DELTA OPIOID ANALGESICS

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Opium, morphine and related natural and synthetic opiates have been used since antiquity, and to the present, for the relief of moderate and severe pain. Morphine and pharmacologically related drugs, however, produced an array of undesired or dangerous side effect that limit their use as analgesics. Prominent among the limiting side effects are constipation, respiratory depression, release of prolactin, and liability for the production of drug dependence.

It was our aim to develop, if possible, a drug or class of drugs with analgesic activity similar to that of morphine, but without the serious side effects associated with morphine. Our overall strategy was to take advantage of advancing knowledge concerning multiple types of opioid receptors, to develop ligands selective for the delta type receptors, to determine whether delta receptor agonists offer advantages over mu agonists, then to design compounds with pharmacokinetic properties compatible with practical therapeutic application. All but the last of these objectives have been realized.

MULTIPLE OPIOID RECEPTORS

At about the same time as the discovery, in 1975 (Hughes et al., 1975), of endogenous opioid substance, Martin and colleagues (Martin et al., 1976) postulated the existence of multiple types of opioid receptors. Studies of natural and synthetic enkephalins in vitro led to confirmation of the multiple receptor hypothesis (Lord et al., 1977). Briefly, morphine and related drugs were postulated to act primarily at mu opioid receptors, ketocyclozocine and related drugs primarily at kappa receptors, and enkephalins at least in part at delta opioid

receptors (Martin et al., 1976; Lord et al., 1977). While relatively selective ligands for mu and kappa opioid receptors were then available, there were no highly (at least 100-fold) selective ligands and certainly none that was selective for the delta opioid receptor.

DESIGN STRATEGY

Substitution of unnatural amino acids in the pentapeptide enkephalin sequence was found to confer resistance to enzymatic degradation and, in some cases, highly mu-selective peptides (Handa et al., 1981). Because linear peptides are highly flexible, our approach was based on creation of constrained cyclic peptide derivative with high affinity for delta opioid receptors, but low affinity for mu and kappa receptors. Synthetic peptides were evaluated in ligand binding and in bioassay with in vitro mouse vas deferens (delta) and guinea pig ileum longitudinal muscle-myenteric plexus (mu and kappa). Continuous progress was made and in 1983 cyclic [D-Pen^2, D-Pen^5] enkephalin (DPDPE) was reported (Mosberg et al., 1993). At about the same time, the highly kappa-selective nonpeptide, U-50,488H, was announced (VonVoigtlander et al., 1983), as well as the mu-selective peptide, PL017 (Chang et al., 1983).

Ligand binding and bioassay experiments confirmed the delta receptor selectivity of DPDPE (Mosberg et al., 1983; Corbett et al., 1984). In bioassays, which assess both receptor affinity and efficacy, DPDPE was found some 1000-fold selective for delta over mu receptors and 40,000-fold selective for delta in comparison with kappa opioid receptors.

DELTA OPIOID ACTIONS

Analgesia

In vivo experiments were conducted in mice and rats to characterize the actions of DPDPE and thereby define, for the first time, those opioid effects that are attributable to agonist actions at delta receptors.

DPDPE was found in rats (Galligan et al., 1984) and mice (Porreca et al., 1984) to produce dose-related analgesia after intracerebroventricular (i.c.v.) injections. The analgesic effects were blocked by naloxone and thus were mediated by naloxone-sensitive opioid receptors.

However, it was necessary to prove, through a series of experiments, that DPDPE produced analgesia by virtue of actions at cerebral delta receptors, not by virtue of its very weak actions at mu receptors. It was found that the actions of DPDPE, but not those of DAMGO (Handa et al., 1981) were blocked by ICI174,864, a selective delta antagonist. On the other hand, analgesic responses to DAMGO, but not to DPDPE, were blocked by a selective mu antagonist, ß-flunaltrexamine (ß-FNA) (Heyman et al., 1987). These experiments established the principle that differential blockade by receptor-selective antagonists could be used as a means of identifying delta opioid actions separately from mu actions.

In experiments in mice it was shown that DPDPE could produce delta opioid receptor-mediated analgesia also after intrathecal (i.t.) administration (Porreca et al., 1984).

Gastrointestinal Transit

Studies in unanesthetized rats (Galligan et al., 1984) and mice (Porreca et al., 1984) demonstrated great qualitative differences among multiple types of supraspinal opioid receptors in regard to inhibition of gastrointestinal transit or propulsion, a useful model of ability of opioids to produce constipation. Mu opioids, such as morphine and DAMGO, produced dose-related inhibition of gastrointestinal transit after i.c.v. injection whereas DPDPE had little or no effect, even when administered in equianalgesic doses. These results gave, for the first time, a clear indication that delta selective opioids could produce analgesia without certain adverse side effects associated with morphine-like opiates.

It was found that DPDPE produced little antitransit effects in mice after i.c.v. or peripheral (s.c.) injection (Porreca et al., 1984; Shook et al., 1987). Moreover, it was shown that a selective mu antagonist, CTP, blocked the peripheral antitransit effects of mu agonists, but of course, had no effect on DPDPE, which was inactive when given peripherally (Shook et al., 1987). When given i.t., DPDPE produced dose-related antitransit effects in mice, showing that brain and spinal delta receptors are linked differently to neural pathways regulating gastrointestinal motility (Porreca et al., 1984).

Gastric Secretion

Before the advent of receptor-selective opioid agonists, the effects of opiates on gastric acid secretion were very confusing (Kromer, 1988). We used DPDPE, DAMGO, PL017 and U-50,488 in pylorus-ligated rats to determine the roles, if any, of brain and peripheral opioid receptors in regulation of gastric secretion in unanesthetized rats (Fox and Burks, 1988). Both locations of receptors and agonist profiles were found to be important. DAMGO, PL017 and morphine, but not DPDPE or U-50,488, inhibited gastric secretion after i.c.v. injection. DAMGO, which does not cross the blood-brain barrier effectively, had no effect after peripheral (i.v.) injection, showing that only brain mu receptors are involved in inhibition of gastric secretion. DPDPE was inactive after i.v. injection, showing that neither brain nor peripheral delta receptors regulate gastric secretion. Finally, U-50,488 given i.v. produced a dose-related stimulation of gastric secretion, suggesting an excitatory effect of peripheral kappa receptors.

Mucosal Transport

Much of the antidiarrheal effect of opiates resides in their ability to inhibit excessive secretion of salt and water from the blood, across the intestinal mucosa, and into the lumen of the intestine (Awouters et al., 1993). In vitro in Ussing chambers, DPDPE and mu agonists (DAMGO, morphine) were found to decrease short-circuit current in mouse isolated jejunum and jejunal mucosa, indicative of antisecretory activity in the intestinal mucosa (Sheldon et al., 1990). In fact, DPDPE was more potent in terms of antisecretory activity than the mu agonists. Experiments with tetrodotoxin showed that the mucosal transport effects of delta opioids are neurally mediated, presumably by actions on secretomotor neurons arising from the submucosal plexus of the enteric nervous system.

These results indicated that while delta receptors may not occur in neural pathways that produce inhibition of intestinal propulsion, they may occur in pathways that regulate mucosal transport, thus suggesting possible antidiarrheal effects.

Antidiarrheal Effects

In mice treated orally with castor oil or given i.p. injections of prostaglandin E_2 (PGE$_2$)

to induce diarrhea, DPDPE produced significant antidiarrheal effects. The delta agonist was effective when administered peripherally (s.c.), i.c.v. or i.t. (Shook et al., 1989; Lemcke et al., 1991). Mu opioids were also active in these models by all three routes of administration and the amplitudes of the mu and delta effects were similar. Kappa opioids, on the other hand, were not effective or only modestly effective in these models. Interestingly, the rank order of potency of both mu and delta opioids in inhibiting PGE_2-induced diarrhea was i.c.v. > i.t. > s.c., indicating that opioid sites in the central nervous system can exert powerful effects on mucosal transport.

The studies also demonstrated separation between opioid antitransit effects and opioid antidiarrheal effects. This is, DPDPE given i.c.v. or s.c. inhibited diarrhea without inhibiting transit, thereby indicating that nonconstipating opioid antidiarrheal actions may be possible. As antipropulsive or constipating effects of mu opioids are undesirable, even in diarrhea (Runkel et al., 1993), the delta opioids may hold advantages over mu opioids as antidiarrheal drugs.

Thermoregulation

While the effects of opioids on body temperature regulation are not of great therapeutic importance, it was of interest to determine whether delta opioids would produce thermoregulatory effects similar to those produced by mu opioids. Nonrestrained rats with implanted radiotelemetry thermistors were employed to study body temperature effects of i.c.v. administered mu, delta and kappa opioids. In these experiments, mu agonists produced dose-related hyperthermia, DPDPE produced dose-related hypothermia, while kappa agonists produced a complex pattern of hypo- and hyperthermia (Spencer et al., 1988). The thermoregulatory effect of mu and delta opioids is one of the few instances in which the two types of opioid receptors appear to mediate opposite responses (Burks, 1991).

Release of Prolactin

Rats received i.c.v. infusions with equianalgesic doses of DAMGO (mu), DPDPE (delta) and U-50,488 (kappa) and blood levels of prolactin were measured (Leadem and Yagenova, 1987). DAMGO produced dose-related elevation of prolactin, U-50,488 produced erratic elevations, and DPDPE had no effect on prolactin levels. This experiment indicates that delta

opioids may not be associated with the undesirable release of prolactin characteristic of mu opioids.

Respiration

Depression of respiration is one of the most serious side effects produced by morphine and allied opiates. The prototype highly selective delta agonist, DPDPE, has not been found to depress respiration in the analgesic dose range. In conscious rabbits, i.c.v. DPDPE did not alter respiratory rate or blood gases (May et al., 1989). In fetal lambs, i.c.v. DPDPE was found to increase respiratory rates (Cheng et al., 1992). In conscious rats, i.c.v. DPDPE produced stimulation of minute volume of respiration (Kramer et al., 1992). Some authors have concluded that delta receptors are involved in opioid respiratory depression, but these conclusions are based on effects of nonselective peptides, such as DADLE (Yeadon and Kitchen, 1989).

Dependence

From both the medical and social points of view, opioid dependence is a significant adverse event associated with mu agonists, such as morphine. Studies to date indicate that DPDPE, like kappa agonists, produces little or no dependence liability as assessed in animals. For example, DPDPE withdrawal does not result in the wet dog shaking behavior characteristic of withdrawal of rats from mu opioids (Lee et al., 1989).

The pharmacological effects of delta, mu and kappa opioids are summarized in Table 1.

DELTA-MU INTERACTIONS

A series of important experiments has shown that DPDPE potentiates (produces supraadditive effects) analgesic responses to morphine, normorphine and codeine (Heyman et al., 1989; Jiang et al., 1990a, 1990b). In these studies, subthreshold doses of DPDPE were administered in conjunction with a complete analgesic dose range of morphine. The dose-response curve was shifted to the left for morphine analgesia in the presence of DPDPE

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Table 1. Summary of opioid actions

Pharmacological endpoint	Mu	Delta	Kappa
Analgesia			
Hot plate	+++	+++	+
Tail flick	+++	+++	+
Abdominal stretch	+++	+++	++
Gastrointestinal propulsion	↓	0	0
Gastric acid secretion	↓	0	↑
Mucosal transport	↓	↓	↓
Antidiarrheal effects	+++	+++	+
Change in body temperative	↑	↓	↑↓
Release of prolactin	↑	0	↑
Respiration	↓	↑	↓
Dependence liability	+++	±	±

treatment. The potentiating effect of DPDPE was selectively blocked by delta receptor antagonists. Moreover, coadministration of DPDPE did not enhance development of tolerance to morphine, thereby raising the possibility that analgesic combinations of DPDPE and morphine, which would contain less morphine than if morphine were used alone, could provide effective analgesia with less tolerance than would be observed with morphine alone.

These data were essentially confirmed in other studies by Adams et al. (1993), who found that the optimal dose ratio of DPDPE to morphine in rats is 20% DPDPE to 80% morphine (by weight).

DELTA RECEPTOR SUBTYPES

A growing body of evidence suggests that there are two subtypes of the delta opioid receptor, δ_1 and δ_2. The pharmacological characteristics of the δ_1 subtype include agonist actions by DPDPE and antagonist actions by [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE). For the δ_2 subtype, the prototype agonist is [D-Ala^2]deltoprhin and antagonists are the benzofuran derivative of naltrindole (NTB) and the 5'-isothiocyanate of naltrindole (NTII) (Porreca and Burks, 1993).

Both subtypes of delta receptors can mediate analgesia (Jiang et al., 1991; Stewart and Hammond, 1993).

NEW DIRECTIONS

A continuing program of chemical design and synthesis has led to modifications of the DPDPE molecule that have resulted in analogues possessing improved selectivity for the delta opioid receptor. For example, it was found that para-halogenation of the benzene ring of the phenylalanine residue in position 4 of DPDPE increased delta selectivity over the parent peptide (Toth et al., 1989). In ligand binding, [p-Cl-Phe^4]DPDPE is approximately 4-fold more selective for delta receptors than DPDPE and, in bioassay, [p-I-Phe^4]DPDPE is some 8-fold more selective than DPDPE. Other Phe^4 modified analogues also have improved selectivity. For example, [(S,S)ß-Me-p-NO$_2$-Phe^4]DPDPE is approximately 3-fold more selective than DPDPE (Hruby et al., 1989). In vivo, all of these analogues produced analgesia similar to that of DPDPE, indicating that analgesic responses are mediated by delta opioid receptors and do not result from action of the compounds at mu receptors.

The pharmacokinetic properties of the peptides related to L?DPE do not favor their use as analgesics given by systemic administration (Weber et al., 1991). Penetration of the blood-brain barrier by these peptides is poor and they are rapidly removed from the circulation by hepatic extraction and are secreted in bile. To avoid the pharmacokinetic disadvantages of peptides, efforts to develop nonpeptide molecules with the pharmacodynamic characteristics of DPDPE have been initiated. Chemical design to date has focused on modifications of the somewhat delta selective molecule, BW373U86, which produces analgesia in laboratory animals (Chang et al., 1993; Wild et al., 1993). The lead compound is only about 7-fold selective for delta over mu receptors in ligand binding assays, but somewhat more selective in bioassays. We are optimistic that improvements in selectivity will be possible.

ACKNOWLEDGEMENTS

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