REVIEW —Current Perspective—

Behavioral Effects of δ -Opioid Receptor Agonists: Potential Antidepressants?[†]

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ABSTRACT—The development of selective δ -opioid receptor agonists has revealed some very intriguing behavioral properties. δ -Opioid agonists have antinociceptive, seizuregenic and convulsive properties. A number of studies have identified a novel behavioral effect of δ -opioid-receptor agonists, implicating a role for the δ -opioid receptor in depression. Early clinical experiments demonstrated that exogenously administered opioid peptides had antidepressant activity in human patients. Also, enkephalinase inhibitors, which prevent the degradation of endogenous enkephalins, produced antidepressant-like effects mediated through the δ -opioid receptor in animal models of depression. More recently, the selective non-peptidic δ -opioid agonists SNC80 and (+)BW373U86 demonstrated antidepressant-like activity in the forced swim assay in rats. These studies propose that the δ -opioid receptor may provide a new therapeutic target for treating human depression.

Keywords: δ -Opioid agonist, Forced swim assay, Depression, Enkephalin

Agonist action at the δ -opioid receptor has some very intriguing pharmacological consequences. Moreover, the development of novel and selective ligands for this receptor has clarified some aspects of δ -opioid agonist actions and their relation to behavior. Certain effects mediated through the δ -opioid receptor are similar to those mediated via μ - and κ -opioid receptors, while others are unique and thus, are specific. Antinociception produced via the δ -opioid receptor is perhaps its most studied effect and is an effect shared with other opioid receptors. A further effect that was seen upon the development of nonpeptidic δ -receptor agonists was a unique and specific convulsant activity. Finally, along with other opioid receptor systems, δ -receptor systems have been implicated as playing a role in depression. Recent studies performed in our laboratory examining nonpeptidic δ -opioid receptor agonists in animal models predictive of antidepressant activity support such observations.

The ability of δ -opioid receptor agonists to produce anti-

nociceptive effects has been reviewed (1, 2) and thus will only be discussed briefly. δ -Opioid receptor agonists are relatively weak at producing antinociception when compared to μ -receptor agonists. Initial studies indicated that δ -selective peptides such as the enkephalin analog [D-Pen², D-Pen⁵]enkephalin (DPDPE) produced antinociception that was reversed by the δ -selective antagonist ICI 174864. More recently, the development of nonpeptidic δ -opioid receptor agonists has produced further evidence for δ -opioid receptor mediated antinociception with the benzamide BW373U86 producing antinociceptive effects upon intrathecal (i.t.) injection in the mouse (3). These effects were reversed by the selective δ -antagonists ICI 174864 and naltrindole. Furthermore, SNC80, the methyl ether derivative of the (+) isomer of BW373U86, was also found to produce naltrindole-reversible antinociception in certain assays (4). Finally, studies using primate models of pain also added to the evidence that nonpeptidic δ -receptor agonists have weak antinociceptive properties (5).

The convulsive effects of opioids as well as their ability to modulate models of convulsions has been debated for some time. Both pro- and anticonvulsive effects have been

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seen with endogenous and exogenous opioid compounds (6). Early studies using morphine showed non-opioid convulsions at high doses of the drug (7). Naloxone-sensitive convulsions were also demonstrated, although high doses of the antagonist were used to reverse the convulsions in most cases (8). Thus, under certain conditions, morphine and other opiates may produce convulsions by both opioid-specific and, at higher doses, nonspecific mechanisms.

The δ -opioid receptor has been featured predominantly in work studying the seizuregenic and convulsive effects of opioid peptides. Initial work performed with centrally administered enkephalins and β -endorphin indicated that these compounds produced epileptiform changes in EEG activity (reviewed by (4)). In some situations, certain physical manifestations of such seizuregenic EEG activity were witnessed (9). These were limited to myoclonic and masticatory jaw movements.

The development of δ -opioid receptor selective peptides allowed the convulsant and seizuregenic properties of δ -opioid receptor stimulation to be studied directly. Tortella and coworkers (10) found that the δ -selective peptides [D-Pen², D-Pen⁵]enkephalin (DPDPE) and [D-Pen², L-Pen⁵]enkephalin (DPLPE) produced a complex EEG response in rats but with no EEG seizure or accompanying physical convulsions. In the same study, DADLE, an opioid peptide with activity at both μ - and δ -opioid receptors was seen to cause non-convulsive EEG seizures that were antagonized

by low doses of naloxone. Consequently, it was concluded that the seizuregenic activity of DADLE was due to activity at μ -opioid receptors.

In contrast to the studies performed by Tortella and colleagues, studies by Haffmans and Dzoljic (11) found seizuregenic activity attributable to the δ -opioid receptor. In these studies, the δ -opioid receptor selective peptide [D-Ser², Leu⁵]enkephalin-Thr (DSLET) was seen to produce epileptiform changes in the electrocorticogram of rats that correlated with myoclonic contractions. This effect was antagonized by the δ -opioid receptor selective antagonist ICI 174864 but not equimolar concentrations of naloxone, indicating that these effects of DSLET were due to δ - rather than μ -opioid receptor activity. The results of this study were strengthened by the fact that De Sarro and colleagues (12) discovered that microinfusion of [D-Ala²]deltorphin, a δ -selective opioid agonist, into the dorsal hippocampus produced naltrindole-reversible epileptogenic effects on the electrocorticogram with associated wet-dog shakes. Thus, the role of the δ -receptor in opioid-mediated seizures and convulsions became more apparent.

The first nonpeptidic δ -opioid receptor agonist, BW373U86 (see Fig. 1a for structure) was examined for convulsive activity by Comer and colleagues (13). After systemic injection, it was found that this compound caused dose-dependent convulsive activity in mice. However, unlike the convulsant agent pentylentetrazol, BW373U86

Fig. 1. The structure of nonpeptidic δ -opioid receptor agonists: a) BW373U86, b) SNC80, c) (+)BW373U86 and d) BU48.

afforded only a single convulsive episode that was not lethal at doses that induced a convulsion in all mice. The convulsion was followed by a short period of Straub tail and subsequently by a few minutes of catalepsy. These effects were antagonized by naltrindole, naltrexone and midazolam clearly indicating the involvement of the δ -opioid receptor and GABAergic systems in producing these effects. Comer and colleagues (13) also discovered that a very rapid tolerance developed to the convulsant effects of BW373U86 and that this tolerance could be blocked by post-convulsant naltrindole given up to 1 h after the initial convulsion. More recently, studies examining SNC80 (see Fig. 1b for structure), the (+) isomer of the methyl ether of BW373U86, showed that this compound had similar convulsant effects to BW373U86 in mice including tolerance development and naltrindole and midazolam reversibility (14). Furthermore, BU48 (see Fig. 1d for structure), a ring constrained buprenorphine analogue that is structurally unrelated to BW373U86, was also found to produce naltrindole-reversible convulsions in mice similar to those produced by BW373U86 and SNC80 (15).

Despite the examination of the convulsant properties of nonpeptidic δ -opioid receptor agonists in mice, the convulsant properties of these compounds has not been well characterized in other species. Nonetheless, convulsant activity was reported upon administration of BW373U86 to rhesus and squirrel monkeys (5, 16), and recent studies in our laboratory demonstrated dose-dependent, naltrindole-sensitive convulsant activity in rats (17).

Studies have also demonstrated a potential role of endogenous opioid systems in the mechanism of action of known antidepressant compounds such as desipramine and imipramine. It was found that chronic administration of imipramine increased the number of [3 H]naloxone binding sites in rat brain (18). Conversely, the opioid antagonist naloxone reversed the antidepressant-like activity of desipramine or clomipramine in the inescapable footshock assay in rats (19). Further interaction between imipramine and opioid systems was evident by a study showing that the antidepressant inhibited enkephalin-degrading aminopeptidase in rat brain homogenate (20). It was also found that chronic administration of a number of known antidepressants to rats increased the levels of immunoreactive enkephalins in regions of the brain (21). Similarly, upregulation of [Met 5]enkephalin was seen in rats given repeated electroconvulsive shock therapy (22). Intriguingly, other studies found that direct inhibitors of enkephalinases produce antidepressant-like profiles in animal models of depression. Baamonde and coworkers (23) discovered that RB101, a mixed enkephalinase inhibitor that inhibits both enzymes thought to be responsible for enkephalin degradation, aminopeptidase N and endopeptidase 24.11, produced an antidepressant-like profile of action in the forced swim

assay in mice. This activity was prevented by naltrindole, thus implicating a role for the δ -opioid receptor. Furthermore, in a rat learned helplessness procedure, a greater naloxone-reversible antidepressant-like effect was found with RB38A, a mixed enkephalinase inhibitor, than with RB38B, a selective endopeptidase 24.11 inhibitor (24). This indicated that a more complete blockade of enkephalin metabolism produces a greater antidepressant-like effect. This effect was later seen to be preventable by the addition of opioid antagonists (25). Finally, BL-2401, a novel, orally active enkephalinase inhibitor, was observed to have antidepressant-like activity that was reversed by naloxone in the forced swim assay in mice (26).

These studies produced substantial evidence for the role of endogenous opioid systems, particularly δ , in animal models of depression. This suggestion was strengthened by the finding indicating that the δ -opioid selective agonist BUBU (Tyr-D-Ser(*O*-*tert*-butyl)-Gly-Phe-Leu-Thr(*O*-*tert*-butyl-OH)) produced antidepressant-like effects in a rat learned helplessness model similar to those seen with RB101 (27). Also, Filliol and colleagues (28) found that, in mice lacking the δ -opioid receptor, altered emotional responses were observed. These included a depressive-like response in the forced swim assay. Thus, it appears as if the δ -opioid receptor system has a role in maintenance of a positive emotional state, at least in rodents. This may partly explain the results indicating that in models of depression, potentiation of this system, either by administering exogenous opioid agonists or agents that inhibit degradation of endogenous opioid agonists, can produce antidepressant-like effects.

A number of lines of evidence have implicated a role for opioid receptor systems in depression, including early studies investigating the potential antidepressant therapy of opioids, opioid antagonists and endogenous peptides in humans. For example, when injected into depressed patients, synthetic β -endorphin was found to have a rapid antidepressant-like action (29). Subsequently, β -endorphins were found to be elevated in patients receiving electroconvulsive shock (ECS) for treatment of depression (30, 31). Thus, it may be possible that part of the antidepressant activity of ECS is due to its effect on endogenous opioid systems. Further evidence for a role of opioid systems in human depression can be inferred from an observation that administration of the opioid antagonist naltrexone to volunteer subjects produced depressive effects (32). Taken together, these data strongly suggest a role of opioid systems in models of depression and possibly even depression in human subjects.

Recent studies performed in our laboratory examined the effect of opioid receptor agonists on the rat forced swim assay (33). This assay involves measuring the immobility, swimming and climbing behaviors of rats upon subsequent

exposures to swimming (34). All currently approved therapeutic antidepressant compounds are active in the assay by decreasing immobility scores compared to vehicle controls (35). Therefore, the assay can be used with predictive validity to screen for novel compounds with potential antidepressant activity.

Like the known antidepressants, desipramine (Fig. 2a) and fluoxetine (Fig. 2b), the δ -opioid receptor agonist (+)BW373U86 produced a decrease in immobility indicating an antidepressant-like effect (Fig. 2c). A δ -opioid mechanism of action in this effect was likely since naltrindole prevented the agonist effect (Fig. 2d). Although changes in locomotor activity are observed with δ -opioid agonists, it is unlikely that these effects are contributing to the changes seen in the forced swim assay as a dose of

10 mg/kg SNC80 produced a significant increase in locomotor activity but no significant effect in the forced swim assay (33). Furthermore, the activity of SNC80 in the forced swim assay remained until 3 h post-injection, whereas locomotor activity levels had returned to control levels after approximately 2 h post-injection. Thus, from these data, it may be concluded that nonpeptidic δ -opioid receptor agonists have an antidepressant-like effect mediated via the δ -opioid receptor.

The convulsion seen upon injection of δ -selective agonists does not appear to play a role in the antidepressant-like activity seen in the forced swim assay (Fig. 3). Although the dose-effect curves for convulsant and antidepressant-like activity overlap and despite the fact that both effects are naltrindole reversible, animals pretreated

Fig. 2. The effects of a) desipramine and b) fluoxetine on activity in the forced swim assay in rats. c) The antidepressant-like profile of action of (+)BW373U86 in the forced swim assay in rats. (+)BW373U86 dose-dependently decreased immobility counts while increasing swimming and climbing counts. d) Naltrindole reverses the antidepressant-like activity of (+)BW373U86, thus implicating the δ -opioid receptor in this effect. The solid bar represents immobility, diagonal single-hatched bar represents swimming and diagonal double-hatched bar represents climbing activity. All groups shown in graph d received 10 mg/kg (+)BW373U86. Data is presented fully in ref. 33 and ref. 17. Numbers in parentheses correspond to n. * P <0.05 and *** P <0.001, when compared to vehicle alone by Dunnett's post-hoc test.

Fig. 3. Midazolam (MDZ) prevents a) the convulsant effects but not b) the antidepressant-like effects of (+)BW373U86. Data is presented fully in ref. 17. The solid bar represents immobility, diagonal single-hatched bar represents swimming and diagonal double-hatched bar represents climbing activity. Treatment groups are as follows: V, MDZ vehicle followed 15 min later by (+)BW373U86 vehicle; MDZ, 0.5 mg/kg MDZ followed by (+)BW373U86 vehicle; (+)BW, MDZ vehicle followed by 10 mg/kg (+)BW373U86; MDZ-(+)BW, 0.5 mg/kg MDZ followed by 10 mg/kg (+)BW373U86. $n=6$ for each group. $*P<0.05$ and $***P<0.001$, when compared to vehicle alone (V) by Dunnett's post-hoc analysis.

with the ultra-short acting benzodiazepine midazolam displayed no convulsant activity but significant antidepressant-like activity in the forced swim assay. In fact, the level of effect in the forced swim assay was identical between animals pretreated with midazolam as those not pretreated with midazolam. Finally, tolerance was also seen to develop to the convulsant effects of (+)BW373U86 without apparently developing to the antidepressant-like effects. Thus, these animals displayed antidepressant-like activity in the absence of convulsant activity, indicating a separation of the two effects.

Concluding remark

Agonists selective for the δ -opioid receptor have some unique and intriguing behavioral effects. The previously

described convulsant activity along with the newly described antidepressant-like effects appear to be characteristic of nonpeptidic δ -opioid selective agonists. These effects, along with effects traditionally associated with opioid ligands such as antinociception and locomotor activity changes, provide an array of behavioral effects by which to measure the activity of these compounds. Further study of these effects may prove to be of significant scientific and possibly therapeutic benefit. Therefore, the δ -opioid receptor, although perhaps the least studied of the three opioid receptors, continues to be an extremely interesting focus for the study of the behavioral effects of opioid ligands.

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