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Dynorphin A_{1-13} Causes Elevation of Serum Levels of Prolactin Through an Opioid Receptor Mechanism in Humans: Gender Differences and Implications for Modulation of Dopaminergic Tone in the Treatment of Addictions

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ABSTRACT

Dynorphin peptides act preferentially at κ- as well as μ- and δ-opioid receptors. This study was conducted to determine whether dynorphin peptides act to lower dopaminergic tone in the tuberoinfundibular system, resulting in elevated serum prolactin levels and, if so, whether such an effect is mediated by the opioid receptors. Dose-related increases in serum prolactin levels were observed after dynorphin A_{1-13} was administered i.v. in doses of 120 and 500 μg/kg to healthy human volunteers with no history of drug or alcohol abuse. Studies were then conducted to determine whether this effect is opioid receptor mediated and, if so, whether at κ- or μ types. Pretreatment with the opioid antagonist nalmefene (30 mg i.v.), which has high affinity at both μ- and κ-opioid receptors, caused a greater attenuation in dynorphin A_{1-13}-stimulated increases in serum prolactin levels than pretreatment with similarly high doses of naloxone, an antagonist with lower affinity for both μ- and κ-opioid receptors. These results suggest dynorphin A_{1-13} lowers tuberoinfundibular dopaminergic tone through action at κ- and possibly μ-opioid receptors. Female subjects were significantly more responsive to the prolactin effects of dynorphin than were male subjects. Dynorphin gene expression, dynorphin peptides, and κ-opioid receptor gene expression and binding have been shown to be altered in response to cocaine administration. Also, both dynorphin peptides and synthetic κ-opioid agonists have been shown to lower dopamine levels in the nucleus accumbens and to attenuate cocaine-induced surges in dopamine levels. Thus, a dynorphin–like compound capable of reaching critical mesolimbic-mesocortical and nigrostriatal dopaminergic systems may be effective in the management of cocaine addiction.

Dynorphin A_{1-17} is one of the natural peptides derived from processed prodynorphin, the single-gene product of one of the three identified mammalian opioid peptide genes, preprodynorphin (Goldstein et al., 1979; Chavkin and Goldstein, 1982). Both dynorphin A_{1-17} and its shortened natural sequence analog dynorphin A_{1-13} have been used in neurobiological studies in various species, including mouse, rat, guinea pig, and nonhuman primates. The processing and further biotransformation of the dynorphin peptides have been studied using various techniques (Chou et al., 1994, 1996). Using the technique of extended range, matrix-assisted laser desorption ionization mass spectrometry, we have studied the ex vivo biotransformation of dynorphin A_{1-17} and dynorphin A_{1-13} in human and in rhesus monkey blood, and the kinetics of the production of the various products (Chou et al., 1994; 1996; Yu et al., 1996). Dynorphin peptides are the natural endogenous ligands of the κ-opioid receptors; these peptides, and especially their shorter biotransformation products, also bind to the μ- and δ-opioid receptors (Chavkin and Goldstein, 1982; Chen et al., 1993).

Several studies in different species have shown that dynorphin A peptides may both provide analgesia and also prevent or reverse opiate withdrawal signs and symptoms in morphine-dependent animals and possibly also in humans (Wen and Ho, 1982; Aceto et al., 1982; Takemori et al., 1993). Studies have shown that both single-dose, acute, and chronic "binge" pattern administration, or self-administration, of cocaine in rodent models leads to a significant increase in levels of gene expression of preprodynorphin with increases in mRNA levels measured by a variety of techniques (Hurd et al., 1992; Spangler et al., 1993; Daunais et al., 1993). These changes have been found exclusively in regions of abundant dopaminergic terminals and specifically in the caudate

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ABBREVIATIONS: ANOVA, analysis of variance; GABA, γ-aminobutyric acid.

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putamen region of the striatum (Spangler et al., 1993; Daunais et al., 1993). Dynorphin peptides have also been reported to be enhanced in the caudate putamen, nucleus accumbens, substantia nigra, and the ventral tegmental area after chronic cocaine administration (Sivam, 1989). Studies from our laboratory using quantitative autoradiography techniques have shown that the densities of κ-opioid receptors, as well as μ-opioid receptors, are significantly increased in the caudate putamen, nucleus accumbens, and other regions of the mesolimbic-mesocortical and nigrostriatal dopaminergic systems where there are abundant dopaminergic terminals, after chronic binge-pattern cocaine administration (Unterwald et al., 1994). Recently, in studies using positron-emission tomography with [^{11}C]carfentanil as the receptor-selective radioligand, a similar finding has been made in cocaine addicts, with increased density in μ-opioid receptors found in specific brain regions (Zubieta et al., 1996). Other studies from our laboratory have shown that the levels of gene expression of the κ-opioid receptor, determined by a modified quantitative solution hybridization RNase protection assay, are significantly reduced in the substantia nigra following chronic binge-pattern cocaine administration in the rat; this may be caused by the significant increase in dynorphin peptides released in that region through the nigrostriatal pathway (Spangler et al., 1996).

Studies from other laboratories have shown that μ-opioid receptor activation may indirectly enhance dopamine release by acting on γ-aminobutyric acid (GABAergic) neurons to inhibit GABA release, thus disinhibiting regulation of dopaminergic neurons in the substantia nigra and ventral tegmental area. In contrast, synthetic κ-opioid receptor ligands may directly reduce dopamine levels in extracellular fluid in the caudate putamen, nucleus accumbens and related specific brain regions, as determined by microdialysis (Spanagel et al., 1990). Recent studies from our laboratory, using microdialysis conducted in awake, freely moving rats, have shown that administration of dynorphin A_{1-17} directly to the nucleus accumbens causes a significant reduction in basal levels of dopamine in the extracellular fluid (Claye et al., 1997).

We therefore have hypothesized that dynorphin peptides may act physiologically to modulate dopaminergic tone in the nigrostriatal, mesolimbic-mesocortical and also in the tuberoinfundibular dopaminergic systems. Changes in levels of activation of the dynorphin and κ-opioid receptor system during binge-pattern cocaine administration is a phenomenon that also may occur in human cocaine addiction, a concept partially supported by the findings of enhanced dynorphin gene expression in brains of cocaine addicts studied postmortem (Hurd and Herkenham, 1993). We have also hypothesized that a natural κ-opioid agonist such as dynorphin A_{1-17} itself, or another synthetic κ-opioid agonist, either a dynorphin A-like peptide or a κ-directed heterocyclic compound, with increased blood-brain permeability, might be effective in managing some aspects of cocaine addiction by modulating cocaine-induced alterations in dopaminergic tone (Kreek et al., 1994; Claye et al., 1997).

This study was conducted to determine whether dynorphin A_{1-13}, administered i.v., may cause an elevation of serum prolactin levels in healthy humans, to confirm and extend our pilot study findings in which such an observation was made initially (Kreek et al., 1994). In humans, prolactin release is primarily under tonic inhibition by dopamine released through the tuberoinfundibular dopaminergic neuron pathways (Moore and Lookingland, 1995). Thus, an elevation in serum prolactin levels would suggest that dynorphin acts in humans to lower dopaminergic tone in the tuberoinfundibular dopaminergic system. This phenomenon might be paralleled by a lowering of basal levels or modulation of induced changes in levels of synaptic and extracellular fluid dopamine in the mesolimbic-mesocortical or nigrostriatal dopaminergic systems, through administration of a different dynorphin-like κ-agonist, which may be capable of reaching these brain regions.

In this study, we also have addressed the related mechanistic question of whether any observed increases in serum prolactin levels are mediated by specific opioid receptors, suggesting action at κ- as well as at μ-opioid receptors, or whether any prolactin releasing effect of dynorphin A_{1-13} is a nonspecific, stress-related effect.

Finally, we have determined that there are gender differences in the effects of dynorphin on serum prolactin levels.

Materials and Methods

Five sets of studies were conducted; all were conducted in 27 normal, healthy volunteer subjects, including 22 males and 5 females, with no history of any drug or alcohol abuse.

The first study was conducted to determine the effects of both a low dose (120 mg/kg b.wt.) of dynorphin A_{1-13} and then a higher dose (500 μg/kg) of dynorphin A_{1-13}, administered i.v., on serum prolactin levels. Ten normal, healthy volunteers participated in this first study. These included 8 males and 2 females, with a mean age of 31 years, ranging from 25 to 48 years.

The second study was conducted to determine whether a moderately high dose of opioid antagonist, naloxone (10 mg), administered i.v. 10 min before the administration of the lower dose of dynorphin A_{1-13} (120 μg/kg), would alter the effects of dynorphin on serum prolactin levels. This dose of naloxone has been generally accepted to be high enough to have full effectiveness at μ-opioid receptors but possibly only partial effectiveness at δ- and κ-opioid receptors. Ten normal, healthy volunteer subjects participated in this second study, including 8 males and 2 females with a mean age of 32 years, ranging from 22 to 42 years.

The third study was conducted to determine whether a very high dose of naloxone (30 mg), administered i.v. 10 min before i.v. administration of a dynorphin A_{1-13} (120 μg/kg), would alter the effects of dynorphin on serum prolactin levels. Because naloxone has its highest affinity at μ-, lower affinity at κ-, and its lowest affinity at δ-opioid receptors, this very high dose (30 mg i.v.) of naloxone also was studied because such a dose might be sufficient to remove all natural endogenous, as well as exogenous, μ- and κ-opioid peptide ligands from specific μ- and κ-opioid receptors. This study was conducted in 10 normal, healthy volunteer subjects, including 9 males and 1 female with a mean age of 33 years, ranging from 28 to 42 years.

The fourth set of studies was conducted to determine whether another opioid antagonist, nalmefene, which has its highest affinity for μ- but also high affinity for κ- and low affinity for δ-opioid receptors, would alter the effects of dynorphin A_{1-13} on serum prolactin levels. Nalmefene was administered at moderately high doses of 10 mg i.v. 10 min before the administration of dynorphin A_{1-13} (120 μg/kg) to determine whether this antagonist would alter the effects of dynorphin A_{1-13} on serum prolactin levels. This study was conducted in 10 subjects including 8 males and 2 females with a mean age of 37 years, ranging in age from 31 to 47 years.

The fifth set of studies was conducted to determine whether a very high dose of nalmefene (30 mg) administered i.v. 10 min before the

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i.v. administration of dynorphin $A_{1–13}$ (120 μg/kg) would alter the effects of dynorphin on serum prolactin levels. Because nalmefene has greater κ-opioid affinity than naloxone, with these very high doses it would be expected that nalmefene would readily remove all κ as well as μ endogenous and exogenous ligands from their specific receptors. This study was conducted in 10 subjects, including 9 males and 1 female with a mean age of 30 years, ranging from 23 to 42 years.

In these five studies, 22 males and 5 females underwent both placebo and low-dose (120 μg/kg) dynorphin $A_{1–13}$ studies. Therefore, the gender differences in response to dynorphin $A_{1–13}$ with respect to the magnitude of prolactin elevation was determined.

All subjects were screened in the outpatient clinic of the Rockefeller University Hospital, an National Institutes of Health-supported General Clinical Research Center, on at least two occasions. All subjects gave both written and oral informed consent to the studies, which followed a protocol approved by the Institutional Review Board of the Rockefeller University Hospital. Studies using dynorphin $A_{1–13}$ were conducted under an approved investigator-initiated Investigational New Drug status application from the United States Food and Drug Administration (held by Mary Jeanne Kreek, M.D., at the Rockefeller University). Dynorphin $A_{1–13}$ was prepared for human use and generously supplied by Neurobiological Technologies Incorporated (Richmond, CA). All subjects were admitted at least the night before each study to the Rockefeller University Hospital General Clinical Research Center. Studies were initiated after a 15-h overnight stabilization in a stress-minimized environment. Thirty minutes before the study, an i.v. indwelling cannula was placed, usually in the antecubital fossa, for administering dynorphin $A_{1–13}$, or placebo, in all studies, and for administering the specific opiate antagonist in studies 2, 3, 4, and 5, as well as for obtaining all blood specimens.

On one study day, in studies 1 to 5, after obtaining baseline specimens, dynorphin $A_{1–13}$, 120 μg/kg ("low dose"), was administered i.v. On a separate study day, in study 1 only, dynorphin $A_{1–13}$ 500 μg/kg ("high dose") was administered i.v. to each subject. The lower dose was always given before the higher dose (at the request of the Food and Drug Administration on the approval of the investigator initiated-Investigational New Drug Application). On a third day of each of the five studies, which was conducted on day 1, 2, or 3, dynorphin placebo was administered i.v. to each study subject. Multiple blood specimens were obtained both before and during the 6 h after dynorphin $A_{1–13}$ or placebo administration. In studies 2, 3, 4, and 5, the specified dose of a specific opiate antagonist was administered 10 min before administration of low-dose dynorphin $A_{1–13}$ (120 μg/kg). In study 2, the antagonist was naloxone, 10 mg; in study 3, naloxone, 30 mg; in study 4, nalmefene, 10 mg; and in study 5, nalmefene, 30 mg, each administered i.v. In each of these antagonist studies, a single dose of dynorphin $A_{1–13,}$ 120 μg/kg, was administered 10 min following the opioid antagonist. On a second study day, dynorphin $A_{1–13}$, at the low dose of 120 μg/kg, was administered alone to each of these study subjects.

Serum prolactin levels were determined by radioimmunoassay (using reagents from Nichols Institute Diagnostics, San Juan Capastrino, CA). Plasma levels of adrenocorticotropin (ACTH) were measured by radioimmunoassay (using reagents from Nichols Institute). Plasma cortisol levels were measured by radioimmunoassay (using reagents from Diagnostic Products Corporation, Los Angeles, CA).

Analyses of variance (ANOVA) with repeated measures followed by Newman-Keuls post hoc tests were used to evaluate the effects of dynorphin both before and after opioid antagonist pretreatment and effects of gender on serum prolactin measurements from the 0- to 90-min blood samples. Later time points, where any differences between the naloxone and nalmefene studies could be caused by the longer half-life of nalmefene in humans, were not used in the statistical analyses.

Results

In the first study (study 1), dynorphin $A_{1–13}$ had a dose-dependent effect on serum prolactin levels as shown in Fig. 1. Both doses of dynorphin $A_{1–13}$, 120 μg/kg and 500 μg/kg administered i.v., significantly elevated serum prolactin levels compared to placebo; there was a significant main effect for condition, $F(2,18) = 14.27$, $p < .0002$. Neuman-Keuls post hoc tests showed that both the low and the high dose each significantly elevated serum prolactin compared to placebo ($p < .001$ and $p < .005$, respectively), and the higher dose led to a significantly greater elevation in prolactin levels than the lower dose of dynorphin $A_{1–13}$ ($p < .05$). The lower dose (120 mg/kg) caused a prompt rise in serum prolactin in all

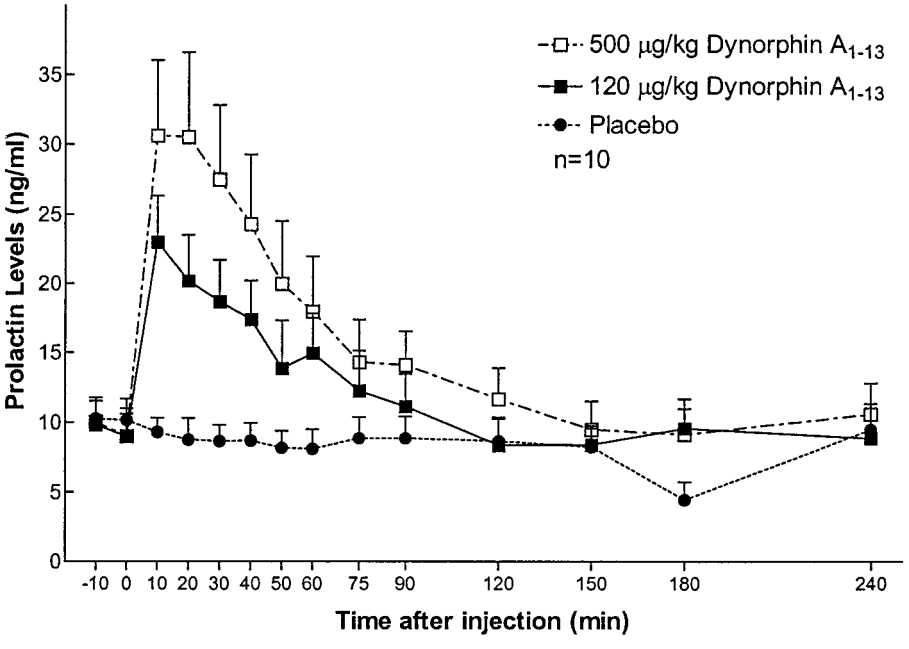

Fig. 1. Dynorphin $A_{1–13}$ at two doses, 120 μg/kg and 500 μg/kg, along with the dynorphin placebo were each administered i.v. on three different days to the same 10 study subjects, all healthy volunteers with no history of drug or alcohol abuse. A dose-dependent elevation of serum prolactin levels compared to placebo was observed; both the low and the high dose each significantly elevated serum prolactin levels compared to placebo ($p < .001$ and $p < .005$, respectively). The higher dose led to a significantly greater elevation in serum prolactin levels than the lower dose of dynorphin $A_{1–13}$ ($p < .05$).

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subjects with a mean peak level of 23 ng/ml, and the increased prolactin levels were significantly higher than placebo levels in these subjects from 10 to 40 min following this i.v. dynorphin administration. The higher dose of dynorphin A_{1-13} (500 μg/kg) similarly caused a prompt rise in serum prolactin levels, which rose to a mean peak of 31 ng/ml, and prolactin levels remained significantly elevated compared to placebo levels from 10 to 75 min. The 500-mg/kg dose of dynorphin A_{1-13} led to significantly higher levels of serum prolactin than the 120-μg/kg dose from 10 to 50 min. The specificity of this effect on serum prolactin can be seen by comparison with the lack of effect of dynorphin A_{1-13} on plasma ACTH or cortisol levels from the same subjects sampled at the same times, shown in Fig. 2.

In study 2, the 120-μg/kg dose of dynorphin A_{1-13}, both with and without pretreatment with the moderately high dose of 10 mg of naloxone, caused an increased level of serum prolactin compared to placebo for these subjects, as shown in Fig. 3a. The mean peak in serum prolactin after 120-μg/kg dynorphin alone was 29 ng/ml, whereas after pretreatment with 10 mg of naloxone, the mean was 25 ng/ml. There was a significant main effect for condition, $F(2,18) = 31.94$, $p < .00002$. Newman-Keuls post hoc tests showed that each dynorphin treatment, with and without naloxone pretreatment, led to significantly higher levels of serum prolactin than found in the placebo condition, $p < .0002$ in each case. Although Neuman-Keuls post hoc tests of condition showed that the pretreatment did not significantly blunt the prolactin rise overall, 10 mg of naloxone significantly blunted the prolactin response compared to dynorphin alone at the 10-, 20-, and 30-min time points (Newman-Keuls post hoc tests of the condition $\times$ time interaction).

In study 3, pretreatment with the higher 30-mg dose of naloxone had a more robust effect in blunting the rise in serum prolactin levels induced by 120 μg/kg dynorphin A_{1-13}, as shown in Fig. 3b. The mean peak of serum prolactin after dynorphin was 29 ng/ml and after pretreatment with 30 mg of naloxone the dynorphin-induced peak was 21 ng/ml. There was a significant main effect of condition, $F(2,18) = 34.95$, $p < .000002$, and Neuman-Keuls post hoc tests showed that

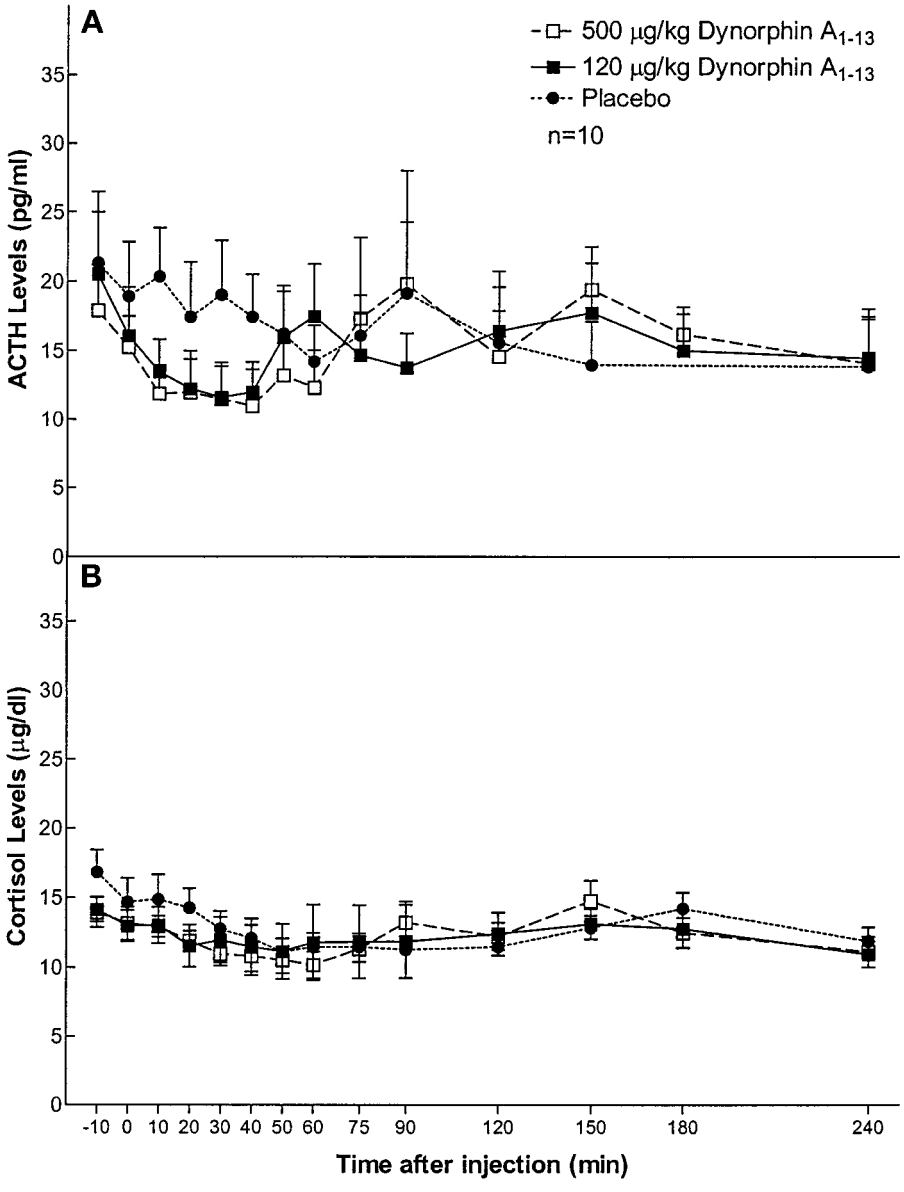

Fig. 2. Dynorphin A_{1-13} at doses of 120 μg/kg and 500 μg/kg administered i.v. had no effects on either plasma ACTH levels (A) or on plasma cortisol levels (B) when compared to levels following administration of dynorphin placebo as measured in the same subjects and blood samples as shown in Fig. 1.

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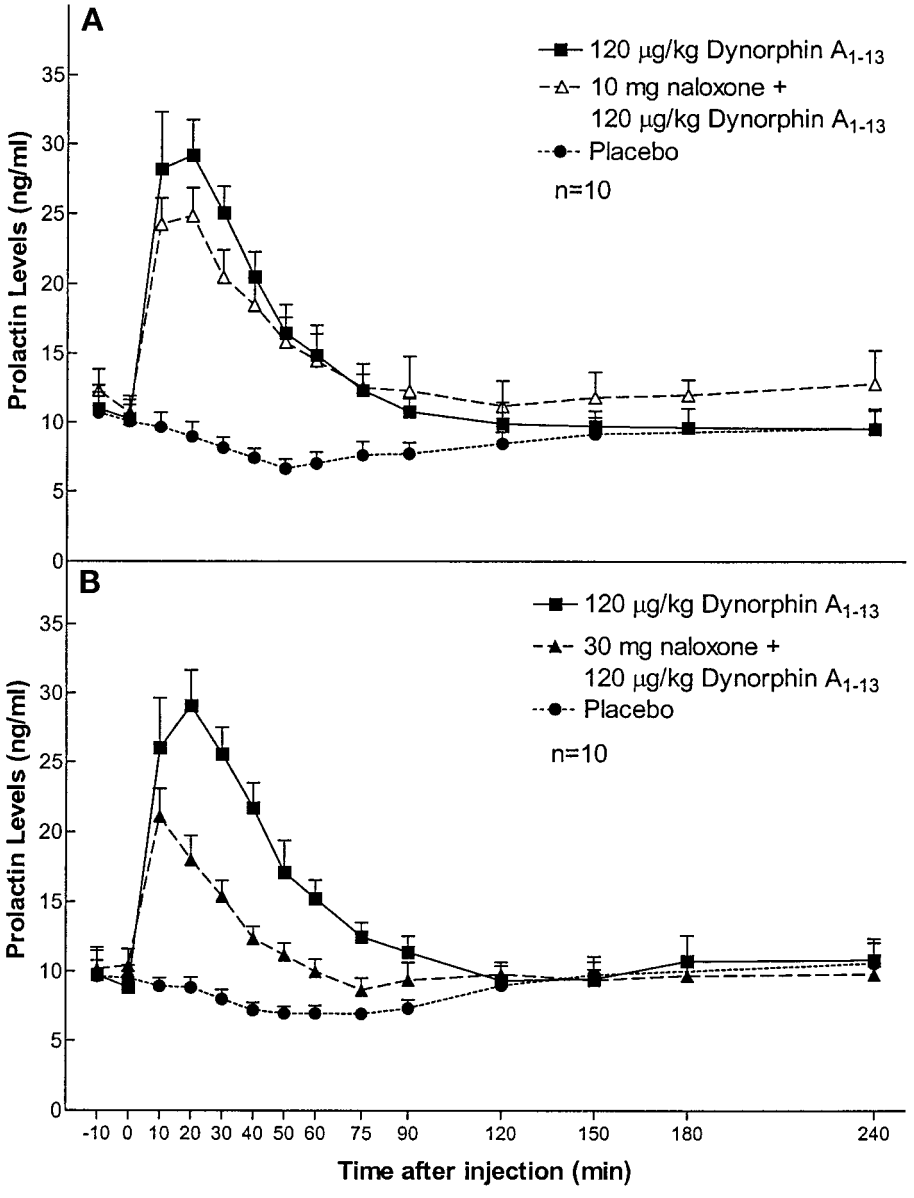

Fig. 3. A, low dose of dynorphin A_{1-13} 120 μg/kg alone or following pretreatment with a moderately high dose, 10 mg, of the specific opioid antagonist naloxone, which is primarily μ-opioid receptor-directed, was compared to a placebo administration to determine whether a moderate dose of a specific opioid antagonist would attenuate the dynorphin A_{1-13}-induced rise in serum prolactin levels. Although pretreatment with moderate dose naloxone did not significantly blunt the prolactin rise overall, this pretreatment did significantly blunt the serum prolactin response at 10-, 20-, and 30-min time points. B, pretreatment with a very high dose of naloxone (30 mg) i.v. significantly blunted the prolactin response overall ($p < .005$) with significant lowering of serum prolactin levels following naloxone pretreatment at 10 min through the 60-min time points.

not only was each dynorphin administration condition significantly elevated compared to placebo in these normal volunteers ($p < .0002$ without, and $p < .002$ with, 30 mg of naloxone), but that the pretreatment with naloxone significantly blunted the prolactin response overall ($p < .005$). The effect of the 30-mg dose of naloxone lasted longer than that of the 10-mg dose: post hoc tests showed that serum prolactin levels were significantly lower with naloxone pretreatment than without, from 10 through 60 min.

In study 4, the effect of pretreatment with 10 mg of nalmefene, an antagonist with high affinity for both μ- and κ-opioid receptors, on the serum prolactin rise elicited by 120 mg/kg dynorphin A_{1-13} can be seen in Fig. 4a. The mean peak in serum prolactin after dynorphin alone was 28 ng/ml and, after pretreatment with 10 mg of nalmefene, 22 ng/ml. As in study 2 with the same dose of naloxone, there was not a significant overall attenuation by nalmefene pretreatment of the prolactin rise after dynorphin, but 10 mg of nalmefene did significantly attenuate the prolactin rise at 10, 20, and 30 min.

The effect of pretreatment with the higher dose of nalmefene, 30 mg, in study 5, is shown in Fig. 4b. The mean peak in serum prolactin levels after dynorphin alone was 25 ng/ml, and after pretreatment with 30 mg of nalmefene, it was 16 ng/ml. This high dose of nalmefene did produce an overall blunting of the dynorphin-induced rise in serum prolactin levels (Newman-Keuls post hoc test, $p < .05$), as did the 30-mg dose of naloxone (see Fig. 3b). The 30-mg dose of nalmefene significantly attenuated the dynorphin-induced rise in serum prolactin levels from 10 although 40 min.

Thus, in these five studies, in each of which 10 healthy volunteers served in each of three conditions within a study, we have shown that i.v. dynorphin A_{1-13} causes a prompt and sustained dose-response elevation of serum prolactin, and that this rise is attenuated significantly by pretreatment with each of the opioid antagonists naloxone and nalmefene. This design gave maximum sensitivity in evaluating the effect of each dose and each opioid antagonist. However, due to the limited total volume of blood that can be sampled from

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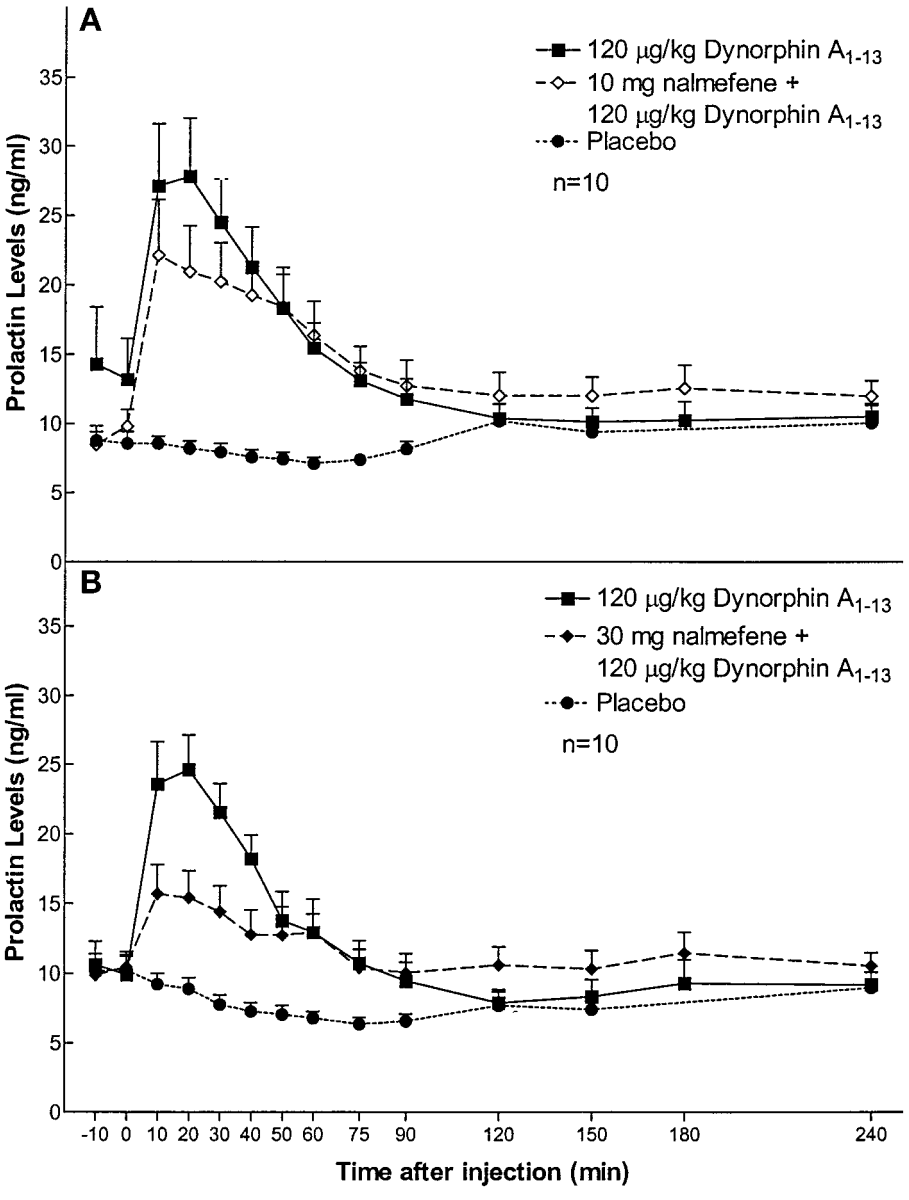

Fig. 4. The effects of a second specific opioid antagonist, nalmefene, which has high affinity for μ- as well as κ-opioid receptors, on attenuating the dynorphin A_{1-13} -induced rise in serum prolactin levels is shown. A, there was not a significant, overall attenuation of prolactin rise when nalmefene at moderate doses (10 mg) was administered, although there was a significant attenuation of prolactin rise at 10-, 20-, and 30-min time points. B, pretreatment with a very high dose of nalmefene (30 mg) produced an overall blunting of the dynorphin-induced rise in serum prolactin levels (Newman-Keuls post hoc test, $p < 0.05$) with a significant lowering of the dynorphin-induced rise in serum prolactin levels from 10 through 40 min.

any one subject, different groups of subjects served in the various different sets of studies.

To determine whether there were differences in the effect of the two opioid antagonists, naloxone and nalmefene, on the serum prolactin response to dynorphin, a two-step analytic approach was used. First, a preliminary ANOVA of data from the placebo and dynorphin A_{1-13} alone conditions of studies 2 to 5 showed that there were no significant differences between the four study groups in their serum prolactin response to 120 μg/kg dynorphin alone. Then, a three-way ANOVA was used to evaluate the possible differences between antagonist and dose using the pretreatment condition from studies 2 to 5: antagonist $\times$ dose $\times$ time, with repeated measures on the last factor. There was a significant main effect of dose as expected from the original analyses of the individual studies, and a significant main effect of time. Of particular interest was the significant antagonist $\times$ time interaction found, $F(8,288) = 5.11$, $p < .00001$. Newman-Keuls post hoc tests showed that nalmefene significantly attenuated the dynorphin-induced rise in serum prolactin more than did naloxone at 10 and 20 min ($p < .0005$ and $p < .005$, respectively). Thus, there was a slightly greater attenuation effect of nalmefene than of naloxone, in the early time period.

Finally, we determined that there are gender differences with respect to the dynorphin A_{1-13}-induced increase in serum prolactin levels. Five females and 22 males had participated in one or more of the five sets of studies and each received both placebo and a low dose (120 μg/kg) of dynorphin A1–13. As shown in Fig. 5, females were far more responsive than males to the low dose of dynorphin, with higher and earlier peak levels of serum prolactin (females: time to peak, 10 min, peak serum prolactin 38 ng/ml; males: 20 min, 23 ng/ml). There was an overall main effect for gender $F(1,25) = 26.41$, $p < .00005$, with the prolactin response to low-dose dynorphin significantly different in females as contrasted to males at the 0 through 90 min time points (Newman-Keuls post hoc tests $p < .05$). Females also had greater prolactin levels in the placebo as well as in the dynorphin conditions (Newman-Keuls post hoc tests, $p < .05$, and $p < .0002$,

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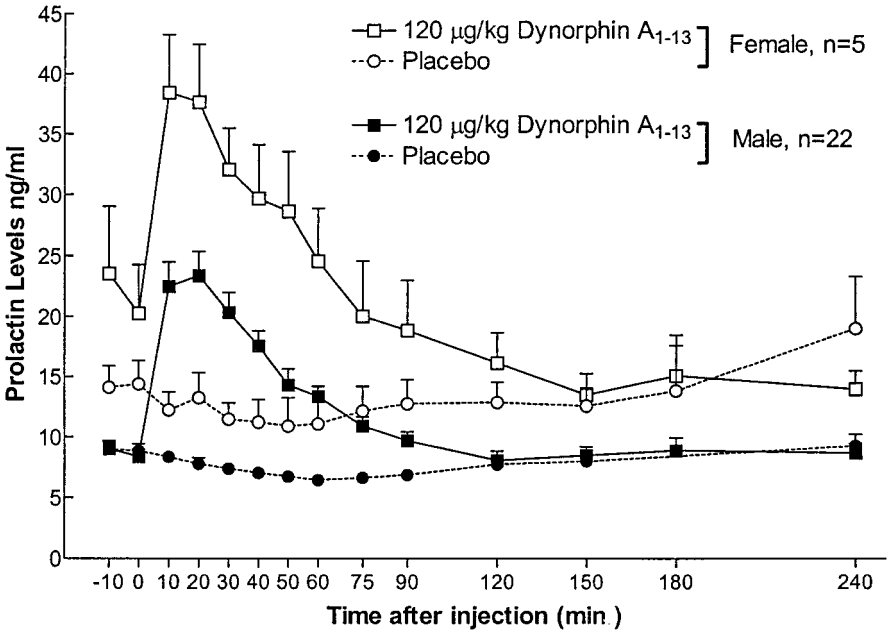

Fig. 5. Females were far more responsive than males to the low dose of dynorphin, with higher and earlier peak levels of serum prolactin. There was an overall main effect for gender, $p < .00005$, with the prolactin response to low-dose dynorphin significantly different at the 0- through 90-min time points (Newman-Keuls post hoc tests, $p < .05$). Also, females had higher prolactin levels in the placebo condition, $p < .05$.

respectively), and the difference in response to dynorphin was shown by a significant interaction (gender × condition) $F(1,25) = 6.47$, $p < .02$.

All study subjects receiving low- or high-dose dynorphin A_{1-13} felt either a "pins and needles" sensation or warmth and/or had observable flushing. Most subjects experienced all three of these symptoms or signs. Onset of these sensations occurred between 10 s and 4 min after i.v. injection of dynorphin A_{1-13}. The offset was between 2 and 15 min, except for three subjects studied in whom symptoms lasted for up to 35 min, and one subject who had symptoms for 90 min. Pretreatment with moderate or very high doses of either specific opioid antagonist did not prevent or ameliorate these signs and symptoms. None of the subjects had any sustained dysphoric response to this natural κ-opioid receptor ligand.

Discussion

In these studies it was shown that dynorphin A_{1-13} administered in doses of 120 μg/kg and 500 μg/kg i.v. to normal, healthy, volunteer humans causes a prompt rise in serum prolactin levels in all subjects, with a significant dose-response effect (see Fig. 1). Peak mean serum prolactin levels were observed 10 to 20 min after dynorphin A_{1-13} administration.

Only three opioid antagonists, naloxone, nalmefene, and naltrexone, which are nonselective for specific opioid receptor types, have been approved for administration to humans. Naloxone and nalmefene were studied with respect to their abilities to attenuate the effects of dynorphin in elevating serum levels of prolactin. These two antagonists have been shown to differ in their pharmacokinetic profiles, in their relative binding affinities at opioid receptor subtypes, and in their potencies and efficacies at these receptors. In a recent report by Kim et al. (1997) ,[^{11}C]carfentanil was used in kinetics studies to demonstrate that the clearance half-life from brain μ-receptors was 28.7 ± 5.9 h for nalmefene compared with 2.0 ± 1.6 h for naloxone. Nalmefene also has a longer plasma apparent terminal half-life in humans than naloxone (7–15 versus 1–2 h; Dixon et al., 1987). Therefore in this study we used only the first 90 min of blood sampling (within one half-life of naloxone) for determination of the relative effects of different doses of naloxone and nalmefene in attenuating dynorphin-induced prolactin release, to avoid differences in serum prolactin levels that might be caused solely by differences in pharmacokinetics of the two antagonists.

A recent study of opioid binding in monkey brain homogenates, using displacement of [^{3}H][D-Ala^2, N-Me-Phe^4, Gly-ol^5]enkephalin, [^{3}H]U69593, and [^{3}H]D-Pen^2, D-Pen^5-enkephalin by a number of opioid ligands to calculate K_is, reported the following values for μ-, κ-, and δ-opioid receptors, respectively (K_is,): nalmefene—0.13, 0.28, 4.56 nM; naloxone—0.62, 1.95, 49.0 nM (Emmerson et al., 1994). This study extended and supported an earlier study of Michel et al. (1985). Extrapolating from these data, nalmefene had a twofold greater affinity for μ- over κ-receptors, and naloxone a 3-fold greater affinity. Furthermore, in comparisons between antagonists, nalmefene had a 5-fold greater affinity for μ-receptors than naloxone,and a 7-fold greater affinity for κ-receptors.

Naloxone had no significant overall attenuation effect when administered in moderately high doses (10 mg) but had a significant attenuating effect on the dynorphin-induced elevation of serum prolactin levels when administered at a very high dose (30 mg; see Fig. 3. Of importance, in a study in which naloxone was administered in a wide range of doses to humans, serum prolactin levels were shown not to have become elevated or reduced (Cohen et al., 1983).

The opioid antagonist, nalmefene, which has high affinity for both μ- and κ-opioid receptors, similarly had a robust effect in attenuating dynorphin-induced elevation in serum prolactin levels (see Fig. 4b) when a very high dose was administered (30 mg) but no significant overall attenuation when moderately high dose (10 mg) was administered (see Fig. 4a).

Studies using cloned rat opioid receptors suggested that dynorphin A_{1-17} and dynorphin A_{1-13} have a preferential

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affinity for κ-receptors. Recently, Zhang et al. (1998) reported that dynorphin A bound to human μ-, δ-, and κ-opioid receptors expressed in *Xenopus* oocytes with the following competition constants K_i: 1.6 nM, 1.25 nM, and 0.05 nM, respectively, or a 32-fold greater affinity for κ- versus μ-opioid receptors, and a 25-fold greater affinity for κ- versus δ-opioid receptors. The results demonstrated that dynorphin was capable of binding to μ- and δ-opioid receptors in the nanomolar range but was a higher affinity ligand for κ-opioid receptors with binding in the subnanomolar range.

The findings of the present studies, which were all conducted in healthy volunteers with no histories of any drug or alcohol abuse, document that dynorphin A_{1-13}, primarily a κ- but also a μ-opioid receptor ligand, causes an elevation of serum prolactin levels, an effect probably mediated through a direct action of dynorphin on κ- (and μ)-opioid receptors to reduce dopaminergic tone in the tuberoinfundibular region of the hypothalamus, since in humans, prolactin release is primarily under tonic inhibition by dopamine. The findings that very high doses (30 mg) of both naloxone and nalmefene could significantly attenuate the dynorphin-induced elevation in serum prolactin levels suggest that the dynorphin effect is mediated in part through specific opioid receptor systems. Moderately high (10-mg) doses of neither naloxone nor nalmefene had a significant overall attenuation effect, although there were effects at specific early time points, whereas very high doses of nalmefene, as well as very high doses of naloxone, robustly attenuated the effect of dynorphin A_{1-13}.

Differences related to gender, reflected in the analgesic effects of the synthetic, κ- and μ-opioid receptor analgesic agents pentazocine, nalbuphine, and butorphanol administered to human subjects, have been reported (Gear et al., 1996). Gender differences have also been reported with respect to changes in tuberoinfundibular dopaminergic activity following administration of the synthetic κ-opioid agonist U50,488 in the rat (Manzanares et al., 1992). In this study, we wanted to determine whether there are any gender differences in serum prolactin elevation effects of natural peptide, dynorphin A_{1-13}. There was a significantly greater response in female than in male subjects. Therefore, these studies show that tuberoinfundibular dopaminergic tone is more readily modulated in females than in males and by a natural peptide that may also be physiologically active in modulating prolactin release. This finding suggests that there are either a greater number of opioid receptors (possibly κ-opioid receptors) in the hypothalamic tuberoinfundibular region in females than in males or, alternatively, that signal transduction systems are more robust in females. These findings have enormous implications for normal physiological function, as well as for the disorders of addictions and chronic pain, and for their management.

In a much earlier study, it was found that another natural opioid peptide, β-endorphin, which acts primarily at μ- and δ-opioid receptors, causes an elevation in serum prolactin levels when administered i.v. in humans (Foley et al., 1979). It is possible for neuropeptides such as β-endorphin or dynorphin A_{1-13} to reach critical sites of action for effecting prolactin release through the tuberoinfundibular dopaminergic system, the projections of which lie along the portal system with its connections to the pituitary, in part, outside the blood-brain barrier. However, it is unlikely that dynorphin A_{1-13} administered by a peripheral i.v. route in humans would have any effects in the nigrostriatal or mesolimbic-mesocortical dopaminergic systems, major sites of action of both opiates and stimulants such as cocaine in the neurolobiological processes related to the development of addiction. Such effects would depend on the ability of this peptide to cross the blood-brain barrier. Studies are in progress in our laboratory, in nonhuman primates, to determine whether dynorphin A_{1-13} and also the natural dynorphin A_{1-17} are able to cross the blood-brain barrier intact (Yu et al., 1996). We have recently shown that another high-affinity κ-opioid receptor peptide agonist, Eisai 2078, crosses the blood-brain barrier in a nonhuman primate model (Yu et al., 1997).

Early studies from our laboratory showed that in humans the responsivity of prolactin release to chronic repeated oral administrations of a μ-opioid receptor-directed agonist, methadone, is a robust effect to which full tolerance or adaptation does not develop, even after years of heroin addiction followed by years of successful steady dose methadone maintenance treatment (Kreek, 1978). These findings document that the dopamine lowering effect of μ-opioid agonists in the tuberoinfundibular region of the hypothalamus is an effect to which tolerance does not fully develop.

Extensive studies have been conducted in our laboratory to determine the differential processing of dynorphin A_{1-13}, the natural, shortened sequence of dynorphin A_{1-17}, which has been used widely in neurobiological studies, especially because it was the initial peptide fully sequenced after the discovery by Goldstein and colleagues (Goldstein et al., 1979; Chavkin et al., 1982). We have found that dynorphin A_{1-13} when added to human blood ex vivo is essentially immediately processed to yield one major opioid peptide, dynorphin A_{1-12} (Chou et al., 1994). This peptide is then rapidly further processed to yield nonopioid species, including primarily dynorphin A_{2-12} and dynorphin A_{4-12} (Chou et al., 1994,1996). To a lesser extent, cleavage also occurs at the 6 to 7 position to yield dynorphin A_{1-6} and dynorphin A_{7-12}. In contrast, the natural dynorphin A_{1-17} peptide is much more slowly processed in both human and rhesus monkey blood, as studied ex vivo, and primarily by cleavage at the 1 to 2 position to yield as the primary biotransformation product dynorphin A_{2-17} (Chou et al., 1994,1996; Yu et al., 1996). Dynorphin A_{2-17} is slowly, further processed to yield smaller, nonpeptide species (Chou et al., 1996; Yu et al., 1996).

The half-life of peptides (and heterocyclic compounds) does not necessarily mirror the "on-time" at the specific receptor, i.e., the time of receptor occupancy. One of the best recent examples of this is the partial agonist buprenorphine, which has a relatively short plasma half-life (rapid clearance) yet a prolonged-opioid receptor occupancy, as measured indirectly by neurochemical and clinical dynamic effects. Nalmefene has a much longer receptor occupancy, as measured by kinetics studies using a radioactive ligand (developed for positron emission tomography), than the apparent terminal half-life in plasma. Dynorphin A_{1-13} is rapidly processed to dynorphin A_{1-12}, an opioid peptide that may share κ-opioid receptor binding documented for dynorphin A_{1-10}, dynorphin A_{1-13}, and dynorphin A_{1-17}. Dynorphin A_{1-12} is then moderately rapidly processed to yield nonopioid peptides, but there is no information available on the receptor occupancy of dynorphin A_{1-12}. It is of interest that in studies of analgesia, amelioration of opioid withdrawal symptoms, as well as in

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this study, the duration of action in humans as well as in animal models is much longer than the plasma half-life of dynorphin $A_{1–13}$.

If we had hypothesized that dynorphin $A_{2–12}$ would cause elevation of serum prolactin levels, we might have expected a much longer elevation than observed in these studies. We have shown that the des-tyr-dynorphin (dynorphin $A_{2–12}$), a congener of the biotransformation product, dynorphin $A_{2–17}$, which is the major product of the natural dynorphin $A_{1–17}$, is more slowly processed than dynorphin $A_{1–13}$ or dynorphin $A_{1–12}$. Because the des-tyr-dynorphins have not been prepared and approved for human use, we cannot test this question directly in humans.

Dynorphin $A_{1–13}$ and dynorphin $A_{1–17}$ have been shown to have effects as analgesic agents and also have been shown to enhance μ-opioid agonist analgesic activity during chronic administration of a μ-agonist, possibly through an attenuation or alteration of expression of opioid tolerance (Aceto et al., 1982; Hooke et al., 1995; Portenoy et al., 1999). Dynorphin $A_{2–17}$ is a primary and nonopioid product of dynorphin $A_{1–17}$ and has been shown to have many effects when administered to living animals, including prevention or reversal of opiate withdrawal symptoms, analgesia, and other behavioral effects (Takemori et al., 1993).

In an early study of the effects of dynorphin $A_{1–13}$ conducted in male Sprague-Dawley rats, serum prolactin elevations were noted at 10 to 120 min, but not at later time points, after dynorphin (of unspecified peptide length) administration at 1- or 10-mg doses by the intracerebroventricular route (Van Vugt et al., 1981). Coadministration of 20 mg of naloxone with 10 mg of dynorphin prevented the dynorphin-induced elevation in serum prolactin levels (Van Vugt et al., 1981). Several other studies have suggested that in the rat, both κ- and μ-opioid receptor synthetic agonists may stimulate prolactin release. In a pair of studies in ovariectomized female and also in male rhesus monkeys, dynorphin $A_{1–13}$, administered i.v. in doses of 1 to120 mg/kg, was found to cause elevations in serum prolactin, attenuated by preadministration of a high dose of naloxone (1 mg/kg) (Gilbeau et al., 1986). However, no significant effects of dynorphin $A_{1–13}$ on plasma levels of thyroid-stimulating hormone, growth hormone, follicle-stimulating hormone, or luteinizing hormone were observed.

This study is the first placebo-controlled study of the effects of administration of dynorphin A peptides in healthy humans with no history of opiate abuse and builds on our earlier pilot study in which a pronounced prolactin responsivity to i.v. administered dynorphin $A_{1–13}$ in humans was found (Kreek et al., 1994). The findings of this study suggest that dynorphin may play a role in normal physiology in the modulation of prolactin release in humans. Also, the findings suggest that females may be more responsive to the effect than males. No effects of dynorphin $A_{1–13}$ on plasma levels of ACTH or cortisol were observed. The findings also suggest that dynorphin $A_{1–13}$ may act through κ- and possibly also μ-opioid receptors in inducing this effect of prolactin release. Because in humans, prolactin release is essentially completely under tonic inhibition by dopamine, the findings of this study provide strong evidence that dynorphin $A_{1–13}$ reduces dopaminergic tone in the tuberoinfudibular dopaminergic system (Moore and Lookingland, 1995).Very recently, we have shown that dynorphin $A_{1–17}$, when administered directly into the nucleus accumbens brain region, causes a significant reduction of basal dopaminergic tone (Claye et al., 1997). These findings, using the natural peptide, support earlier studies in which synthetic nonpeptide κ-opioid receptor agonists instilled directly into the nucleus accumbens had been shown to also significantly lower dopaminergic tone and also attenuate induced increases in dopamine levels (Spanagel et al., 1990). However the same κ-agonists instilled directly into the ventral tegmental area had no effect on dopaminergic tone in the nucleus accumbens. In contrast, μ-opioid agonists were found to have the opposite effect when instilled directly into the ventral tegmental area, with elevations in dopamine levels in the nucleus accumbens, presumably by disinhibiting the normal tonic inhibition of dopamine by GABAergic neurons.

Other studies from our laboratory have shown that dynorphin gene expression is abundant in the hypothalamus. Also, using both quantitative autoradiography and the solution hybridization RNase protection assay, we have found that both κ- and μ-opioid receptor gene expression and functional receptors are abundant in the hypothalamus. Natural endogenous dynorphin peptides acting at κ- as well as at μ-opioid receptors in these regions may serve to modulate prolactin release and may also physiologically modulate dopamine release through the tuberoinfundibular dopamine system. These findings suggest that a natural dynorphin peptide or, more likely, a synthetic peptide or synthetic compound with dynorphin-like κ-opioid agonist activity, with greater access to critical limbic and striatal brain regions [where the mesolimbic-mesocortical and nigrostriatal dopaminergic nerve terminals (which have been implicated as the primary sites of rewarding effects of drugs of abuse) and the tuberoinfundibular dopamine system studied herein are localized] may have considerable value not only in the management of chronic pain, but also in specific aspects of specific addictive diseases. Dynorphin $A_{1–13}$ peptides have been shown to be effective in preventing or reversing signs and symptoms of opioid withdrawal in animal models. Recent preliminary studies suggest dynorphin peptides may have similar effectiveness in managing opiate withdrawal symptoms in μ-agonist narcotic-dependent individuals, may cause "good" or "bad" subjective effects in former opioid-dependent persons and also may cause subjective effects in healthy volunteers with no history of drug abuse (Wen and Ho, 1982; Specker et al., 1998; Greenwald et al., 1997; King et al., 1998).

Of special interest at this time, dynorphin, or more likely a dynorphin-like peptide or a related κ-opioid agonist, may be effective in managing some aspects of cocaine dependence. Such an agent could possibly attenuate the dopaminergic surge that occurs after each acute and chronic administration of cocaine, caused by the blockade of the dopamine transporter that enhances synaptic and overall extracellular concentrations of dopamine, identified by many laboratories as probably the major reinforcing or "rewarding" effect of cocaine. The increases in dynorphin gene expression and peptides following cocaine administration may serve a counter-regulatory role resulting in the observed lowered basal levels of dopamine following chronic cocaine administration (Spangler et al., 1993, 1996; Claye et al., 1997). A κ-agonist, such as a dynorphin-like peptide, that is capable of reaching the mesolimbic-mesocortical and nigrostriatal dopaminergic systems, might be effective in reducing the

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sequellae following chronic long-term cocaine exposure, which may contribute to the so-called "craving" or drug hunger and thus relapse to or continued cocaine use (Kreek, 1996).

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