potency
1t	36.0 ± 6.6	18.9
1m	7.6 ± 1.3	4.0
3	1.9 ± 0.3	1.0
	antagonist activity	
compd	IC_{50} ratio[c]	rel potency
2t	181 ± 34	33.5
2m	7.8 ± 1.7	1.4
4	5.4 ± 0.6	1.0

[a] Prepared by the method of H. B. Rang (*Br. J. Pharmacol.* **1964**, *22*, 356) as described previously.[3] [b] The morphine IC_{50} divided by agonist IC_{50} in same tissue. The ratios represent the means of three to six determinations. [c] The IC_{50} of morphine in the presence of antagonist (10 nM) divided by morphine IC_{50} in same tissue. The ratios represent the means of three to six determinations.



1t, R = R' = (−)-α-Oxy
1m, R = (−)-α-Oxy; R' = (+)-α-Oxy
2t, R = R' = (−)-β-Nal
2m, R = (−)-β-Nal; R' = (+)-β-Nal
5, R = R' = OH
6, R = R' = $ON(COCH_2)_2$
7, R = OH; R' = $OCH_2C_6H_5$
8, R = (−)-α-Oxy; R' = $OCH_2C_6H_5$
9, R = (−)-α-Oxy; R' = OH
10, R = (−)-β-Nal; R' = $OCH_2C_6H_5$
11, R = (−)-β-Nal; R' = OH

3, R = (−)-α-Oxy
4, R = (−)-β-Nal



R and R' = α-Oxy(G = CH_3) or β-Nal [G = $CH_2CH(CH_2)_2$]

The specific bivalent ligands that were investigated contain pharmacophores that confer either agonist (α-oxymorphamine,[16] $G = CH_3$) or antagonist (β-naltrexamine,[17] $G = CH_2$-Δ (Δ = cyclopropyl)) activities. The spacer length for these compounds was dictated by the peak potency that was observed when the diglycyl unit is incorporated into each half of the molecule.[4] The threo isomers[18] 1t and **2t** were prepared by condensing the

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N-hydroxysuccinimide diester **6** of succinylbis(glycylglycine)[19] with either (−)-α-oxymorphamine or (−)-β-naltrexamine in DMF. The meso isomers[20] **1m** and **2m** were synthesized by coupling the (−)-opiates to succinylbis(glycylglycine) monobenzyl ester **7**[21] to afford the opiate intermediates **8** and **10**, which, after catalytic hydrogenolysis to the corresponding acids **9** and **11**, were coupled to the corresponding (+)-enantiomers.[22] The monovalent ligands **3** and **4** were obtained by coupling the (−)-opiates to *N*-acetylglycylglycine.[23]

The activities of the bivalent and monovalent ligands on the guinea pig ileal longitudinal muscle (GPI) are presented in Table I. These data represent agonist or antagonist potencies mediated principally through μ receptors.[24] It can be seen that the threo isomers **1t** and **2t** are more potent than the corresponding monovalent analogues **3** and **4** by factors of 18.9 and 33.5, respectively.[25] In contrast, the meso isomers **1m** and **2m** exhibit considerably smaller potency enhancements, being only 4.0- and 1.4-fold more potent than the monomers. Thus the threo isomers are approximately 5 and 24 times more potent than the corresponding meso compounds.

With use of guinea pig brains for the μ receptor binding assay,[25] good correlation was observed between the relative affinities and agonist potencies of the α-oxymorphamine compounds. However, no significant differences in binding were noted among the antagonist β-naltrexamines. These results may reflect possible differences between sites that bind agonists and those that bind antagonists.[26] Other possible explanations for the discrepancy between binding and antagonist potency include differences between the organization of the μ receptor system in the ileum and brain of guinea pigs and differences between the interaction of antagonists with intact cell membranes and membrane fragments.

The stereoselectivity of the potency enhancements supports the idea that vicinal opioid receptors are the recognition sites involved in the bridging of bivalent ligands **1t** and **2t**. Thus, a pharmacophore having the same absolute stereochemistry as morphine facilitates effective bridging. Further, we suggest that the greater potency of the meso bivalent ligands **1m** and **2m** relative to the corresponding monomers **3** and **4** is related to nonspecific interaction between the tethered (+)-opiate and a vicinal opioid receptor or other vicinal membrane component.

These results suggest that it may be possible to design highly selective ligands for an opioid receptor type by adjusting the spacer length if different interreceptor distances exist for different receptor types. Indeed, such differences in selectivity have been observed among antagonist bivalent ligands in the β-naltrexamine series,[3] and a highly selective κ antagonist[27] has been developed from this approach.

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Registry No. **1m**, 97133-82-9; **1m**·2HCl, 97168-81-5; **1t**, 97133-81-8; **1t**·2HCl, 97168-80-4; **2m**, 97133-83-0; **2m**·2HCl, 97168-82-6; **2t**, 97073-81-9; **2t**·2HCl, 97134-60-6; **3**·, 97073-83-1; **3**·HCl, 97073-82-0; **4**, 97073-85-3; **4**·HCl, 97073-84-2; **5**, 97073-86-4; **6**, 97073-87-5; **7**, 97073-88-6; **8**, 97073-89-7; **9**, 97073-90-0; **10**, 97073-91-1; **11**, 97073-92-2.

(18) **1t**·2HCl: mp 273 °C; R_f 0.07 (silica gel, EtOAc–EtOH–H_2O–NH_4OH, 10:10:10:1); anal. ($C_{46}H_{58}N_8O_{12}$·2HCl·5H_2O) C, H, N. **2t**·2HCl: mp 235 °C; R_f 0.69 (silica gel, EtOAc–EtOH–H_2O–NH_4OH, 8:8:8:1); anal. ($C_{52}H_{66}N_8O_{12}$·2HCl·3H_2O) C, H, N.

(19) The active ester **6** was synthesized by condensing Gly-Gly with succinyl chloride to afford diacid **5**, which was esterified with *N*-hydroxysuccinimide in the presence of DCC.

(20) **1m**·2HCl: mp >260 °C; R_f 0.1 (silica gel, EtOAc–MeOH–H_2O–NH_4OH, 8:2:0.7:0.3); anal. ($C_{46}H_{58}N_8O_{12}$·2HCl·5.5H_2O) C, H, N. **2m**: mp >260 °C; R_f 0.37 (silica gel, CH_3CN–H_2O–NH_4OH, 95:5:3); anal. ($C_{52}H_{66}N_8O_{12}$·2HCl).

(21) The monobenzyl ester **7** was prepared by reacting Gly-Gly benzyl ester with succinic anhydride in CH_2Cl_2, esterification of the product ($HOOCCH_2CH_2CO$-Gly-GlyOBz) with C_6Cl_5OH in the presence of DCC, and condensing this intermediate with Gly-Gly.

(22) Intermediate **8** was prepared by coupling **7** to (−)-α-oxymorphamine with the aid of HOBt/DCC in DMF. Hydrogenolysis of **8** in the presence of 10% Pd on carbon gave the acid **9**, which then was coupled to (+)-α-oxymorphamine with use of HOBt/DCC in DMF. Intermediate **11** was prepared from (+)-β-naltrexamine by an analogous route. Both (+)-α-oxymorphamine and (+)-β-naltrexamine were prepared by reductive amination[17] ($NaCNBH_3$ and NH_4OAc in MeOH) of (+)-oxymorphone and (+)-naltrexone obtained by total synthesis from *m*-methoxyphenethylamine via (+)-dihydrocodeinone (Rice, K. C. In "Problems of Drug Dependence 1981"; Harris, L. S., Ed.; NIDA Research Monograph 41, Washington, DC, 1982, p 99), (+)-7-bromodihydrocodeinone dimethyl ketal (Iijima, I.; Minamikawa, J.-I.; Jacobson, A. E.; Brossi, A.; Rice, K. C. *J. Org. Chem.* **1978**, *21*, 398), and (+)-thebaine.[14] N-Demethylation of (+)-oxymorphone as described earlier afforded (+)-noroxymorphone,[14] which was alkylated with cyclopropylmethyl bromide to afford (+)-naltrexone. The samples of (+)-oxymorphone and (+)-naltrexone were identical in all respects (TLC, NMR, MS, mp, IR) with authentic samples of the corresponding (−)-enantiomers except for optical rotation. Separation of the 6α- and 6β-epimers was effected by column chromatography (silica gel, CH_3CN–acetone–MeOH–H_2O–Me_3N, 77:20:2:1:2).

(23) Monovalent ligand **3**: mp 230 °C; R_f 0.31 (silica gel, EtOAc–MeOH–H_2O–NH_4OH, 8:2:1:0.3); anal. ($C_{23}H_{30}N_4O_6$·HCl) C, H, N. **4**: mp 253 °C; R_f 0.60 (silica gel, EtOAc–EtOH–H_2O–NH_4OH, 10:10:5:1); anal. ($C_{26}H_{34}N_4O_6$·HCl·1.5H_2O) C, H, N. Both **3** and **4** were prepared by coupling (−)-α-oxymorphamine or (−)-β-naltrexamine with Ac-Gly-Gly through the *N*-hydroxysuccinimide active ester in DMF.

(24) The agonist effect of the ligands in the α-oxymorphamine series were reduced by approximately 97% after the GPI was pretreated with the highly selective irreversible μ antagonist β-FNA (Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* **1982**, *80*, 377). Also the β-naltrexamine series antagonized morphine (μ agonist) 5–10 times more effectively than ethylketazocine (κ agonist).

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