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Endorphinergic Attenuation of Distress by Concomitantly Enhancing Endogenous Opioid Release and Switching Opioid Receptor Signaling from an Excessively Excitatory to a Normal Inhibitory Mode

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ABSTRACT

The endogenous opioid system plays a significant role in the modulation of distress in many psychiatric, neurologic, and neurodevelopmental disorders. Many clinical distress symptoms show similarities to the excitatory autonomic withdrawal effects in chronic opioid-dependent animals and humans, as well as to the "quasi-morphine withdrawal syndrome" evoked in naïve rodents shortly after acute systemic injection of cyclic AMP-phosphodiesterase (cAMP-PDE) inhibitors. These symptoms result from excessive excitatory opioid receptor signaling and increased endorphin release. Pharmacologic analyses of the remarkably plastic bimodal (excitatory/inhibitory) signaling functions of opioid receptors have utilized microelectrode recordings from opioid-sensitive neurons in tissue cultures of mouse sensory ganglia and hot-water tail-flick assays in mice. These studies led to development of specific chemical formulations that switch opioid receptor signaling from an excessively excitatory to a normal inhibitory mode. Critical combinations of cAMP-PDE inhibitors that release endorphins plus specific agents that switch opioid receptors from excitatory G_s-coupled to inhibitory G_i/G_o-coupled signaling were shown to attenuate hyperalgesia and distress evoked by diverse chemical stressors in mouse tail-flick assays. Both the "quasi-morphine withdrawal syndrome" in naïve rodents as well as the excitatory withdrawal effects in chronic, opioid-dependent animals and humans may be manifestations of a common Endorphinergic Distress Syndrome (EDS). We suggest that many distress symptoms are caused by EDS, a dysfunctional imbalance in the endogenous opioid system, consisting of abnormal endorphin levels, together with opioid receptors predominately in their excitatory mode. Therefore, concomitantly enhancing endogenous opioid release and switching excessive excitatory opioid receptor signaling to inhibitory signaling can attenuate these distress symptoms. Trials of a critically formulated oral preparation, containing both endorphin enhancers and opioid receptor switchers, have resulted in long-term anxiolytic efficacy and enhanced calm and mental clarity in large numbers of individuals with distress symptoms. These endorphinergic formulations may provide treatment for the emotional and physical distress associated with many psychiatric, neurologic, and neurodevelopmental disorders.

Keywords: Endorphins; Bimodal Opioid Receptors; Distress; Opioid Analgesia; Hyperalgesia; Tolerance; Dependence; Endorphinergic Distress Disorder

1. Introduction

In a recent study of the effects of cyclic AMP-phosphodiesterase (cAMP-PDE) inhibitors in mice, Crain and Shen [1] emphasized Collier *et al*.'s [2,3] pioneering studies demonstrating that acute systemic injection of naïve rodents with a cAMP-PDE inhibitor, e.g., caffeine or theophylline, rapidly evokes a "quasi-morphine withdrawal syndrome" that is remarkably similar to naloxone-precipitated excitatory withdrawal effects in chronic morphine-dependent animals. Collier *et al*. [3] postulated that the opioid dependence-like effects of administration of these cAMP-PDE inhibitors to naïve mice may be mediated by "hypertrophy of a neuronal cAMP system [that] releases endogenous opioid".

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The "quasi-morphine withdrawal syndrome" in rodents evoked by cAMP-PDE inhibitors included characteristic hyperexcitability behavior, e.g. jumping, teeth chattering, hyperalgesia, and body shakes [2]. During the 20-year period following Collier *et al*.'s studies [1,2], Crain and Shen (Sections 2 - 4) carried out an extensive series of pharmacologic analyses of opioid receptor functions that have provided significant insight into possible mechanisms that may facilitate counteracting both this acute "quasi-morphine withdrawal syndrome" as well as the characteristic excitatory withdrawal effects in chronic opioid-dependent animals and humans.

2. Bimodal (Excitatory/Inhibitory) Opioid Receptor Signaling

Crain *et al*. [4] demonstrated that sensory-evoked dorsal-horn synaptic networks discharges in organotypic tissue cultures of mouse spinal cord with attached dorsal root ganglia were selectively depressed by perfusion with a series of exogenous and endogenous opioids, at concentrations remarkably proportionate to their potency in the intact animal. Electrophysiologic analyses of opioid effects on dorsal-root ganglion neurons in these cord-ganglion tissue cultures, utilizing intracellular microelectrode techniques, revealed that opioids could evoke not only well-known inhibitory signaling effects, *i.e*., shortening of the duration of the action potential, but also novel *excitatory* signaling, *i.e*., prolongation of the duration of the action potential of the same sensory neuron [5]. These excitatory effects were shown to be selectively elicited by >1000-fold lower opioid concentrations than required to elicit inhibitory effects. Furthermore, these excitatory opioid effects were selectively enhanced by application of pharmacologic agents, e.g. forskolin, that increase the cyclic AMP levels in these neurons.

Shortening by opioids of the action potential duration (APD) of dorsal-root ganglion (DRG) perikarya has generally been considered to be a useful model of their inhibition of Ca^{2+} influx and transmitter release at presynaptic ganglion terminals [6]. Similarly the delayed repolarization associated with opioid-induced prolongation of the APD provides evidence of *excitatory* effects of opioids on dorsal-root ganglion neuron perikarya and implies increased Ca^{2+} influx and stimulation of Ca^{2+}-dependent transmitter release in presynaptic ganglion terminals in the spinal cord [5]. Since these neurons are devoid of synaptic inputs, excitatory modulation by opioids of their APD is clearly a *direct* action, distinct from the dis-inhibitory mechanisms that may mediate some of the excitatory effects of opioids in the brain [7].

Since nanomolar concentrations of specific μ-, δ- and κ-opioid agonists evoke prolongation of the APD that can be prevented by the specific opioid receptor antagonists, naloxone and diprenorphine (1 - 10 nM), Crain and Shen [7] postulated that these excitatory effects are mediated by *high-affinity excitatory opioid receptors*. Opioid-induced APD prolongation in dorsal-root ganglion neurons was also shown to be selectively blocked by cholera toxin Asubunit (which ADP-ribosylates G_s and attenuates ligand activation of associated receptors), whereas opioid-induced shortening was blocked by pertussis toxin (which ADP ribosylates G_i and G_o and interferes with inhibitory receptor signaling) [7]. These data suggested that excitatory effects of opioids on these neurons are mediated by opioid receptors that are positively coupled via a G_s-like protein to adenylyl cyclase (AC) and cAMP-dependent voltage-sensitive ionic calcium conductances (resembling, for example, G_s-coupled β-adrenoceptors). By contrast, inhibitory effects are mediated by opioid receptors linked to G_i/G_o (resembling α-adrenoceptors). Crain and Shen's evidence of bimodal excitatory/inhibitory opioid receptor signaling [7] is consistent with an animal model of persistent pain (arthritic rats), in which Kayser *et al.* found that exceedingly low doses of morphine elicit a naloxone-reversible paradoxical hyperalgesia whereas increased doses are highly effective in producing analgesia. Crain and Shen [7] emphasized that excitatory effects mediated by bi-modally acting opioid receptor signaling may provide a novel mechanism to account for some of the paradoxical hyperalgesic, aversive (e.g., itching) and euphoric effects of opioids in the central and peripheral nervous systems.

Low concentrations of opioids prolong the APD potentials in about 80% of the DRG neurons tested in ganglion-cord explants, whereas opioid-induced APD shortening is observed in about 50% of the cells [5]. Since cells that show opioid-induced action potential shortening in culture mimic the opioid-sensitive properties of nociceptive primary afferent neurons *in situ*, this raises the possibility that excitatory effects of opioids may occur in non-nociceptive as well as nociceptive neurons [7].

These acute electrophysiologic microelectrode analyses demonstrating bimodal excitatory/inhibitory opioid receptor signaling in sensory DRG neurons *in vitro* were extended to studies showing that *chronic* opioid treatment of these neurons in culture results in *progressive sensitization of excitatory opioid receptors signaling* [8]. This is in contrast to characteristic *desensitization* of G_s-coupled β-adrenergic receptors, many inhibitory opioid receptors and most other G protein-coupled receptors during sustained agonist exposure (see review by Crain and Shen) [9]. Correlative *in vivo* studies were then carried out utilizing hot-water tail-flick assays in mice [10]. Moderate doses of morphine (ca. 1 mg/kg, s.c.) prolonged the tail-flick latency for several hours, as expected, in this mouse model of analgesia and hyperalgesia. In contrast, >1000-fold lower doses of morphine (ca. 1 μg/kg) elicited significant rapid onset (ca. 1 - 2 sec) *de-*

creases in tail-flick latency which lasted for >3-hr after drug injections [11]. This low-dose opioid-induced hyperalgesia was especially prominent when the temperature of the water in the tail-flick assay was reduced from the usual 55˚C to 52˚C so that the tail immersion stimulus was completely innocuous in control tests [11]. Crain and Shen [11] considered this low-dose opioid-induced decrease in tail-flick latency to be a reasonable animal model of opioid hyperalgesia. We suggest that it may also be viewed as a low-dose opioid-induced *distress* response that can be rapidly converted to an *anxiolytic*, *calming* response (Sect. 5, 6) by cotreatment with an opioid receptor switcher, e.g., ultra-low-dose naltrexone. (see Section 3.1 and Section 6).

Coordinated studies of chronic high-dose opioid treatment of DRG neurons *in vitro* and mice *in vivo* showed expected onset of tolerance that was attributable to the onset and progressive sensitization of excitatory G_s-coupled opioid receptor signaling ("protracted dependence"), thereby counteracting the initial inhibitory opioid receptor signaling that mediates analgesia and distress relief [9,10]. Studies were therefore begun to determine treatments that could be utilized to selectively block excitatory opioid receptor signaling and thereby enhance concomitant inhibitory opioid receptor signaling. Guided by evidence that excitatory opioid receptor signaling is mediated by high-efficacy excitatory G_s-coupled opioid receptors, Crain and Shen [9,10] carried out systematic assays *in vitro* and *in vivo* to determine if *ultra-low doses* of specific antagonists of opioid receptors might selectively block excitatory, but not inhibitory signaling. This led to the discovery of ultra-low-dose naltrexone or naloxone (ULDN) as an effective Opioid Receptor Switcher [10]. Chronic cotreatment of mice (s.c) with morphine together with ULDN not only enhanced the antinociceptive potency of morphine in hot-water tail-flick assays but also attenuated development of tolerance and withdrawal symptoms [12]. The reliability and the validity of this antinociceptive assay and its specificity for analyzing opioid receptor signaling functions have been confirmed by elegant studies utilizing intra-spinal infusion of ultra-low-dose naltrexone in morphine-treated rats [13,14] and correlative biochemical analyses of ultra-low-dose naloxone in morphine-treated rats [15,16]. These studies in mice provided the scientific rationale for a successful-Phase III clinical trial on >700 chronic low-back pain patients with high-dose oxycodone plus ULDN (Oxytrex™), resulting in >50% decrease in physical dependence following sudden withdrawal of opioids after 12 weeks [17,18].

3. Opioid Receptor Switchers

3.1. ULDN and CTX-B

In addition to the development of ultra-low-dose naltrexone and naloxone as effective Opioid Receptor Switchers (Section 2), Shen and Crain [19] had already discovered an alternative switcher during their intracellular microelectrode recordings from sensory DRG neurons *in vitro*. As noted above, cholera toxin-Asubunit selectively blocks opioid excitatory signaling, indicating mediation of this excitatory effect by G_s-coupled opioid receptors. Surprisingly, brief treatment with purified preparations of the non-toxic *B-subunit* of cholera toxin also selectively blocked opioid excitatory receptor signaling. Since the B-subunit is known to bind selectively, with high specificity, to the monosialoganglioside glycolipid, GM1 which is abundantly distributed on the external surface of neuronal cell membranes [20,21], these results suggested that GM1 *ganglioside may regulate* G_s*-coupled excitatory opioid receptor signaling* in sensory and perhaps other types of neurons [9,19].

Tail-flick assays in mice confirmed this hypothesis by demonstrating that cotreatment of mice with a low dose of CTX-B rapidly switched low-dose morphine-induced thermal hyperalgesia to a prominent analgesia lasting for several hours, remarkably similar to the effect of cotreatment with ULDN [22]. Furthermore, chronic cotreatment of mice for 5 days with CTX-B plus high-dose morphine blocked the development of opioid tolerance, similar to the effect of chronic ULDN plus morphine. Finally, tail-flick assays were also utilized to evaluate opioid dependence. A low-dose of naloxone, 10 μg/kg, administered acutely to chronic morphine-treated mice evoked hyperalgesia, which resembles naloxone-precipitated autonomic withdrawal symptoms (including characteristic hyperalgesia) in humans. This excitatory dependence effect was completely prevented by cotreatment with either ULDN or CTX-B [22].

In view of the remarkable efficacy of CTX-B to switch opioid receptor signaling from an excitatory to an inhibitory mode, comparable to the effects of ULDN, Crain and Shen [22] postulated that CTX may bind to and block an allosteric GM1-modulating site on opioid receptors, whereas ULDN acts as a selective competitive antagonist at excitatory opioid receptors. Furthermore, Wu *et al*. [23,24] demonstrated that a homogenous population of cloned δ opioid receptors transfected in CHO cells in culture, which normally show only opioid inhibition of adenylate cyclase via pertussis toxin-sensitive G_i coupling, can be rapidly *switched* to an excitatory mode by treatment with GM1 (ca. 1 μM for 30 min); other gangliosides were ineffective. Application of the same opioid agonist to the GM1-treated CHO cells then results in *stimulation* of adenylate cyclase, suggesting that these cloned opioid receptors had undergone a conformational change that switched their coupling from G_i to G_s, thereby converting these receptors from an inhibitory to excitatory mode.

This study led Crain and Shen [9,25] to conclude that in neurons with relatively low levels of GM1, most opioid receptors are evidently coupled to G_i and G_o, and are therefore in an inhibitory mode. Binding of GM1 to a putative allosteric site on opioid receptors elicits a conformational change in the receptor-G protein association that results in switching of receptor-coupling from G_i/G_o to G_s, thereby converting the receptor from an inhibitory to an excitatory mode. GM1 treatment of DRG neurons can therefore increase not only the efficacy but also the *numbers* of G_s-coupled opioid receptors in these cells. These GM1-regulated bimodal properties of opioid receptors are in sharp contrast to most monoaminergic and other G-coupled receptor systems where molecularly distinct receptor subtypes are preferentially coupled to *either* G_s or G_i/G_o, e.g., G_s-coupled β-adrenergic vs. G_i/G_o-coupled α-adrenergic receptors [9,25]. These studies on the critical role of GM1 in binding to opioid receptors and switching them from an inhibitory to an excitatory mode led Crain and Shen [9] to propose that opioid tolerance or dependence is mediated not only by upregulation of the G_s-AC-cAMP system but also by elevation of the concentration of GM1 ganglioside, following activation of the cAMP-dependent glycosyltransferase that synthesizes GM1. Coordination of these processes provides a positive-feedback phosphorylation cycle that could amplify the sensitivity of GM1-regulated, G_s-coupled, excitatory opioid receptors to remarkably low levels of endogenous opioids and thereby account for the *protracted dependence* (e.g., naloxone supersensitivity) observed for months after the withdrawal of chronic exogenous opioids *in vitro* as well as *in vivo* [26].

3.2. Neuraminidase Inhibitors

Although CTX-B is an effective agent for rapidly switching opioid receptors from an excessively excitatory to a normal inhibitory mode, it may not be useful for chronic treatment because it may interfere with other important long-term functions of GM1 ganglioside in the nervous system. Wu *et al*. [27] have shown, for example, that GM1 may play a critical role in the prevention of Parkinson's disease. Crain and Shen, therefore, have attempted to develop agents that may produce more graded attenuation of excessively excitatory signaling by opioid receptors. Neuraminidase inhibitors are quite promising agents for this purpose.

3.2.1. Oseltamivir

GM1 ganglioside plays a significant role in nociceptive neurons in regulating opioid receptor excitatory signaling that has been demonstrated to mediate "paradoxical" morphine hyperalgesia and to contribute to opioid tolerance/dependence (Section 3.1). Neuraminidase (sialidase) increases levels of GM1 in neurons by enzymatic removal of sialic acid from abundant polysialylated gangliosides. Acute treatment of mice with the neuraminidase inhibitor, oseltamivir markedly enhanced morphine analgesia [28]. Acute oseltamivir also reversed "paradoxical" hyperalgesia induced by a low dose of morphine, unmasking potent analgesia. In chronic studies, co-administration of oseltamivir with morphine prevented and reversed the hyperalgesia associated with morphine tolerance. These results provided the first evidence indicating that treatment with a neuraminidase inhibitor, oseltamivir, blocks morphine's hyperalgesic effects by decreasing neuronal levels of GM1. The study further implicates GM1 in modulating morphine analgesia and tolerance, via its effects on the underlying excitatory signaling of G_s-coupled opioid receptors. Incidentally, this work suggests a remarkable, previously unrecognized effect of oseltamivir ("Tamiflu"), which is widely used clinically as an antiviral agent against influenza, on glycolipid regulation of opioid receptor signaling in nociceptive neurons.

3.2.2. Sulfates

Based upon studies demonstrating that inorganic sulfate inhibits sialidases in various mammalian cell types [29], Crain and Shen carried out preliminary tests on the effect of sodium sulfate on opioid receptor signaling in mouse tail-flick assays. Acute treatment of mice with low doses of morphine (1 - 3 mg/kg) resulted in relatively small increases in tail-flick latency, whereas cotreatment with morphine plus sodium sulfate (at doses ranging from 1 - 10 mg/kg) elicited remarkable increases (ca. 10-fold) in tail-flick latency. Treatment with these doses of sodium sulfate alone had no significant effect on tail-flick latency. These surprising increases in tail-flick latency during cotreatment with morphine plus sodium sulfate were maintained even after a 5-day test period during which time the mice treated with morphine alone developed the usual opioid tolerance and dependence (Crain and Shen, unpublished; US Patent Application #2010-0015147). These effects of sodium sulfate are consistent with abundant clinical evidence that cotreatment with morphine plus magnesium sulfate potentiates opioid analgesia [e.g. 30]. However, this potentiation has, heretofore, been attributed to specific effects of magnesium, rather than Crain and Shen's postulate that inorganic sulfate acts as a neuraminidase inhibitor. These effects of sodium sulfate were particularly unexpected because the morphine utilized in Crain and Shen's studies was, in fact, morphine sulfate!

Pressure of other research projects with oxycodone and rolipram precluded more systematic analyses of this interesting effect of sodium sulfate. Nevertheless, Crain and Shen's studies with sodium sulfate stimulated development of the use of dietary sulfated agents as novel

types of neuraminidase inhibitors that could be utilized to switch opioid receptor signaling from an excitatory to inhibitory mode. Pending further correlative biochemical and molecular pharmacologic analyses, Crain and Shen's evidence that cotreatment with sodium sulfate markedly enhances morphine's analgesic effects in mouse tail-flick assays could be due not only to possible neuraminidase-inhibition of GM1 synthesis, but also to sulfation effects that may interfere with GM1-binding to opioid receptors (analogous to the role of cholesterol sulfate in modulating specific protein-binding functions [31]. N-acetylcysteine (NAC) appears to be a particularly appropriate agent as an Opioid Receptor Switcher because it has been shown to provide an *in vivo* source of inorganic sodium sulfate [32] and possesses many other therapeutic benefits, e.g. significant analgesic effects on capsaicin-evoked inflammatory pain in mice, enhancing metabolic synthesis of glutathione, protection against acetaminophen-induced hepatotoxicity (see Section 6). Magnesium or sodium sulfate are, of course, other useful sulfates.

4. Cotreatment with Endorphin Enhancers and Opioid Receptor Switchers

As noted above (Section 1), Collier *et al*. [3] postulated that the opioid dependence-like effects of acute injection of a cAMP-PDE inhibitor, e.g. caffeine or the ophylline, in naïve rodents may be mediated by "hypertrophy of a neuronal cAMP system [that] releases endogenous opioid." Crain and Shen [1] confirmed this research by demonstrating that systemic (s.c.) injection in *naïve* mice of IBMX or caffeine (10 mg/kg) as well as a much lower dose (1 μg/kg) of a more specific cAMP-PDE inhibitor, rolipram [33], rapidly evokes thermal hyperalgesic effects (lasting > 5 h) that appear to be mediated by *enhanced excitatory opioid receptor signaling*, as occurs during withdrawal in opioid-dependent mice. Furthermore, co-treatment of mice with any one of these cAMP-PDE inhibitors plus ultra-low-dose naltrexone (NTX) (0.1 ng/kg^{-1} pg/kg, s.c.) results in prominent opioid enhanced *analgesia* (lasting >4 hrs)—even when the dose of rolipram is reduced to as low as 1 pg/kg.

Crain and Shen [1] suggested that these excitatory effects of cAMP-PDE inhibitors in naïve mice can best be accounted for by cAMP-enhanced release of small amounts of endogenous bimodally-acting (excitatory/inhibitory) opioid agonists by neurons in nociceptive networks in the spinal cord (as well as other regions of the central nervous system). Cotreatment with ultra-low-dose NTX selectively antagonizes high-efficacy excitatory Gs-coupled opioid receptor-mediated signaling [9,10,12] activated by the putative cAMP-induced release of endogenous opioid agonists. Cotreatment with ultra-low-dose NTX results in rapid conversion to inhibitory Gi/Go-coupled opioid receptor-mediated signaling, which normally requires activation by much higher doses of opioid agonists [34].

As noted above (Section 2), low doses of *exogenous* morphine, dynorphin or other bimodally-acting opioid agonists (0.1 - 1 μg/kg, s.c.) rapidly evoke prominent opioid receptor-mediated thermal hyperalgesia in naïve mice, lasting for several hours [11,35]. Cotreatment of these mice with ultra-low-dose NTX (0.1 ng/kg) blocks this hyperalgesia and results in rapid conversion to potent analgesia lasting >3 hours. These correlative data provide a pharmacologic bioassay, which may help to estimate the amount of *endogenous* opioid-agonist release that could account for the hyperalgesia observed in Crain and Shen's [1] study of cAMP-PDE inhibitors in *naïve* mice. Furthermore, cotreatment with a subanalgesic dose of kelatorphan [36], an inhibitor of multiple endogenous opioid peptide-degrading enzymes, appears to stabilize endogenous excitatory amounts of opioid agonists released by low doses of cAMP-PDE inhibitors, resulting in conversion of the usual hyperalgesia to prominent analgesia without requiring selective blockade of excitatory opioid receptor signaling [1].

Cotreatment of naïve mice with 1 pg/kg rolipram plus another selective blocker of excitatory opioid receptor signaling, CTX-B (10 μg/kg) (see Section 3.1) also resulted in prominent opioid analgesia [1]. Administration of CTX-B alone had no effect on baseline tail-flick latency. In other mice cotreated with rolipram (1 μg/kg) plus CTX-B (10 μg/kg), the prominent unmasked analgesia was rapidly blocked by delayed injection of high-dose NTX (1 mg/kg) at 3 hours after initial drug treatment. This result provides further evidence that the analgesia unmasked by CTX-B is mediated by endogenous inhibitory opioid receptor signaling.

Maintenance of opioid receptor-mediated hyperalgesia for many hours after injection of cAMP-PDE inhibitors is interesting in view of the relatively rapid metabolic degradation of the small quantities of endogenous opioid peptide agonists [37] that appear to be released so as to activate excitatory opioid receptor signaling in mice. Rolipramor caffeine-induced elevation of cAMP may increase the efficacy and duration of the released endogenous opioid agonists as a result of the cAMP-*dependent glycosyltransferase* [38], *positive-feedback phosphorylation cycle* [8,9,12] that rapidly increases synthesis of GM1 and thereby markedly prolongs activation of progressively sensitized excitatory GM1-regulated, G_s-coupled opioid receptors (see Section 3) [12,23-25]. In addition to the release of endogenous opioid peptides by rolipram as suggested by Crain and Shen's [1] study, *endogenous morphine*, which is widely distributed in low concentrations throughout the nervous system [39], may also be released [40], although no direct assays have

been carried out in mouse tail-flick or related stress tests.

5. Endorphinergic Attenuation of Distress

In order to apply these intriguing mouse tail-flick findings (Sections 3 and 4) to humans, a series of double-blind pilot studies were completed to test cotreatments with Endorphin Enhancers (e.g. caffeine, forskolin) and Opioid Receptor Switchers (e.g. NAC, magnesium sulfate), using a "cold pressor" pain tolerance assay [41,42]. Prior cold pressor tests carried out by LaVincente *et al*. [43] demonstrated that cotreatment of healthy human subjects with the exogenous opioid, buprenorphine, together with ultra-low-dose naloxone or ultra-low-dose naltrexone markedly enhanced opioid analgesia, confirming earlier mouse tail-flick assays by Crain and Shen (Section 3). In Crain *et al*.'s "cold pressor" studies [42], each nutraceutical-Endorphin Enhancer and Opioid Receptor Switcher was administered to subjects separately as well in combination. Pain tolerance was not increased more than placebo by the separate administration of each agent; in fact, the Endorphin Enhancers alone generally produced a *decrease* in pain tolerance. Remarkably, the combination of an Endorphin Enhancer with an Opioid Receptor Switcher produced a dramatic *increase* in pain tolerance for most subjects, It is important to note that these endorphinergic formulations effectively and safely increased pain tolerance even though they contained no conventional analgesics [41,42,44].

These clinical studies are consistent with Crain and Shen's preclinical research demonstrating that the combination of an Endorphin Enhancer with an Opioid Receptor Switcher produces increased pain tolerance by enhancing *inhibitory* opioid receptor signaling by *endogenous* rather than *exogenous* opioids (Section 4). By combining an Endorphin Enhancer (e.g., caffeine, forskolin) with an Opioid Receptor Switcher (e.g., NAC, magnesium sulfate), a promising new class of remarkably safe and potentially effective pain relief formulations has been discovered. Furthermore, these studies reveal that the addition of an Opioid Receptor Switcher reverses the pain hypersensitivity typically seen when Endorphin Enhancers are administered alone. Therefore, a simple and safe method to reverse hyperalgesia typically produced by cAMP-PDE inhibitors, such as caffeine, has also been revealed. Recent studies on the analgesic effects of NAC on capsaicin-evoked inflammatory pain in mice [45], which can be blocked by naloxone, provide further support for our use of this sulfation agent as an Opioid Receptor Switcher. However, the molecular mechanism of action of NAC on bimodal opioid receptor functions (see Sect. 3.2) requires further pharmacologic analyses. Interestingly, intrathecal capsaicin injections in rats have been shown to release β-endorphin in the brain [46]. This indicates that capsaicin can act as an Endorphin Enhancer and that co-treatment with NAC may, indeed, result in switching from excitatory to inhibitory opioid receptor signaling as suggested by Crain and Shen (Sections 3 and 4).

Clinical observations of the emotional state of the subjects in these "cold pressor" studies revealed unexpected, dramatic, and meaningful changes in the *emotional distress* they experienced when facing this stress-inducing situation [41,42]. When subjects were administered a combination of an Endorphin Enhancer (e.g., caffeine, forskolin) and an Opioid Receptor Switcher (e.g., NAC, magnesium sulfate), a remarkable reduction in emotional and physical distress, including anxiety and agitation as well as anger and irritability, was evident in the majority of subjects. Most subjects, when given this combination of agents, were clearly more relaxed and calmer with a greater sense of well-being and a more adaptive approach to the stress-induced "cold pressor" paradigm. In discussing their experience in each experimental condition, it became clear that the subjects' higher pain tolerance, *i.e*., their ability to keep their hands in the ice-cold water longer, when administered the combination formulation, was generally due to their reduced emotional and physical distress more than a significant decrease in nociceptive pain.

Therefore, the combination of an Endorphin Enhancer (e.g., caffeine, forskolin) and an Opioid Receptor Switcher (e.g., NAC, magnesium sulfate) may provide a promising method to safely relieve emotional and physical distress and to enhance an adaptive response to stressful situations. These findings suggest a new understanding of earlier preclinical studies conducted by Crain and Shen [1] using an animal tail-flick paradigm, such that this novel method to simultaneously enhance endorphins and rebalance opioid receptor signaling relieves *distress even more than directly attenuating nociceptive pain*. In fact, increasing evidence suggests that exogenous opioids, such as morphine, function more by reducing emotional and physical distress than the actual elimination of nociceptive pain [47]. By amplifying the natural distress-relieving benefits of *endogenous* opioids rather than administering *exogenous* opioid drugs, this evidence suggests that endorphins triggering inhibitory signaling may provide a sense of calm, comfort, and well-being without the serious problems seen with *exogenous* opioid drugs, including tolerance, dependence, and other noxious side-effects such as cognitive and gastrointestinal symptoms.

In order to evaluate the potential clinical benefits of this novel nutraceutical formulation, a 26-month clinical research project was conducted consisting of 203 case studies [41,42]. Combinations of one or more Endorphin Enhancers (e.g., caffeine, forskolin) and Opioid Receptor Switchers (e.g., NAC, magnesium sulfate), with and without synergizing agents (e.g., l-theanine, white willow bark), were administered to patients suffering from a

wide variety of emotional and physical distress dysfunction disorders including chronic anxiety and agitation, anger and irritability, cravings and addictions, aches and pains, as well as affective, autism spectrum, and attention-deficit, and dementia disorders. A remarkable and reliable set of clinical benefits of the endorphinergic formulations were reported by patients as well as observed by their psychotherapists and family members. Most patients felt a sense of calm, comfort, well-being, and positive mood while simultaneously experiencing enhanced mental clarity, energy, memory, and attention. A consistent effect among patients was a reduction in underlying agitation and restlessness, anxieties and worries, obsessions and compulsions, anger and irritability, cravings and substance abuse, as well as motivation and attention deficits. As a result, they were more able to constructively change their beliefs and behaviors, and often developed healthier lifestyles, with greater control over binge-eating as well drug and alcohol use, obsessions and compulsions, emotional outbursts, and social withdrawal. These clinical observations were consistent with self-reported improvements revealed on the Distress Dysfunction Inventory anonymously administered at the end of each case study [42].

These clinical findings are compelling and consistent with the decades of preclinical nerve tissue culture and animal research on the endogenous opioid system summarized above (Sections 3 and 4). Furthermore, this clinical support for our novel method to balance and enhance the endogenous opioid system is particularly significant since it is well accepted in the clinical and research literature that caffeine is counter-indicated for anxiety disorders because it directly stimulates anxiety and pain sensitivity [48,49]. Paradoxically, by combining caffeine with NAC and/or magnesium sulfate, as predicted by the bimodal theory of the endogenous opioid receptor system, the anxiety-stimulating and hyperalgesic effects of caffeine are transformed into anxiolytic and pain relief benefits. This reversal of caffeine's effect on emotional distress and pain sensitivity is consistent with, and supports the principles that sulfated agents can switch opioid receptors from the excitatory to the inhibitory mode, thereby reducing anxiety and pain. Without an Opioid Receptor Switcher, administration of an Endorphin Enhancer alone is more likely to produce the opposite effects, *i.e*. emotional and physical distress.

6. Endorphinergic Distress Syndrome

Our preclinical and clinical studies provide consistent support for the bimodal nature of opioid receptors and the critical role of excessive excitatory opioid receptor signaling, together with abnormal endorphin levels, in producing a wide variety of emotional and physical distress symptoms that are reminiscent of Collier's [2,3] "quasi-morphine withdrawal syndrome," including exaggerated fears and anxiety, irritability and anger, cravings and addictions, as well as hyperalgesia and pain. Since the endogenous opioid system appears to play a central role in the mediation of an adaptive response to stress, we postulate that imbalances in this system are among the neurophysiologic dysfunctions responsible for many of the symptoms of emotional and physical distress, which are manifested in a variety of psychiatric, neurologic, and neurodevelopmental disorders including chronic anxiety, addiction, anger, hyperalgesia, and affective, personality, autism spectrum, attention-deficit, and dementia disorders as well as stress-related illnesses.

On the basis of our preclinical and clinical evidence we propose the existence of an *Endorphinergic Distress Syndrome* (EDS), manifested clinically by a variety of emotional and physical distress symptoms, created and maintained, at least in part, by a dysfunctional imbalance in bimodal opioid receptor signaling and abnormal endogenous opioid availability. The remarkably consistent clinical benefits of the novel, critically formulated endorphinergic preparations used in our studies, based on our new insights into bimodal opioid receptors and endorphin science, provide significant support for the critical role of endogenous opioid system imbalances in causing and maintaining these distress conditions. The elusive quest for the central mediating system underlying the emotional and physical distress symptoms of many psychiatric, neurologic and neurodevelopmental syndromes as well as stress-related medical disorders may be closer than ever.

Furthermore, our clinical studies suggest that effective treatment of EDS may be provided by the oral administration of critically formulated nutraceutical formulations consisting of one or more Endorphin Enhancers (e.g., caffeine, forskolin) with one or more Opioid Receptor Switchers (e.g., NAC, magnesium sulfate). These safe endorphinergic formulations have been shown to significantly reduce a wide variety of emotional and physical distress symptoms, including chronic anxiety, anger, addiction, and hyperalgesia. Therefore, these formulations should be considered as part of a comprehensive treatment plan for distress-related psychiatric, neurologic, and neurodevelopmental disorders. Our clinical evidence suggests that by reducing emotional and physical distress, caused by endogenous opioid system imbalances, our endorphinergic formulations can facilitate the effectiveness of more conventional treatments for these disorders. Further treatment considerations for EDS are more thoroughly explored elsewhere [42].

7. EDS Due to Excessive Opioid Peptides, Excitatory Amino Acids, and Sugars in Common Foods

Our research suggests that EDS may be due, in part, to

excessive dietary intake of opioid peptides and excitatory amino acids in a variety of popular foods. In addition to the opioid peptides that can be released by endorphin enhancers, e.g., caffeine, rolipram, and other cAMP-PDE inhibitors, as reviewed in Sect. 4, evidence has been obtained that opioid peptides, known as exorphins, are also present in many foods [50]. Pepsin hydrolysates of wheat gluten and casein have been shown to contain specific opioid peptides, e.g., gluteomorphin and casomorphin that appear to be able to cross the blood brain barrier [51,52]. These and many other opioid peptides have been extracted from bread, milk, cheeses, etc. In view of the > 1000-fold greater opioid sensitivity of excitatory vs inhibitory opioid receptor signaling (Sect. 2), and the progressive excitatory sensitization that occurs after initial activation, excessive *exorphins* circulating after intake of these processed foods may well be responsible for exacerbating low-dose opioid-induced hyperalgesia, protracted dependence, and the widespread societal increase in EDS conditions that are triggered by *endorphinergic* activation of excitatory opioid receptor signaling (Sect. 3, 4). Furthermore, mouse tail-flick assays by Crain and Shen [41] demonstrated additional types of dietary agents that can selectively activate G_S-coupled excitatory opioid receptors so as to evoke hyperalgesia, e.g. excitatory amino acids, such as glutamic acid and its salts, e.g. monosodium glutamate (MSG). These preclinical studies have helped to guide development of endorphinergic formulations for the treatment of EDS.

Interestingly, increasing evidence indicates that excessive dietary *sugar* intake can cause "*endogenous opioid dependence*" [53,54]. Various biochemical and pharmacologic mechanisms have been suggested to account for this unexpected "sugar addiction" [54], but no conclusive, direct evidence has been demonstrated. In view of the studies by Crain and Shen (Sect. 3.1) on the crucial role of GM1 ganglioside in regulating G_S-coupled excitatory opioid receptor signaling, we suggest that these opioid dependence and addictive effects of dietary sugars may be a *direct* effect of their enhancement of the biosynthesis of gangliosides which, as emphasized by Fishman and Brady [55], "proceeds by the ordered sequential addition of sugars to the lipid moiety" of these glycolipids. Alternatively, sugars may enhance the binding affinity of GM1 ganglioside to the putative allosteric site on opioid receptors discussed in Sect. 3.1. These opioid effects of dietary sugars are remarkably consonant with the *endogenous opioid hyperalgesia and dependence* elicited in *naïve* mice by Crain and Shen [56] shortly after systemic injection of a low dose of GM1 ganglioside (10 μg/kg, s.c.).

We suggest, therefore, that excessive dietary use of these common foods and additives, including wheat gluten, milk casein, sugar, and MSG, may have deleterious effects on the endogenous opioid system, specifically producing excessive excitatory opioid receptor signaling. Given the widespread consumption of these foods, they may play a role in the development of EDS, producing cravings and dependence as well as emotional and physical distress, more generally. The use of our endorphinergic formulations become all the more important, both to: 1) restore balance to the endogenous opioid system created by these common foods, as well as by other substances that create endorphinergic imbalances, e.g. exogenous opiate drugs, and 2) to facilitate withdrawal from them with minimal tolerance/dependence side effects.

8. Future Research Implications

The favorable clinical results obtained following treatment with our present nutraceutical formulations (Sect. 5) are, indeed, quite remarkable in view of the diverse arrays of endorphins that may be released in many different regions of the central and peripheral nervous system by our preliminary selection of specific nutraceuticals. Nevertheless, our present research project provides significant support for the central role of endogenous opioid system imbalances in the development and maintenance of EDS associated with a wide range of psychiatric, neurologic, and neurodevelopmental, as well as distress-related medical conditions [42]. Further research is needed to confirm these findings and to correlate specific symptom patterns and syndromes with neuroimaging and biochemical assays, which have been developed to assess neurotransmitter systems, including specific endogenous opioids, as well as other relevant bioassays, such as sulfate, GM1, and cortisol levels.

Our preliminary studies also support the importance of further clinical research designed to systematically investigate the specific imbalances in the endogenous opioid system that underlie each EDS cluster to determine whether specific neurophysiologic profiles exist. Once these specific profiles are determined, and assessment methods are established, more critically targeted endorphinergic formulations can be developed. While we have carefully selected specific Endorphin Enhancers and Opioid Receptor Switchers for our preclinical and clinical studies, other nutraceutical as well as pharmaceutical agents should also be evaluated (Sections 2 and 3). Systematic double-blind-dosing clinical studies, using a variety of functionally appropriate agents, will determine the most therapeutic formulations for EDS disorders. Neuroimaging and biochemical assays should also be done to assess the effects of these endorphinergic formulations on neurotransmitters and other bioassays throughout the CNS and peripheral nervous system, as well as activation of relevant brain regions.

In summary, three decades of preclinical and clinical

research on the endogenous opioid system suggests that the principles used to develop these novel formulations, which combine one or more Endorphin Enhancers, with one or more Opioid Receptor Switchers, perhaps with other synergizing agents, may be remarkably effective towards the development of a new class of endorphinergic nutraceuticals, as well as pharmaceuticals, for the prevention and treatment of EDS. These endorphinergic formulations are intended to attenuate dysfunctional emotional and physical distress by concomitantly enhancing endogenous opioid release and switching opioid receptor signaling from an excessively excitatory to a normal inhibitory mode. Systematic large-scale clinical studies will help validate and refine the use of these novel critically formulated endorphinergic therapeutics for the prevention and treatment of emotional and physical distress symptoms associated with a wide variety of psychiatric, neurologic, neurodevelopmental and other distress-related medical disorders, as well as a safe method to enhance well-being, mental clarity, positive mood, and adaptive emotional, cognitive, behavioral, and physiological functioning.

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In Memoriam of Dr. Ke-Fei Shen —Died on November 17, 2012

by Dr. Stanley M. Crain, Professor Emeritus, Albert Einstein College of Medicine

I met Ke-Fei in 1980 at the "Shanghai Workshop on Neurobiology", an historic conference at the Brain Research Institute in Shanghai. The Chinese National Academy of Sciences had invited me, along with a dozen US and European neuroscientists, in order to update 100 young Chinese neuroscientists (including Dr. Shen) on recent trends in Western neuroscience research. This conference was co-sponsored by the US National Academy of Sciences and provided the first official contact between US and Chinese neuroscientists after many years of intellectual isolation during the brutal Chinese "cultural revolution!"

I was about 57 years old during my visit to Shanghai and among the bright young Chinese neuroscientists at the conference was Dr. Ke-Fei Shen, who was then 48 and already established as a prominent leader of the Shanghai Brain Research Institute. He was evidently inspired by my lecture about utilizing electrophysiologic analyses of nerve tissue cultures to analyze the mechanisms of action of opioids. In collaboration with Dr. Eric Simon (co-discoverer of opioid receptors), we had recently demonstrated that sensory-evoked dorsal-horn synaptic networks discharges in organotypic tissue cultures of mouse spinal cord with attached dorsal root ganglia were selectively depressed by perfusion with a series of exogenous and endogenous opioids, at concentrations remarkably proportionate to their potency in the intact animal (Crain *et al*., *Brain Research*, 1978).

Several years later, I received a letter from the director of the Shanghai Brain Research Institute indicating that Dr. Shen would like to visit my laboratory at the Albert Einstein College of Medicine en route from a 6-month visiting research scholarship in France. Dr. Shen arrived in my lab around 1985 and he was so impressed with our opioid research projects that he decided to prolong his visit for "a while". Ke-Fei and I interacted so well together that he finally made a crucial decision to join my laboratory and cancel his prior commitment to return to the Shanghai Brain Research Institute!

Dr. Shen skillfully initiated a series of elegant electrophysiologic analyses of opioid effects on dorsal-root ganglion neurons in our organotypic cord-ganglion tissue cultures, utilizing intracellular microelectrode techniques that I had developed many years earlier (Crain, *Journal of Comparative Neurology*, 1956). Within a year we were able to obtain significant experimental evidence that opioids could evoke not only well-known inhibitory signaling effects but also novel excitatory signaling in the action potential of the same sensory neuron. These excitatory effects were shown to be selectively elicited by >1000-fold lower opioid concentrations than required to elicit inhibitory effects. Excitatory opioid effects were selectively enhanced by application of pharmacologic agents that increase the cyclic AMP levels in these neurons (Shen & Crain, *Brain Research*, 1989). This seminal paper stimulated a remarkable series of pharmacologic and biochemical studies initially aimed at molecular mechanisms underlying these novel opioid-evoked excitatory effects in sensory neurons and subsequently applied to broader problems, e.g. opioid-induced hyperalgesia, tolerance and dependence (published in *Brain Research*, *PNAS*, *Pain* and other prominent journals). After a decade of microelectrode analyses of opioid effects on sensory neurons in vitro, Dr. Shen and I began in 1995 another decade of significant pharmacologic behavioral studies of analgesia and hyperalgesia in mice, utilizing antinociception and hyperalgesia hot-water tail-flick assays. These studies led to our discovery of novel methods to attenuate tolerance and dependence during chronic opioid treatment of mice as well as pain patients by co-treatment with ultra-low-dose naltrexone or neuraminidase inhibitors of GMI ganglioside.

Finally, we demonstrated that a significant degree of opioid-like analgesia could be elicited in mice without the use of exogenous opioids. Naïve mice were injected with cyclic AMP-phosphodiesterase inhibitors, e.g., rolipram or caffeine, resulting in hyperalgesia and the release of *endogenous* opioids, *i.e*., endorphins. Subsequent injection of ultra-low-dose naltrexone resulted in the rapid onset of opioid-mediated (naltrexone-reversible) analgesia (Crain & Shen, *Brain Research*, 2008).

Dr. Shen and I were, indeed, delighted to have had the opportunity to demonstrate this animal model of *endorphinergic analgesia* as the culmination of our research projects at the Albert Einstein College of Medicine! This study also provided the biochemical and pharmacologic rationale for our discovery of an *Endorphinergic Distress Syndrome* and the development of novel clinical treatments of severe emotional and physical distress dysfunction disorders, including chronic anxiety, addiction, and pain, in collaboration with my son, Dr. Steven Crain, a neuropsychotherapist. Successful pilot studies have recently been carried out with a nutraceutical endorphinergic formulation (Endorphinate®) developed by Pondera Pharmaceuticals Inc.

Dr. Shen's pioneering intracellular microelectrode analyses of the bimodal excitatory/inhibitory functions of opioid receptors in sensory neurons in tissue culture have, indeed, provided remarkably fruitful insights into complex behavioral activities of the entire nervous system, far beyond our wildest expectations when we started this collaborative project in the 1980's!