Two New Opioid Delta-Receptor Ligands: A Highly Selective Agonist and a Potent Selective Antagonist in in Vitro Isolated Preparations

Masaaki UEKI*, Kazuko AO0, Midori KAJIWARA§, Kozo SHINOZAKI*, Hideyuki INOUE* and Tetsuo OKA§•†

*Department of Pharmacology, School of Medicine, Tokai University, Isehara 259-11, Japan
§Department of Applied Chemistry, Science University of Tokyo, Tokyo 162, Japan
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Abstract—N,N-Diallyl derivatives of enkephalin analogues were chemically synthesized, and their biological activities were estimated in in vitro isolated preparations. N,N-Diallyl-[D-Ala², D-Leu⁵]-enkephalin [test compound I] at doses up to 10 μM did not inhibit the electrically-evoked contractions of guinea-pig ileum, which had been suggested to contain opioid mu- and kappa-receptors, but it significantly depressed the contractions of mouse vas deferens, which had been indicated to contain mu-, kappa- and delta-receptors, suggesting that test compound I did not act on both mu- and kappa-receptors, but acted on delta-receptors. Additionally, the Ke (equilibrium dissociation constant) values against test compound I of naloxone were approximately 30 nM and similar to those of Mr 2266, also indicating that test compound I acted as a delta agonist. Moreover, the Ke values of ICI 154129 against compound I were approximately 340 nM, strongly suggesting that test compound I acted as a delta agonist. The Ke values of bis-[N,N-diallyl-[D-Ala², Leu⁵]-enkephaly]-cystine [test compound II] against [D-Ala², D-Leu⁵]-enkephalin in mouse vas deferens and morphine or ethylketocyclazocine in guinea-pig ileum were 44.9 nM and 5.00 or 11.3 μM, respectively, showing that test compound II was a potent selective opioid delta antagonist. In conclusion, among compounds synthesized, two new opioid delta-receptor ligands, one being a highly selective agonist and the other being a potent selective antagonist in in vitro isolated preparations, were found in the present study.

Non-peptide opioid agonists are known to have higher affinity to either mu- or kappa-receptors than delta-receptors. The substitution of a larger moiety such as an allyl, cyclopropylmethyl or furylmethyl group for the N-methyl group on a non-peptide opioid is known to yield an antagonist with higher affinity to either mu- or kappa-receptors than delta-receptors. Thus, substitution of an allyl group for the N-hydrogen group on the tyrosine of enkephalins or their synthetic analogues, which have higher affinity to delta-receptors than either mu- or kappa-receptors, is expected to yield antagonists with higher affinity to delta-receptors than either mu- or kappa-receptors. It has been shown, however, that the N-monoallyl derivative of either [D-Ala², Leu⁵]-enkephalin, [D-Ala², D-Leu⁵]-enkephalin or [D-Ala², Met⁵]-enkephalin-Thr⁶ is a weak mixed agonist-antagonist in in vitro isolated preparations such as guinea-pig ileum and mouse vas deferens and is not a selective delta antagonist (1).

In contrast to N-monoallyl substitution, N,N-diallyl substitution of enkephalin analogues has been reported to exhibit a selective antagonism at the delta-receptor (2).
However, the effectiveness of such delta antagonists as ICI 139462 and ICI 154129 to antagonize the agonist action of delta agonists like enkephalins was approximately ten times lower than that of naloxone (2). In the present investigation, therefore, N,N-diallyl derivatives of potent delta agonists were chemically synthesized, and their biological activities were estimated in order to find a potent delta antagonist.

An unexpected compound, a highly selective delta agonist as well as an anticipated compound, a potent delta antagonist, the effectiveness of which was approximately the same as that of naloxone, were found in the present study.

Materials and Methods

Chemicals: Gifts of compounds which were gratefully received were naloxone-HCl from Sankyo Company (Tokyo); Mr 2266 [(−)-2-(3-furylmethyl)-5, 9-diethyl-2'-hydroxy-6,7-benzomorphan] from Nippon C. H. Boehringer Sohn Co., Ltd. (Osaka); ICI 154129 (N,N-diallyl-Tyr-Gly-Gly-o-(CH2S)-Phe-Leu) (where ψ-(CH2S) signifies replacement of the amide CO-NH bond by CH2S) from Dr. J. W. Holaday, Walter Reed Army Inst. Res. (Washington, D.C., U.S.A.); and ethylketocyclazocine from Sterling-Winthrop Res. Inst. (Rensselaer, New York, U.S.A.). Morphine-HCl was purchased from Takeda Chemical Ind., Ltd. Opioid peptides such as [D-Ala², D-Leu⁵]-enkephalin, N,N-diallyl-[D-Ala², D-Leu⁵]-enkephalin [test compound I] and bis-[N,N-diallyl-[D-Ala², Leu⁵]-enkephaly]-cystine [test compound II] were synthesized by the dimethylphosphinothioic mixed anhydride method in solution (3). The final products were purified by preparative thin layer chromatography and by gel chromatography on Sephadex LH-20 in methanol. The purities of these products were established by elemental analysis and thin layer chromatography.

In vitro isolated preparations: Male ICR-JCL mice weighing 30–40 g and male Hartley guinea-pigs weighing 300–500 g were used for this study. The myenteric plexus-longitudinal muscle strip of guinea-pig ileum and the mouse vas deferens were prepared, and the preparations were set up for electrical stimulation as described previously (4). The % inhibition of the stimulated muscle twitch produced by a drug was plotted against the log concentration of the drug to estimate the IC50 (concentration of the drug to produce 50% inhibition of the twitch). The Kₐ (equilibrium dissociation constant) values of opioid antagonists against opioid agonists were determined by the ‘single’ dose method of Kosterlitz and Watt (5).

Results

Agonist action on isolated preparations: The preliminary experiment showed that both agonistic potency and antagonistic effectiveness of bis-[N,N-diallyl-[D-Ala², Leu⁵]-enkephaly]-cystine [test compound II] were increased by the pretreatment of isolated preparations with peptidase inhibitors such as bestatin, captopril and thiorphan, while those of both ICI 154129 and N,N-diallyl-[D-Ala², D-Leu⁵]-enkephalin [test compound I] were not augmented by these inhibitors. Therefore, all comparative experiments were carried out on the preparation pretreated with 100 nM of bestatin, 1 μM of captopril and 1 μM of thiorphan (6).

ICI 154129 at doses up to 10 μM did not inhibit the electrically-evoked contractions of either mouse vas deferens or guinea-pig ileum (Table 1). In contrast to ICI 154129, test compound I significantly inhibited the contractions of mouse vas deferens. Test compound I at doses up to 10 μM, however, did not inhibit the contractions of guinea-pig ileum (Table 1). On the other hand, test compound II at doses up to 50 nM did not inhibit the contractions of mouse vas deferens. However, it produced the naloxone-reversible slight inhibition of contractions of mouse vas deferens with doses ranging from 0.1 to 0.5 μM. The magnitude of the inhibition produced at the dose of 0.5 μM was approximately 20% and was not significantly different from that induced at the dose of 0.2 μM, indicating that test compound II acted on mouse vas deferens as a weak partial agonist. Test compound II at doses up to 10 μM, however, did not inhibit the contractions of guinea-pig ileum (Table 1).

The Kₐ values against test compound I of naloxone were approximately 30 nM and...
similar to those of Mr 2266 in mouse vas deferens (Table 2), indicating that test compound I acted as a delta agonist on mouse vas deferens (7). Additionally, the Ke values of ICI 154129 against test compound I were approximately 340 nM (Table 2) and similar to those against [D-Ala², D-Leu⁵]-enkephalin (Table 3), also suggesting that test compound I acted as a delta agonist on mouse vas deferens.

**Antagonistic action on isolated preparations** The antagonistic effectiveness of either ICI 154129 or test compounds I and II against either a delta-, mu- or kappa-agonist was estimated in either mouse vas deferens or guinea pig ileum.

The inhibitory action of [D-Ala², D-Leu⁵]-enkephalin, a delta agonist, on mouse vas deferens was antagonized by both ICI 154129 and test compound II. The Ke value of ICI 154129 or test compound II against [D-Ala², D-Leu⁵]-enkephalin was 363 or 44.9 nM, respectively (Table 3). In contrast to test compound II, test compound I at doses up to 200 nM showed no antagonism against the agonist action of [D-Ala², D-Leu⁵]-enkephalin. Additionally, the antagonistic effectiveness of test compound I at doses more than 500 nM could not be estimated due to its agonist action on mouse vas deferens.

The inhibitory action of morphine, a mu agonist, on guinea-pig ileum was antagonized only by high doses of either ICI 154129 or

### Table 1. Agonist actions of ICI 154129 and test compounds I and II on mouse vas deferens and guinea-pig ileum

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mouse vas deferens</th>
<th>Guinea-pig ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>IC₅₀ (nM)</td>
</tr>
<tr>
<td>ICI 154129</td>
<td>5</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Test compound I</td>
<td>8</td>
<td>405±36</td>
</tr>
<tr>
<td>Test compound II</td>
<td>6</td>
<td>&gt;500⁴</td>
</tr>
</tbody>
</table>

All preparations were pretreated with 100 μM of bestatin, 1 μM of captopril and 1 μM of thiophan. 

⁴N.N-Diallyl-[D-Ala², D-Leu⁵]-enkephalin.

⁴Bis-[N,N-diallyl-[D-Ala², Leu⁵]-enkephalin]-cystine.

Mean±S.E. The magnitude of the inhibition produced at the dose of 500 nM was approximately 20% and similar to that induced at the dose of 200 nM.

### Table 2. The Ke values of naloxone, Mr 2266 and ICI 154129 against test compound I in mouse vas deferens

<table>
<thead>
<tr>
<th>Opioid</th>
<th>Ke (nM)</th>
<th>Mr 2266</th>
<th>ICI 154129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naloxone</td>
<td>30.2±3.1</td>
<td>27.3±0.72</td>
<td>336±34</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 4 experiments.

### Table 3. Antagonistic effectiveness of ICI 154129 and test compounds I and II against agonist action of [D-Ala², D-Leu⁵]-enkephalin on mouse vas deferens

<table>
<thead>
<tr>
<th>Compounds</th>
<th>n</th>
<th>Ke (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICI 154129</td>
<td>3</td>
<td>363±49</td>
</tr>
<tr>
<td>Test compound I</td>
<td>4</td>
<td>No antagonism at 200 nM⁵</td>
</tr>
<tr>
<td>Test compound II</td>
<td>4</td>
<td>44.9±3.1⁶</td>
</tr>
</tbody>
</table>

All preparations were pretreated with 100 μM of bestatin, 1 μM of captopril and 1 μM of thiophan. 

⁵Mean±S.E. ⁶The effect of doses more than 200 nM could not be determined due to the agonist action.
test compound II (Table 4). Additionally, higher doses of either ICI 154129 or test compound II were required to antagonize the inhibitory action of ethylketocyclazocine, a kappa agonist, than that of morphine.

### Discussion

Both a highly selective agonist and a potent selective antagonist to opioid delta receptors were found in the present investigation.

The fact that test compound I does not have an agonist activity in guinea-pig ileum, which has been indicated to contain both opioid mu- and kappa-receptors (8), but have a naloxone-reversible agonist activity in mouse vas deferens, which have been suggested to contain opioid mu-, kappa- and delta-receptors (4, 7, 9), suggests that test compound I does not act on either mu- or kappa-receptors as an agonist, but acts on delta-receptors as an agonist. Moreover, the fact that the agonist action of test compound I on mouse vas deferens is antagonized by either naloxone or Mr 2266 only at the relatively high \( K_e \) values (27–31 nM) also suggests that test compound I acts as a delta agonist on mouse vas deferens, since naloxone has been shown to have a high affinity to mu-receptors (\( K_e \) values ranging from 1 to 5 nM) and relatively low affinity to both kappa- and delta-receptors (\( K_e \) values ranging from 10 to 50 nM) (7, 9), while Mr 2266 has high affinity to both mu- and kappa-receptors (\( K_e \) values ranging from 1 to 5 nM) and relatively low affinity to delta-receptors (\( K_e \) values ranging from 10 to 50 nM) (7). Finally, the observation that the agonist action of test compound I on mouse vas deferens is antagonized by ICI 154129, which is a weak but highly selective antagonist of delta-receptors (2), with an average \( K_e \) value of 336 nM, strongly indicates that test compound I acts as a delta agonist on mouse vas deferens, since the range of the \( K_e \) values of ICI 154129 against delta agonists has been reported to be from 250 to 900 nM (2). In contrast to two highly selective delta agonists, deltakkephalin (10) and [D-Pen\(^2\), D-Pen\(^5\)]-enkephalin (11), the characteristic feature of test compound I is that it has no agonist action at all on guinea-pig ileum at doses up to 10 
M, while the two already reported delta agonists have been shown to have agonist actions on guinea-pig ileum, IC\(_{50}\) values of deltakkephalin and [D-Pen\(^2\), D-Pen\(^5\)]-enkephalin being 0.46 and 6.9 
M, respectively (10, 11).

The fact that the \( K_e \) value of test compound II against [D-Ala\(^2\), D-Leu\(^5\)]-enkephalin in mouse vas deferens is 44.9 nM shows that the effectiveness to antagonize the agonist action of the delta agonist of compound II is high enough and similar to that of naloxone.

Additionally, the fact that the \( K_e \) value of test compound II against either a mu or kappa agonist is more than 100 times higher than that against a delta agonist shows that the selectivity of test compound II is quite high, since the \( K_e \) value of naloxone against either a kappa or delta agonist is approximately 10 times higher than that against a mu agonist (7–9). While this manuscript was in preparation, it was reported that a new selective delta antagonist, ICI 174864, was equipotent with naloxone (12). Thus, test compound II seems to be equipotent to ICI 174864 as a delta antagonist.

It is apparent that both selective agonists and antagonists are quite useful for studying...
the physiological and pathophysiological roles of opioid delta receptors. By using test compound I, effects of sodium and 5'-guanylylimidodiphosphate (Gpp(NH)p), a nonhydrolyzable derivative of GTP, on the inhibition of [3H]-naloxone binding to guinea-pig brain membrane preparations were recently studied. The ratio of the concentration required to produce a 50% inhibition of [3H]-naloxone binding in the presence of both Gpp(NH)p and sodium to that in the absence of both Gpp(NH)p and sodium was less than 1 in antagonists, from 3 to 10 in mixed agonist-antagonists, from 16 to 300 in agonists other than test compound II, and less than 1 in test compound I (13), indicating the quite interesting possibility that opioid delta-receptors are not coupled to adenylate cyclase, while both mu- and kappa-receptors are coupled to this enzyme (14). More detailed experiments are obviously required, however, to determine whether or not opioid delta receptors are coupled to adenylate cyclase.

References


