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Review

Development of Delta Opioid Peptides as Nonaddicting Analgesics

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Although much effort has been devoted to opioid research since the identification of enkephalins, understanding of the physiological importance and mechanisms of action of endogenous opioids lags behind understanding of opiate alkaloids such as morphine. In recent years, several novel approaches have been refined with promise for the successful development of the long-awaited nonaddicting analgesics that act at the opioid delta receptor. The present communication reviews these efforts.

KEY WORDS: delta opioid peptides; nonaddicting analgesics; drug development; peptide drugs.

INTRODUCTION

Nearly 15 years after the initial euphoria associated with the discovery of the endogenous opioids (1), the immediate promise of novel therapeutic agents derived from these substances has yet to be fulfilled, and many pharmaceutical companies have canceled their long-standing opioid research programs. Does such pessimism reflect the realities of the development of peptides as drugs, or is there still cause to envision useful compounds emerging from the initial discovery of endogenous opioids? Recent developments in the synthesis of peptide analogues, peptidomimetics, and nonpeptides suggest that abandoning the effort for novel applications of opioid peptides is premature. Indeed, it would be gratifying if peptides could be developed as drugs because of their unique properties. Opioid peptides will be unique therapeutic agents (2) when compared to opiates for the following reasons.

1. It is anticipated that peptide analogues of enkephalins and related peptides will not be able to cross the placenta because of degradation by placental enzymes and, hence, could serve as obstetric medications, providing analgesia for the mother without exposing the fetus (3,4). Thus, peptides that degrade during their placental passage could be superior to the commonly used opiates (meperidine).

   Further,, the opioid delta receptor cannot be demonstrated in human fetal brain tissue. Thus, unlike mu and kappa opioids, delta agonists offer an additional level of safety for the fetus.
2. On metabolic degradation, peptides will be hydrolyzed to their constituent amino acids, and the metabolic end products, unlike the opiates, are polar, easily eliminated from the body, and unlikely to cause liver or kidney damage.
3. From the drug design aspects, peptides offer special advantages. As peptides are made up of subunits, amino acid residues, virtually an unlimited number of analogues can be synthesized. As peptides are conformationally labile, the three-dimensional architecture of the peptides can be altered by incorporating various structural modifications (such as unnatural amino acids, *N*-methyl substituents, peptide bond replacements, formation of cyclic structure, etc.) to achieve a desired pharmacophoric conformation. Thus, manipulation of three dimensional structure to obtain a desired bioactivity is easier with opioid peptides than with structurally rigid opiates.
4. Peptides are endogenous. They serve as better models for studies on biosynthesis and conformation. As peptides are polar molecules, solution studies can be performed in different solvents and with different ions to understand the effects of solvents, etc., on conformation. In addition, techniques for peptide synthesis have been simplified and are automated (solid phase method).
5. Peptide agonists for the opioid delta receptor are likely to have decreased dependence/abuse liability and lower reinforcing efficacy.
6. Peptide agonists for the opioid delta receptor do not demonstrate significant analgesic cross-tolerance to opiates acting at the mu receptor such as morphine; thus, they are likely to be useful pain relievers in patients undergoing prolonged therapy or high-dose treatments with mu opiates (5).

Hence, design of biologically active peptides as analgesics would be highly desirable.

BACKGROUND

Efforts to gain insight into the pharmacological rele-

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vance of the various opioid receptor types have been especially hampered by the lack of stability of the endogenous opioid peptides. Thus, it was not possible to simply inject these peptide substances and measure a robust pharmacological response. This situation has delayed clear understanding of the relevance and potential therapeutic importance of the opioid delta receptor. This opioid receptor was postulated on the basis of rank order of potency of enkephalins and opiate alkaloids in two classical bioassays exclusively used by Hans Kosterlitz (Aberdeen, Scotland) and his colleagues, the guinea pig isolated ileum (GPI) and the mouse isolated vas deferens (MVD) (6). It was suggested that a new receptor type was present in the MVD and termed the delta receptor after the d in deferens (6). Later, this receptor was identified using radioligand binding approaches in nervous tissue (7).

Administration of the endogenous ligands of the receptor, [Leu^5] and [Met^5]enkephalin, produces a few measurable actions. The reason for the relative inactivity of these substances is thought to be due to the rapid degradation of these simple, short, straight-chain peptides by endopeptidases present throughout the body (8). Attempts to circumvent this problem generally have involved the incorporation of unnatural D-amino acids along the peptide sequence. Unfortunately, the modification of the peptide backbone changed the selective activity at the delta receptor and possible cross-reactivity at other receptor types (i.e., the mu and kappa receptors) (for reviews see Refs. 9–13).

A variety of approaches has been used to overcome the concern of cross-reactivity at other receptor subtypes of stabilized enkephalin analogues (13). One of the most successful of these approaches has involved the stabilization of the peptide molecule in a conformation which prefers the desired receptor by incorporation of conformational restrictions (14). This approach has been exploited by Victor Hruby (Tucson, Arizona) and Henry Mosberg (Ann Arbor, Michigan) as well as by the group of Peter Schiller (Montreal, Canada) (see Ref. 15) and others. The introduction of conformational restrictions such as unnatural bulky synthetic amino acids and techniques of cyclization of the peptide chain has resulted in the synthesis of highly selective peptides such as [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (16). The introduction of this peptide in 1983 provided the first tool for investigating the opioid delta receptor. An equally important development occurred at about the same time when the group at ICI (Macclesfield, U.K.) introduced the first highly selective delta receptor antagonist, ICI 174,864 (17). With these tools, opioid researchers could finally begin to study the pharmacological importance and potential physiological involvement of delta receptors.

DIRECT INVOLVEMENT OF OPIOID DELTA RECEPTORS IN ANTINOCICEPTION

Initial studies were done with the highly selective cyclic delta agonist, DPDPE in the rat (18) and in the mouse (19). These studies showed that central (intracerebroventricular, icv) administration of this compound produced effective analgesia in the hot-plate test in the rat and in the hot-plate and tail-flick tests in the mouse. In addition, unlike mu agonists, over the effective analgesic dose range and higher, DPDPE did not inhibit propulsion of contents along the gastrointestinal tract. In contrast, agonists at the opioid mu receptor were shown to produce both analgesia and inhibition of intestinal motility. Thus, it was concluded that cerebral delta receptors could be activated to produce analgesia and that these receptors were not involved in the central regulation of gastrointestinal motility.

The concept that supraspinal delta receptors could be implicated in the direct production of analgesia was met with a good deal of skepticism, mainly because of the fact that although the ligand studied was highly delta selective, the possibility still existed that it produced its effects through cross-reactivity at the mu receptor. Additionally, it had long been accepted that substances that produced opiate-like analgesia within the brain did so by actions at the mu receptor, a view that was reinforced by noting the strong correlation between clinically effective analgesic doses of mu agonists and activity in the guinea pig isolated ileum. Confirmation of the possible involvement of the delta receptor in antinociceptive processes was important because of the potential therapeutic implications of a novel class of substantially nonaddicting analgesic drugs.

Efforts to establish firmly the possibility of delta receptor involvement in analgesia focused on a series of studies emphasizing the use of DPDPE in conjunction with the highly selective delta antagonist, ICI 174,864 (20), and antagonists at the mu opioid receptor such as beta-funaltrexamine (beta-FNA) (21) and naloxonazine (22–24). The concern of DPDPE action at opioid kappa receptors was minimal since this agonist had no measurable affinity for the kappa receptor in radioligand binding assays (16,25,26) or in the rabbit vas deferens (27), a bioassay for opioid kappa receptors (28).

Using the mouse tail-flick test, icv DPDPE produced analgesia which was blocked in a dose-related fashion by ICI 174,864 (29). The doses of ICI 174,864 needed to antagonize the analgesic actions of icv. DPDPE were not themselves analgesic and did not antagonize the analgesic actions of morphine or of [D-Ala^2, NMPhe^4, Gly-ol]enkephalin, DAMGO (29), a highly selective mu receptor agonist (30). Further, no cross-tolerance could be demonstrated between morphine and DPDPE following icv administration in the mouse. The demonstration of differential antagonism using a delta antagonist, ICI 174,864, provided strong evidence for the independent participation of delta receptors in the production of analgesia at supraspinal levels. Additional evidence stemmed from studies with mu receptor antagonists such as beta-FNA and naloxonazine. In mice pretreated with beta-FNA, the morphine and DAMGO dose–response lines were displaced significantly to the right, indicating blockade of mu receptors. In contrast, the analgesic dose–response line of DPDPE was totally unchanged in these beta-FNA-pretreated mice (31). Furthermore, while ICI 174,864 could not antagonize the analgesic effects of morphine in control mice, this delta antagonist was able to antagonize morphine in beta-FNA-pretreated mice (31). This finding suggested that when mu receptors were blocked, morphine produced its analgesic actions by acting at other available receptors, such as the delta receptor. The demonstration that morphine could produce analgesia at sites other than the

mu receptor *in vivo* was additionally demonstrated by the work of Takemori and Portoghese (32), who showed that the analgesic effects of morphine could be antagonized by either ICI 174,864 or by the kappa antagonist, nor-binaltorphimine (33), in mice pretreated with beta-FNA but not in control mice. Thus, differential antagonism of analgesia produced by mu and delta agonists was demonstrated using antagonists selective for the mu receptor as well (31,32).

Another approach supporting the concept of delta-mediated analgesia came from the use of naloxonazine, an antagonist at the postulated mu_1 receptor (22,24). The importance of testing with naloxonazine is that DPDPE has been reported to be the only compound to date which does not bind to the high-affinity mu_1 binding site (34). The established approach of pretreating animals with sc naloxonazine 24 hr prior to testing for analgesia with icv DAMGO or DPDPE, with morphine being used as reference mu agonist, was used, and additionally, the ability of the agonists to inhibit gastrointestinal propulsion was studied (35). The results again supported the concept of delta-mediated analgesia, with the supraspinal actions of morphine and DAMGO being antagonized by naloxonazine pretreatment, but those of DPDPE being unaffected (35). Although the analgesic actions of morphine and DAMGO were blocked by naloxonazine pretreatment, the actions of these mu agonists to inhibit gastrointestinal propulsion were not; DPDPE had been previously demonstrated to have no effects on propulsion following icv administration (19,36). The differentiation between effects on propulsion in the intestine and the production of analgesia suggested differences in mu receptor function at the supraspinal level. Importantly, however, differential antagonism was again demonstrated in that the analgesic effects of DPDPE were unaltered by this postulated mu_1 antagonist, while the effects of the mu agonists were blocked, again supporting the supraspinal involvement of delta receptors, as well as mu_1 receptor, in the production of analgesia (35).

Other approaches also supported the concept of delta receptor involvement in the production of analgesia. An important finding was that of Vaught and colleagues, who used specific mouse strains which had been previously demonstrated to be deficient in opioid mu receptors using autoradiography techniques (37). In these CXBK mice, the analgesic potency of opioid mu agonists such as DAMGO or morphine was found to be greatly decreased; the ED_{50} for morphine was found to be approximately 10-fold higher in the CXBK compared to the progenitor strain, while DAMGO was particularly inactive, having an ED_{50} which was difficult to estimate (38). Critically, however, the potency of DPLPE, a selective delta agonist, was found to be unchanged, providing direct evidence of delta receptor involvement in analgesia (38). This finding was also confirmed in a second species of mouse shown to be deficient in mu receptors, the C57BL/6J-bg^i (beige) mice (38). Additionally, Jensen and Yaksh (39) used microinjection techniques to demonstrate that while both mu and delta opioid agonists were active in many sites in the brain, there were sites, notably the medullary reticular formation, which would respond to [D-Thr^2, Leu^5, Thr^6]enkephalin (DTLET) but not to morphine in the rat. These lines of evidence also supported the concept of delta analgesia at supraspinal sites. While the concept of supraspinal delta receptor involvement in the production of analgesia remains somewhat controversial, an examination of the data suggests that the most direct evidence favors the involvement of cerebral delta receptors in this effect.

It is also important that the spinal cord has been demonstrated to be an important target site for opioid analgesia (23,29,35,40–44). In this regard, the participation of opioid delta receptors in spinal analgesia is not controversial. The existence of delta receptors at the spinal level is critical, however, in that these sites may be different from the delta sites within the brain. Support for this suggestion stems from the observation that DPDPE and other delta agonists have minimal gastrointestinal effects after administration to supraspinal sites but are effective in this end point at the spinal level (19,36,45).

Further, while naloxonazine antagonizes the analgesic actions of icv DAMGO and morphine, this compound does not block the analgesic actions of DAMGO, morphine, or DPDPE given by the intrathecal (ith) route. Additionally, naloxonazine does not antagonize the gastrointestinal effects of icv DAMGO and morphine but does block the gut effects of ith DAMGO, morphine, and DPDPE. Thus, opioid mu and delta receptors in the brain and spinal cord may be independent targets for different agonists. As differences in opioid delta receptors have been suggested (46,47), it may be that the delta receptors in the spinal cord are more similar to those in the MVD than those in the brain, offering opportunities for the independent development of therapeutically unique agonists. Most of the evidence supporting a lack of involvement of cerebral delta receptors in analgesia is based on the relative potencies of agonists at mu and delta receptors in producing analgesia. It is indeed the case that DPDPE is generally less potent than mu agonists, but several recent compounds under development at the Arizona Group show increased potency of 6–10 times (compared to DPDPE), while maintaining an equivalent degree of selectivity, and it is clear the future substances will achieve equivalent potency with mu agonists.

The importance of analgesics that act directly at the delta opioid receptor is further emphasized by noting a recent paper by Magnan and Tiberi (48). Studying opioid receptor binding sites in human fetal brain, they have identified the presence of mu and kappa sites, but not delta sites. Therefore a delta analgesic could be useful during pregnancy, providing pain relief for the mother with greatly reduced risk for the fetus.

DEPENDENCE LIABILITY OF OPIOID DELTA AGONISTS

One of the aims of opioid research has always been to develop strong analgesics which are devoid of dependence liability associated with compounds such as morphine. Following the development of DPDPE, the question of dependence liability at the delta receptor was obvious and was investigated by Cowan and colleagues (49). These experiments tested whether opioid antagonist-induced physical dependence signs would result following prolonged exposure to DPDPE and have focused on the dependence liability at the delta receptor. The model used was well established pre-

viously by Wei and colleagues (50–52) and the study was designed to evaluate the development of physical dependence relative to agonists selective at mu and kappa receptors. For this purpose, development of physical dependence was evaluated following infusion of equianalgesic doses of DPDPE, DAMGO, morphine, and U50,488H into the aqueduct of Sylvius of the rat. The animals received infusions of the compounds for a period of 70 hr. At the time of testing, each rat received a sc injection of an opiate antagonist (i.e., nonspecific antagonist such as naloxone or a delta antagonist, ICI 174,864) and signs of withdrawal were evaluated. The results demonstrated three levels of abstinence following administration of antagonists. First, rats receiving infusions of water showed negligible abstinence scores; second, rats receiving DPDPE showed low to moderate abstinence scores; and third, rats receiving DAMGO or morphine showed high abstinence scores indicating a severe degree of physical dependence. Thus, while the data were somewhat disappointing in that the DPDPE-infused rats showed an appreciable level of physical dependence, the data were clear that agonists at delta receptors provide clear advantage relative to agonists at mu receptors in that a much lower degree of physical dependence is produced. It is possible that in spite of the relatively high degree of selectivity for DPDPE at delta receptors, this compound may, nevertheless, have some actions at mu sites following prolonged infusion. It remains to be seen whether agonists with selectivity for the delta receptor of 10–100 times greater than that of DPDPE will produce even lower dependence liability or a different form of dependence associated strictly with the delta opioid receptor. Furthermore, the possibility of subtypes of opioid delta receptors also raises the possibility that other agonists at these sites will produce negligible dependence liability.

MODULATION OF MU AGONIST ACTION BY DELTA AGONISTS

In addition to studies describing the direct analgesic properties of agonists at opioid delta receptors, recent evidence suggests that under some conditions, opioid mu and delta receptors may be functionally coupled (53,54). An initial report of such functional modulation came from the observation of Vaught and Takemori (55), who showed that Leu^5-enkephalin given icv at subantinociceptive doses could potentiate the analgesic actions of morphine. This observation was immediately followed by work of Lee and colleagues, who showed that in contrast to the actions of Leu^5-enkephalin, Met^5-enkephalin could, in nonanalgesic doses, antagonize the analgesic effects of morphine (56,57). Based on these observations *in vivo,* Vaught and colleagues postulated the existence of a supraspinal mu–delta complex (57). Further evidence for such a complex has been provided on the basis of mathematical analyses of radioligand binding data from preparations of rat brain membranes (58–62) as well as mouse brain (63,64). In the proposed model, agonists at delta receptors could modulate the actions of mu agonists, while not producing direct effects alone. The observation of both positive modulation (i.e., Leu^5-enkephalin/morphine) and negative modulation (i.e., Met^5-enkephalin/morphine) has never been fully explained, however, and may serve as a key observation in the identification of subtypes of opioid delta receptors, a suggestion that has recently been made by the Herz group (46,47). It is important to note that in the current thinking of the hypothesized mu–delta receptor complex, opioid mu and delta receptors can exist either separately or in complexed form. Indeed, such a distinction may form the basis for the existence of delta receptor subtypes. The possibility of mu receptor subtypes has been suggested previously and supported by a variety of evidence including the differential antagonism of DAMGO and morphine analgesia and gastrointestinal propulsion effects by naloxonazine (35). Finally, the hypothesis of functional coupling between mu and delta receptors is not limited to the mediation of antinociception. Data reported from the laboratory of Dr. John Holaday have suggested such interactions in the modulation of endotoxic shock in the rat (65,66) and other work has shown modulation of mu-mediated changes in urinary bladder motility (67).

Recent work has extended these early observations *in vivo* by demonstrating that subanalgesic doses of icv DPDPE potentiate the analgesic actions of morphine, while subanalgesic doses of icv [D-Ala^2, Met^5]enkephalinamide (DAMA) antagonize the analgesic actions of morphine, and that both the potentiation and the antagonism are blocked by ICI 174,864 (29,35,43,44). Critically, it must be emphasized that ICI 174,864 had no analgesic effects alone and did not directly antagonize the analgesia produced by morphine. These data correlate well with the findings of Vaught *et al.* (57), who showed that Met-enkephalin could prevent the potentiation of morphine analgesia produced by Leu-enkephalin, reinforcing the view that the modulatory actions occurred through the delta receptor. Additionally, it has been demonstrated that a higher dose of icv DPDPE is not required to produce the potentiation of morphine analgesia in situations of morphine tolerance, suggesting that indeed the enkephalins and enkephalin analogs may serve in a physiologically important role to modulate the action of (endogenous) agonists at the mu receptor. Another important observation was that ICI 174,864 did not potentiate or antagonize morphine analgesia alone, suggesting that mere occupation of the delta receptor, or perhaps displacement of any morphine binding to the delta receptor making effectively higher concentrations of morphine available, was not sufficient to produce modulation. The delta receptor must be occupied by an agonist in order for modulation to occur.

In recent studies it has been discovered that not all mu agonists are susceptible to modulation by DPDPE (43). Thus, mu agonists of diverse structure such as morphine, normorphine, and levorphanol appear to be modulated, while compounds such as DAMGO, PL017, and sufentanil are not. These observations raise the possibility that some mu agonists may selectively interact with the mu-complexed receptor, while others do not (44). Such suggestions have also been recently supported using radioligand binding techniques and autoradiography studies (61,62,68) providing suggestions of mu receptors inside of, and outside of, the hypothesized receptor complex. In addition to concepts of differences in mu receptors, such studies provide suggestions of differences in delta receptors (i.e., complexed and noncomplexed). Indeed, such suggestions have been made on the basis of compounds with high activity within the brain but with minimal activity for the delta receptors in the MVD

(46,47). Studies from our laboratory with antagonists have also shown that modulation can be disrupted by beta-FNA but not naloxonazine (44). Thus, while the identity of the hypothesized mu-complexed receptor awaits investigation, it would not appear to be a mu_1 receptor (69). The inability of DPDPE or DAMA to modulate the actions of mu agonists in beta-FNA-pretreated animals is subject to various interpretations, including the suggestion that this antagonist actually disrupts the receptor complex (70).

The complex nature of such interactions require a great deal of additional investigation using techniques *in vivo* and *in vitro* before firm conclusions can be drawn. However, certain points can be emphasized. First, both the potency and the efficacy of mu agonists can be increased by some delta agonists. Second, modulation of the analgesic responses to these mu agonists does not mean that the negative effects of the mu agonists will also be potentiated—such negative effects (i.e., development of physical dependence, respiratory depression, and gastrointestinal effects) may be mediated via mu-noncomplexed receptors. Third, use of negative modulators may prevent the development of tolerance and dependence on some mu agonists. Such possibilities would allow for the use of mu agonists of lower efficacy and increased safety (such as, for example, codeine) in order to allow for equivalent relief of pain without the rapid development of tolerance and much lower physical dependence and abuse potential. Alternatively, the use of negative modulators may selectively prevent the expression of the negative aspects of traditional mu agonists, while nevertheless providing sufficient levels of pain relief. Finally, it may be possible to achieve a delta agonist which can substitute for traditional mu opioids which provide effective pain relief without abuse liability or development of physical dependence.

Related to the above aspects is the recent work by Chang and colleagues, who explored the strategy of alternated mu and delta receptor activation for limiting opioid tolerance (5,7). By alternated chronic administrations of DADLE and morphine, relatively selective delta and mu agonists, respectively, a significant regression of analgesic tolerance for both morphine and DADLE was obtained in rats. It should be noted, however, that the favorable degrees of cross-tolerance described by these authors was dependent on judicious dose selections for DADLE and morphine so as to enhance receptor selectively. Additional useful information would be provided from similar studies in which more selective delta agonists are alternately administered with more selective mu agonists. Therefore, as their studies suggest, it may be possible to modulate the extent of opioid tolerance *in vivo* and success of this process will depend on the development of non-mu opioid receptor agonists that are stable *in vivo,* possess a high specificity and intrinsic analgesic activity, and preferably are without the usual side effects of the opioids. (5).

SYNTHESIS OF RECEPTOR SPECIFIC PEPTIDES FOR DRUG DEVELOPMENT

As discussed earlier one of the main problems associated with the peptides in drug development is ease of their degradation. Many of these peptides undergo easy degradation in the GI tract and strategies for development of orally active opioid peptides have only resulted in partial success. Even after parenteral administration, stability of these in plasma and their ability to cross the blood–brain barrier are primary concerns. A number of synthetic modifications, especially the development of "peptidomimetics," offer a viable alternative to new drug design.

Development of highly specific mu and delta receptor-specific peptides is essential for drug development. Use of "conformational restriction" for the synthesis of mu- and delta-specific peptides has been reviewed by Schiller (73) and Hruby (74). Generally, one of the main problems associated with many of the conformationally restricted peptides is lowered receptor affinity.

In spite of the extensive studies on the conformation-receptor activity, conformational requirements for receptor selectivity and affinity are poorly understood (75). Some of the earlier straight-chain or linear delta-specific opioid peptides analogues with moderate affinities were reported by Roques and colleagues (76,77) and two of these peptides that are widely used in biding studies are H-Tyr-Ser-Gly-Phe-Leu-Thr-OH (DSLET) and H-Tyr-Thr-Gly-Phe-Leu-Thr-OH (DTLET). Recently this group reported (78) the synthesis of [D-Ser^2(*o*-t-butyl, Leu^5]-enkephalinyl-Thr^6 (DSTBULET), [D-Ser^2 (*o*-t-butyl), Leu^5]-enkephalinyl-Thr^6 (*o*-t-butyl) (BUBU), and [D-Thr^2 (*o*-t-butyl), Leu^5]-enkephalinyl-Thr^6 (DTTBULET). DSTBULET displays an even higher affinity to delta receptors than DSTLET and, unlike Met^5-enkephalin, is resistant to degradation. BUBU is even more delta-selective than DSTBULET, whereas DTTBULET is inactive. Apparently the bulky t-butyl groups induce large conformational changes that affect the affinity and selectivity. These peptides are examples of straight-chain peptides that exhibit both a high selectivity and a high affinity.

Several mu selective opioids have been synthesized to date. H-Tyr-D-Ala-Gly-(N,Me)-Phe-Gly-ol (DAMGO) was one of the first highly selective mu opioid peptide analogues developed. Schiller and colleagues utilized both conformational restriction and linear analogue approaches to develop mu receptor-specific peptides, and one of the earliest successful mu-selective synthetic peptide was H-Tyr-Cyclo-[-D-A_2Bu-Gly-Phe-Leu-] (79). Extensive structure–activity studies carried out by this group culminated in developing another conformationally restricted highly mu receptor-selective tetrapeptide, H-Tyr-D-Orn-Phe-Asp-NH^2 (80). Following that, Schiller and colleagues reported (81) a linear dermorphin analogue, H-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA), which appears to be one of the most mu-selective peptides reported to date (at least 10 times more selective than DAGO).

Recently, Hruby and colleagues reported (82) the synthesis of a halogenated analogue of DPDPE and this peptide has a high affinity for the delta receptor, presumably due to the lipophilic halogen atom. Hruby and colleagues (74) have earlier reported the synthesis of highly mu receptor-specific peptides using a somatostatin peptide fragment, H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol, as a template. A number of highly mu receptor-selective peptides such as CTAP, CTOP, and several related tetrahydroisoquinoline (Tic) analogues were designed as highly selective mu-

receptor antagonists. Other strategies employed by medicinal chemists to design either highly selective and/or stable peptides are incorporation of alpha, beta dehydro amino acids and synthesis of retroinverso peptides (83–85).

To summarize, through both the linear analogue synthesis approach and conformational restriction, a number of delta and mu receptor-specific peptides were synthesized, and only through extensive SAR studies is it possible to select the most fruitful approach.

Valuable information for the design of novel opioid peptides has also been obtained from the recent reports on deltorphins (86–88). Richter *et al.* (86), utilizing cDNA techniques, reported that the gene that codes for dermorphin also codes for another peptide and named it the dermorphin gene-associated peptide (DGAP). Lazarus *et al.* (86) studied the interaction of DGAP and [D-Met^2]-DGAP (a synthetic peptide) with opioid receptors and found them to be delta selective peptides. Eraspamer and colleagues have recently isolated two heptapeptides, H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2 and H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2, from the skin extracts of phyllomedusa bicolor and named them deltorphins (88). These deltorphins exhibited a high affinity and selectivity for delta receptors. These naturally occurring peptides might serve as templates for the design of peptide with a high selectivity, and more importantly, high affinity and efficacy, in drug design.

SUMMARY

Recent advances in the development of novel and highly selective agonists for the opioid delta receptor reveal two general approaches which might be exploited clinically. Thus, opioid delta receptors may serve as (i) independent target sites for the direct action of novel and selective delta agonists or (ii) target sites where the actions of opioids binding to the mu-complexed sites can indirectly be modulated. In order to apply these concepts, the research effort must focus on the evaluation of the pharmacology of novel opioid delta agonists in three ways. First, they must be studied as directly useful and applicable compounds which can substitute for traditional mu opioids in the initiation of beneficial effects such as pain relief, but with significantly decreased hazards of physical dependence. Analogues of DPDPE have been synthesized with even greater selectivity than DPDPE, i.e., with essentially no mu agonist activity at all. Additionally, while most analgesic studies in the past have focused on selectivity ratios based on potency, few, if any, have focused on efficacy (i.e., the maximal achievable effect). Therefore, a major goal for the clinical development of these compounds will be to evaluate the analgesic efficacy of delta opioids and to incorporate efficacy as a design consideration. In this way, it would seem feasible to design delta agonists which are both potent and efficacious analgesics. Second, delta agonists must be studied as positive modulators (enhancers) of the actions of opioid mu agonists; the benefits of positive modulation of mu agonists include the increase in potency and efficacy of mu agonists, without increasing the rate of development of tolerance to analgesia or incidence of physical dependence. Third, delta agonists must be studied as negative modulators (attenuators) of the actions of opioid mu agonists; the benefits of negative modulation (particularly of compounds of high efficacy) may be related to inhibiting the development of sedation, analgesic tolerance, and physical dependence and sedation, while maintaining adequate analgesia.

Other hurdles need to be overcome as well. The delivery form of the opioid delta agonist still needs to be established. However, some progress has been made in that some delta opioids, such as metkephamid, have appreciable ability to cross the blood–brain barrier. The problems of creating delta analgesics are not limited to the blood–brain barrier, as acceptable forms for oral delivery also need to be stable and absorbed across the gastrointestinal mucosa. Some of these hurdles may become lessened, if peripheral sites of opioid analgesia become relevant during pain states (72). Finally, a potentially limiting factor in the delivery of peptides will be the cost involved. Nevertheless, as the structure of the molecule which produces the desired properties is elucidated, the design and synthesis of nonpeptide compounds with similar attributes can eventually be achieved. It seems clear from the evidence that agonists at the delta opioid receptor have great potential in delivering the desired therapeutic agent that opioid researchers and physicians have been seeking for so long.

Finally, advances in conformational analysis techniques, computer-aided drug design, synthetic methodologies, and valuable information form genetic research provide hope that drugs for treatment of addictive disorders will be developed.

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