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Molecular Brain Research 66 (1999) 184–187

MOLECULAR BRAIN RESEARCH

Short communication

Acute intermittent morphine increases preprodynorphin and kappa opioid receptor mRNA levels in the rat brain

Xiao-Min Wang [b], Yan Zhou [a], Rudolph Spangler [a,*], Ann Ho [a], Ji-Shen Han [b], Mary Jeanne Kreek [a]

[a] *Laboratory of the Biology of Addictive Diseases, The Rockefeller University, Box 171, 1230 York Avenue, New York, NY 10021, USA*
[b] *Neuroscience Research Institute, Beijing Medical University, Beijing 100083, China*

Accepted 15 December 1998

Abstract

We determined the effects of morphine on mRNA levels for the opioid ligands preprodynorphin (PPD) and preproenkephalin (PPE) and the kappa opioid receptor (KOR). Rats received six injections of morphine (6.25 mg/kg/injection) every 2 h, and were sacrificed 30 min later. mRNA levels were measured in brain tissue after removal of the cortex, cerebellum and brainstem. There were increases in PPD and KOR mRNA levels ($P < 0.05$ and $P < 0.005$, respectively), with no alteration of PPE. These alterations in the kappa/dynorphin system may counter morphine-induced effects on the brain.

Keywords: Morphine; Preprodynorphin; Preproenkephalin; Kappa opioid receptor

It has been speculated that opiate-induced decreases in endogenous opioid receptors might play a role in opiate tolerance; i.e., that a decrease in opiate receptor number or affinity after prolonged exposure leads to decreased effect upon subsequent exposure to the opiate. However, studies on the regulation of opioid receptors by repeated and chronic morphine administration indicate that this simple mechanism cannot explain tolerance [3,4].

In the case of a different drug of abuse, cocaine, many of its effects are mediated by increased synaptic dopamine levels due to inhibition of dopamine re-uptake [8]. Repeated cocaine injections in a 'binge' pattern increase striatal dopamine levels [12]. This pattern of cocaine administration also increases kappa opioid receptor (KOR) density, as measured by quantitative autoradiography, especially in dopaminergically innervated regions of the rat brain [27]. 'Binge' pattern cocaine administration consistently increases levels of preprodynorphin (PPD) mRNA [21], while preproenkephalin (PPE) mRNA is only increased on the second and third day of 'binge' cocaine administration [2,22].

Like cocaine, morphine elevates synaptic dopamine levels in the brain [6]. Dopaminergic neurons are under tonic inhibition by the inhibitory neurotransmitter GABA. Opiates increase the activity of dopaminergic neurons by suppressing the release of GABA [10]. The resulting increases in dopamine possibly mediate at least part of the analgesic effect of morphine [1].

Trujillo et al. [24] reported that morphine causes an increase in dynorphin-derived peptides, with no increase in enkephalin-derived peptides. The effects of systemic morphine injections on PPD and PPE mRNA levels have not been clearly established [7,9,26]. No studies have reported effects of morphine tolerance on kappa opioid receptors in the brain.

The regulation of kappa opioid receptors and PPD mRNA levels by 'binge' pattern cocaine injections, and the fact that both cocaine and morphine increase dopamine levels, led us to examine the effects of acute intermittent morphine injections on opioid mRNA levels. We employed a quantitative RNase protection assay to determine levels of PPD, PPE and KOR mRNA.

Male Wistar rats (190–220 g) were individually housed in a stress-minimized facility with free access to food and water. Animals were adapted to a standard 12-h light/dark cycle (lights on from 0900 to 2100) for 7 days. Before

* Corresponding author. Fax: +1-212-327-8574; E-mail: spanglr@rockvax.rockefeller.edu

0169-328X/99/$ - see front matter
PII: S0169-328X(99)00021-2

drug administration, all animals were handled and received s.c. injections of saline for 6 days in order to minimize injection-induced stress. Two cohorts of six rats each, on different days, were randomly assigned to treatment groups, three to a group on each day. On day 7, animals received six subcutaneous injections of morphine sulfate (6.25 mg/kg/inj) or an equal volume of saline (6×1 ml/kg) every 2 h starting 1.5 h after the lights came on. Rats were decapitated following 15-s exposure to CO_2, 30 min after the last injection. After removal of cerebellum and cortex, brains were homogenized for RNA extraction.

Quantitation of RNA was carried out by trichloroacetic acid precipitation of ribonuclease-protected hybrids [2], using probes and standards previously described [20]. Each value was expressed as a percent of the mean control value. Student's *t*-tests were carried out for each mRNA studied in order to evaluate the statistical significance of differences between treatment groups. Data in graphs are presented as mean ± S.E.M.

PPD mRNA levels were significantly elevated in extracts from the brains of rats which had received six injections of morphine at 2 h intervals and sacrificed 30 min after the last injection ($t = 2.56$, $p < 0.05$; Fig. 1A). This increase was approximately 90% more than the mean saline control level of 1.27 pg PPD mRNA/μg total RNA.

Shown in Fig. 1B are the relative KOR mRNA levels for rats treated with saline and morphine. There was a significant increase in KOR mRNA levels in brain extracts from rats treated with morphine compared to saline controls ($t = 7.26$, $p < 0.005$). This increase was approximately 70% above the mean saline control level of 0.50 pg KOR mRNA/μg total RNA.

Relative PPE mRNA levels are shown in Fig. 1C. There were no significant alterations in PPE mRNA levels from the mean saline control level of 7.91 pg PPE mRNA/μg total RNA.

Both cocaine and morphine increase extracellular dopamine in the brain. 'Binge' cocaine increases PPD mRNA at all time-points tested, and elevates PPE mRNA less robustly and only during a narrow time window [2,22]. 'Binge' cocaine also increases kappa opioid receptor binding [27]. This study sought to determine whether alterations in the endogenous opioid system known to take place following 'binge' cocaine administration, might also be caused by administration of morphine in an acute intermittent pattern.

As with 'binge' cocaine administration, there was a significant increase in PPD mRNA levels in the rat brain after repeated morphine administration (Fig. 1A). This observation is consistent with the finding that preprodynorphin-derived peptide concentrations are increased by morphine [24].

Similar to the effect of 'binge' cocaine on kappa opioid receptor binding levels, there was a significant increase in KOR mRNA levels in the rat brain after repeated morphine administration (Fig. 1B). 'Binge' cocaine does not cause a significant increase in mean KOR mRNA levels in the caudate-putamen, and causes a decrease in KOR mRNA in the substantia nigra [20]. However, the substantia nigra contains a small portion of the total KOR mRNA in the brain; and in the caudate-putamen, a region where

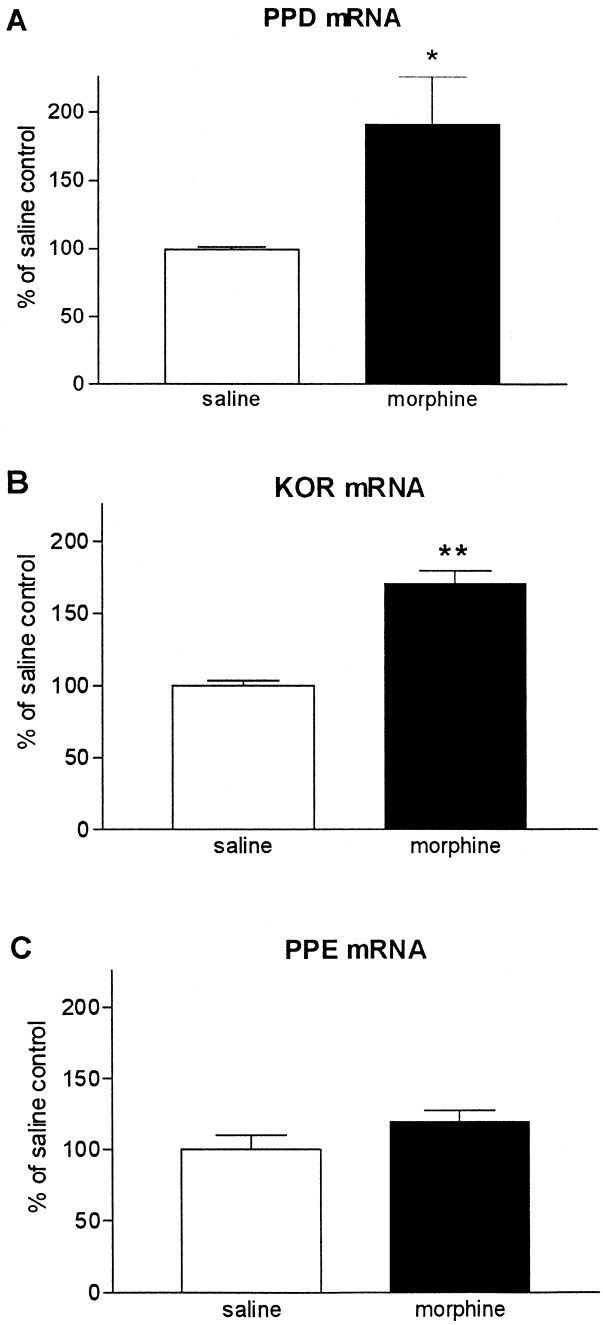

Fig. 1. Effects of repeated injections of morphine on opioid mRNA levels (expressed as percent of mean saline control, ±S.E.M.). (A) Effects of repeated injections of morphine sulfate (6.25 mg/kg, s.c.) on preprodynorphin (PPD) mRNA levels in the rat brain; $n = 6$; *, $p < 0.05$ vs. mean saline control level. (B) Effects of repeated injections of morphine on kappa opioid receptor (KOR) mRNA levels in the rat brain; $n = 3$; **, $p < 0.005$ vs. mean saline control level. (C) Effects of repeated injections of morphine on preproenkephalin (PPE) mRNA levels in the rat brain; $n = 6$.

increased PPD mRNA is found following 'binge' cocaine administration, there was a positive correlation on an animal-by-animal basis between PPD and KOR mRNAs [20]. Thus, while the mean KOR mRNA levels are not significantly increased in the caudate-putamen by 'binge' cocaine, the fact that PPD is increased, and that KOR mRNA levels correlate with PPD mRNA levels, indicates that KOR is regulated in conjunction with PPD in this large region. The mechanism of this difference in regulation between the forebrain and the midbrain is not clear; however, the substantia nigra is the major target region for dynorphinergic neurons projecting back from the caudate-putamen. KOR mRNA transcribed in the substantia nigra is possibly in dopaminergic neurons, while KOR mRNA in the caudate-putamen is presumably transcribed in GABAergic projection neurons which themselves are synthesizing opioid peptides, possibly dynorphins.

A transient increase in PPE, which does not persist with chronic treatment [2], is seen after some cocaine administration paradigms [22,23]. We did not observe any changes in PPE mRNA levels in the rat brain after acute intermittent morphine injections (Fig. 1C). While one group reported no changes in striatal PPE mRNA following morphine administration [11], another group reported decreased PPE mRNA levels [26]. Another study showed that morphine could block cAMP-induced increases in PPE, but had no significant effect alone [9]. The finding of no alteration in PPE mRNA following acute intermittent morphine administration is in accord with the report of Trujillo et al., [24] which showed no changes in preproenkephalin-derived peptides following repeated injections of morphine.

The analgesic effects of opiates are associated in part with the release of dopamine [1]. However, increased dopamine also activates the dynorphin/kappa opioid system. While ligands thought to act at mu or delta opioid receptors lead to increased dopamine release when applied locally at dopamine terminals, kappa opioid receptor ligands cause decreased dopamine release: both synthetic and natural peptide kappa opioid receptor agonists decrease evoked dopamine release in slices [14,28], or when applied through a probe directly to forebrain tissue in vivo [5,6,13,19].

Kappa opioid receptor agonists block some of the behavioral effects of cocaine, for example, conditioned place preference [18]. Likewise, the kappa opioid receptor agonist dynorphin A_{1-13}, when injected centrally, counters certain morphine-induced responses [16]. Dynorphin A_{1-13}, administered into the ventricles of the rat brain, dose-dependently decreases the analgesic effect of morphine [25]. In contrast, dynorphin A_{1-13} increases the analgesic effect of morphine in tolerant rats [25]. Dynorphin A_{1-17}, administered into the rat spinal cord, decreases the analgesic effect of morphine in naive animals at the three doses tested, while intrathecal dynorphin A_{1-17} increases the analgesic effect of morphine in tolerant rats [17]. Dynorphin apparently counters morphine effects by acting in selected regions of the brain, decreasing analgesia in naive rats and decreasing expression of tolerance in tolerant rats. The cellular mechanism for the anti-analgesic action of agonists of the kappa opioid receptor is beginning to be elucidated [15].

The data in the present study indicate that mRNA for both the ligand and the receptor of the dynorphin/kappa system are increased following repeated morphine administration. Following the model of counter-regulation proposed by others, the two elements of the dynorphin/kappa system might both be responding to decrease morphine effects. This finding has implications for the development of adjunctive therapies for pain management, as well as addiction.

Acknowledgements

We thank Dr. Anne Marie Mathieu-Kia for valuable comments on the manuscript.

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