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 \( \kappa \) as well as \( \mu \)- and \( \delta \)-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 \( \mu \)g/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 \( \kappa \) - or \( \delta \)-types. Pretreatment with the opioid antagonist nalmefene (30 mg i.v.), which has high affinity at both \( \mu \)- and \( \kappa \)-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 \( \mu \)- and \( \kappa \)-opioid receptors. These results suggest dynorphin A_1–13 lowers tuberoinfundibular dopaminergic tone through action at \( \kappa \) - and possibly \( \mu \)-opioid receptors. Female subjects were significantly more responsive to the prolactin effects of dynorphin than were male subjects. Dynorphin gene expression, dynorphin peptides, and opioid receptor gene expression and binding have been shown to be altered in response to cocaine administration. Also, both dynorphin peptides and synthetic \( \kappa \)-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 \( \kappa \)-opioid receptors; these peptides, and especially their shorter biotransformation products, also bind to the \( \mu \)- and \( \delta \)-opioid receptors (Chavkin and Goldstein, 1982; Chen et al., 1993).

Several studies in different species have shown that dynorphin \( \Lambda \) 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.

ABBREVIATIONS: ANOVA, analysis of variance; GABA, \( \gamma \)-aminobutyric acid.
putamen region of the striatum (Spangler et al., 1993; Dau-
nais et al., 1993). Dynorphin peptides have also been re-
ported to be enhanced in the caudate putamen, nucleus ac-
cumbens, 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 \( \kappa \)-opioid recep-
tors, as well as \( \mu \)-opioid receptors, are significantly increased
in the caudate putamen, nucleus accumbens, and other re-
gions of the mesolimbic-mesocortical and nigrostriatal dopa-
mnergic systems where there are abundant dopaminergic
terminals, after chronic binge-pattern cocaine administra-
tion (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 \( \mu \)-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 \( \kappa \)-opioid receptor, determined
by a modified quantitative solution hybridization RNase pro-
tection 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 ni-
grostriatal pathway (Spangler et al., 1996).

Studies from other laboratories have shown that \( \mu \)-opioid
receptor activation may indirectly enhance dopamine release
by acting on \( \gamma \)-aminobutyric acid (GABAergic) neurons to
inhibit GABA release, thus disinhibiting regulation of dopa-
mnergic neurons in the substantia nigra and ventral teg-
mental area. In contrast, synthetic \( \kappa \)-opioid receptor ligands
may directly reduce dopamine levels in extracellular fluid in
the caudate putamen, nucleus accumbens and related spe-
cific brain regions, as determined by microdialysis (Spanagel
et al., 1990). Recent studies from our laboratory, using mi-
crodialysis conducted in awake, freely moving rats, have
shown that administration of dynorphin \( \text{A}_{1-17} \) 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 tu-
beroinfundibular dopaminergic systems. Changes in levels of
activation of the dynorphin and \( \kappa \)-opioid receptor system
during binge-pattern cocaine administration is a phenome-
non that also may occur in human cocaine addiction, a con-
cept partially supported by the findings of enhanced dynor-
phin gene expression in brains of cocaine addicts studied
earlier (Kreek et al., 1994; Claye et al., 1997).

This study was conducted to determine whether dynorphin
\( \text{A}_{1-13} \), 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 re-
leased 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 tuberoinfund-
ibular 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 do-
paminergic systems, through administration of a different
dynorphin-like \( \kappa \)-agonist, which may be capable of reaching
these brain regions.

In this study, we also have addressed the related mecha-
nistic question of whether any observed increases in serum
prolactin levels are mediated by specific opioid receptors,
suggesting action at \( \kappa \)- as well as at \( \mu \)-opioid receptors, or
whether any prolactin releasing effect of dynorphin \( \text{A}_{1-13} \) is a
nonspecific, stress-related effect.

Finally, we have determined that there are gender differ-
ences 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 fe-
males, 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.w.t.) of dynorphin \( \text{A}_{1-13} \) and then a higher dose
(500 \( \mu \)g/kg) of dynorphin \( \text{A}_{1-13} \), 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 moder-
ately high dose of opioid antagonist, naloxone (10 mg), adminis-
tered i.v. 10 min before the administration of the lower dose of dynorphin
\( \text{A}_{1-13} \) (120 \( \mu \)g/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 \( \mu \)-opioid receptors but
possibly only partial effectiveness at \( \delta \) - and \( \kappa \)-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. admin-
istration of a dynorphin \( \text{A}_{1-13} \) (120 \( \mu \)g/kg), would alter the effects of
dynorphin on serum prolactin levels. Because naloxone has its high-
est affinity at \( \mu \)-, lower affinity at \( \kappa \)-, and its lowest affinity at
\( \delta \)-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, \( \mu \)- and \( \kappa \)-opioid peptide
ligands from specific \( \mu \)- and \( \kappa \)-opioid receptors. This study was con-
ducted 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 \( \mu \)- but also high affinity for \( \kappa \) - and low affinity for \( \delta \)-opioid
receptors, would alter the effects of dynorphin \( \text{A}_{1-13} \) on serum prolactin
levels. Nalmefene was administered at moderately high doses
of 10 mg i.v. 10 min before the administration of dynorphin \( \text{A}_{1-13} \)
(120 \( \mu \)g/kg) to determine whether this antagonist would alter the
effects of dynorphin \( \text{A}_{1-13} \) 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
i.v. administration of dynorphin A₁₋₁₃ (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 A₁₋₁₃ studies. Therefore, the gender differences in response to dynorphin A₁₋₁₃ 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 A₁₋₁₃ 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 A₁₋₁₃ 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 A₁₋₁₃ 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 A₁₋₁₃, 120 µg/kg (“low dose”), was administered i.v. On a separate study day, in study 1 only, dynorphin A₁₋₁₃ 500 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 A₁₋₁₃ 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 A₁₋₁₃ (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 A₁₋₁₃, 120 µg/kg, was administered 10 min following the opioid antagonist. On a second study day, dynorphin A₁₋₁₃, 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 Capistrano, 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 A₁₋₁₃ had a dose-dependent effect on serum prolactin levels as shown in Fig. 1. Both doses of dynorphin A₁₋₁₃, 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 A₁₋₁₃ (p < .05). The lower dose (120 µg/kg) caused a prompt rise in serum prolactin in all
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 A1-13 (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 A1-13 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 A1-13 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 A1-13, 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 (Neuman-Keuls post hoc tests of the condition × 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 A1-13, 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
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 $\mu$- and $\kappa$-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, $p < .005$), 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
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 A1–13 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 × dose × 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 × 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 A1–13-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.
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 A1–13 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 A1–13. 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 A1–13 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 A1–13 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 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 A1–13 administration.

Studies using cloned rat opioid receptors suggested that dynorphin A1–17 and dynorphin A1–13 have a preferential

![Fig. 5.](image_url)
affinity for k-receptors. Recently, Zhang et al. (1998) reported that dynorphin A bound to human µ-, δ-, and k-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 k- versus µ-opioid receptors, and a 25-fold greater affinity for k- 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 k-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 A1–13, primarily a k- 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 k- (and µ)-opoid 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 A1–13.

Differences related to gender, reflected in the analgesic effects of the synthetic, k- 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 k-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 A1–13. 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 k-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 A1–13 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 A1–13 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 neurobiological 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 A1–13 and also the natural dynorphin A1–17 are able to cross the blood-brain barrier intact (Yu et al., 1996). We have recently shown that another high-affinity k-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 responsibility 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 A1–13, the natural, shortened sequence of dynorphin A1–17, 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 A1–13 when added to human blood ex vivo is essentially immediately processed to yield one major opioid peptide, dynorphin A1–12 (Chou et al., 1994). This peptide is then rapidly further processed to yield nonopioid species, including primarily dynorphin A2–12 and dynorphin A4–12 (Chou et al., 1994, 1996). To a lesser extent, cleavage also occurs at the 6 to 7 position to yield dynorphin A1–6 and dynorphin A1–7. In contrast, the natural dynorphin A1–17 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 A2–17 (Chou et al., 1994, 1996; Yu et al., 1996). Dynorphin A2–17 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 A1–13 is rapidly processed to dynorphin A1–12, an opioid peptide that may share k-opioid receptor binding documented for dynorphin A1–10, dynorphin A1–13, and dynorphin A1–17. Dynorphin A1–12 is then moderately rapidly processed to yield nonopioid peptides, but there is no information available on the receptor occupancy of dynorphin A1–12. It is of interest that in studies of analgesia, amelioration of opioid withdrawal symptoms, as well as in
this study, the duration of action in humans as well as in animal models is much longer than the plasma half-life of dynorphin A₁₋₁₃.

If we had hypothesized that dynorphin A₂₋₁₂ 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 A₇₋₁₂), a congener of the biotransformation product, dynorphin A₁₋₁₇, which is the major product of the natural dynorphin A₁₋₁₇, is more slowly processed than dynorphin A₁₋₁₃ or dynorphin A₁₋₁₂. Because the des-tyr-dynorphins have not been prepared and approved for human use, we cannot test this question directly in humans.

Dynorphin A₁₋₁₃ and dynorphin A₁₋₁₇ 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 A₂₋₁₇ is a primary and nonopiod product of dynorphin A₁₋₁₇, 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 A₁₋₁₃ 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 naltroxone 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 A₁₋₁₃, administered i.v. in doses of 1 to 120 mg/kg, was found to cause elevations in serum prolactin, attenuated by preadministration of a high dose of naltroxone (1 mg/kg) (Gilbeau et al., 1986). However, no significant effects of dynorphin A₁₋₁₃ on plasma levels of thyroid-stimulating hormone, growth hormone, follicle-stimulating hormone, or luteinating 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 responsiveness to i.v. administered dynorphin A₁₋₁₃ 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 A₁₋₁₃ on plasma levels of ACTH or cortisol were observed. The findings also suggest that dynorphin A₁₋₁₃ 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 A₁₋₁₃ reduces dopaminergic tone in the tuberoinfundibular dopaminergic system (Moore and Lookingland, 1995). Very recently, we have shown that dynorphin A₁₋₁₇, when administered directly into the nucleus accumbens brain region, causes a significant reduction of basal dopaminergic tone, causes a decrease in the mesolimbic-mesocortical and nigrostriatal dopaminergic systems, and may be effective in reducing the

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 in which the mesolimbic-mesocortical and nigrostriatal dopaminergic nerve terminals (where 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 A₁₋₁₃ 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-opioid receptor 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-opioid 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
sequeleae 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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References


