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The effect of Gly–Gln [ß-endorphin$_{30-31}$] on morphine-evoked serotonin and GABA efflux in the nucleus accumbens of conscious rats

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ABSTRACT

Glycyl-L-glutamine (Gly–Gln; β-endorphin$_{30-31}$) is an endogenous dipeptide synthesized through the post-translational processing of β-endorphin$_{1-31}$. Central Gly–Gln administration inhibits the rewarding properties of morphine and attenuates morphine tolerance, dependence and withdrawal although it does not interfere with morphine analgesia. In an earlier study, we found that Gly–Gln inhibits morphine-induced dopamine efflux in the nucleus accumbens (NAc), consistent with its ability to inhibit morphine reward. To further investigate the mechanism responsible for its central effects we tested whether i.c.v. Gly–Gln administration influences the rise in extracellular serotonin and GABA concentrations evoked by morphine in the NAc. Conscious rats were treated with Gly–Gln (100 nmol/5 μl) or saline i.c.v. followed, 2 min later, by morphine (2.5 mg/kg) or saline i.p. and extracellular serotonin and GABA concentrations were analyzed by microdialysis and HPLC. Morphine administration increased extracellular serotonin and GABA concentrations significantly within 20 min, as shown previously. Unexpectedly, Gly–Gln also increased extracellular serotonin concentrations significantly in control animals. Combined treatment with Gly–Gln + morphine also elevated extracellular serotonin concentrations although the magnitude of the response did not differ significantly from the effect of Gly–Gln or morphine, given alone suggesting that Gly–Gln suppressed morphine induced serotonin efflux. Gly–Gln abolished the morphine-induced rise in extracellular GABA concentrations but had no effect on extracellular GABA when given alone to otherwise untreated animals. These data show that Gly–Gln stimulates NAc serotonin efflux and, together with earlier studies, support the hypothesis that Gly–Gln inhibits the rewarding effects of morphine by modulating morphine induced dopamine, GABA and serotonin efflux in the NAc.

1. Introduction

The discovery of β-endorphin$_{1-31}$ and other opioid peptides in the mid-1970s was a major step toward understanding why morphine and other opioids relieve pain (Brownstein, 1993; Corbett et al., 2006). Although often overlooked, the initial analyses of β-endorphin$_{1-31}$ isolated not one, but a series of structurally related β-endorphin peptides in brain and peripheral tissues (Dennis et al., 1983; Zakarian and Smyth, 1979). These other peptides, which were synthesized through the post-translational processing of β-endorphin$_{1-31}$, included β-endorphin$_{1-27}$, β-endorphin$_{1-26}$ and the N-terminally acetylated derivatives of all three peptides (Loh, 1992). The post-translational processing of β-endorphin is of interest because both C-terminal proteolysis and N-terminal acetylation essentially abolish the opioid receptor affinity of β-endorphin$_{1-31}$ (Akil et al., 1981; Loh, 1992). β-Endorphin$_{1-27}$ is a notable exception to this generalization, however: A potent opioid receptor antagonist, β-endorphin$_{1-27}$ inhibits the analgesic activity (Deakin et al., 1980; Hong et al., 1993; Nicolas and Li, 1985) and reinforcing effects (Bals-Kubik et al., 1992; Spanagel et al., 1991) of β-endorphin$_{1-31}$ and other opioids. Thus, post-translational processing converts β-endorphin$_{1-31}$ from a potent opioid receptor agonist to an opioid receptor antagonist and inactive forms of the peptide (Loh, 1992).

The post-translational conversion of β-endorphin$_{1-31}$ to β-endorphin$_{1-27}$ also generates a dipeptide, β-endorphin$_{30-31}$ or glycyl-L-glutamine (Gly–Gln) (Loh, 1992; Parish et al., 1983). Gly–Gln is a biologically active peptide that inhibits some of the pharmacological effects of opioids, as one might predict from its co-synthesis with β-endorphin$_{1-27}$. Central Gly–Gln administration inhibits the cardiovascular and respiratory depression caused by morphine, for example, although it does not inhibit morphine or β-endorphin$_{1-31}$ analgesia and has no affinity for opioid receptors (Owen et al., 2000; Unal et al., 1994, 1997). Gly–Gln also attenuates the addictive properties of morphine. It essentially abolishes acquisition and expression of conditioned place preference, a test for morphine's incentive or rewarding effects (Tzschentke, 2007), and inhibits the

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development of morphine tolerance, dependence and withdrawal (Cavun et al., 2005). Gly–Gln thus exhibits a number of potentially valuable pharmacological properties although it's mechanism of action remains unknown.

The ability of Gly–Gln to inhibit opiate reward prompted us to investigate whether the dipeptide modulates morphine-evoked dopamine efflux in the nucleus accumbens (NAc). We found that intracerebroventricular (i.c.v.; 1–100 nmol) Gly–Gln administration inhibited the morphine-induced rise in extracellular dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in the NAc in a dose-related manner although it did not affect extracellular dopamine or DOPAC concentrations in otherwise untreated animals (Basaran et al., 2010). These data are consistent with the hypothesis that Gly–Gln abolishes the rewarding properties of morphine by inhibiting the ability of morphine to stimulate dopamine release in the NAc.

Opioids are classically thought to stimulate NAc dopamine release by inhibiting GABA release from interneurons in the ventral tegmental area (VTA) that tonically inhibit mesolimbic dopamine neurons (Creed et al., 2014; Johnson and North, 1992; Ting-a-Kee and van der Kooy, 2012). Direct injection of mu receptor agonists into the NAc also stimulates dopamine efflux, however, which indicates that opioids modulate dopamine release by acting in both the NAc and VTA (Aono et al., 2008; Saigusa et al., 2008; Yoshida et al., 1999). Opiates also stimulate serotonin (Tao and Auerbach, 1995, 2002a) and GABA (Sun et al., 2011) release in the NAc and there is evidence that both neurotransmitters contribute to the opioid-stimulated release of dopamine (Aono et al., 2008; Guan and McBride, 1989; Parsons and Justice, 1993; Xi and Stein, 2002; Yoshida et al., 1997). The present study therefore investigated whether Gly–Gln antagonizes the morphine-induced efflux of serotonin or GABA in the NAc.

2. Experimental procedures

2.1. Animal and surgical procedures

Male Sprague–Dawley rats (250–350 g; Experimental Animals Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) were housed under a 12-h light/dark cycle with free access to food and water and were allowed to acclimate to the animal facility for 5 days before each experiment. Rats were anaesthetized with 2–4% sevoflurane (Sigma-Aldrich, Inc., St. Louis, MO) in 100% O_2 and placed in a stereotaxic frame and anesthesia was maintained with supplemental administration of sevoflurane as required to suppress the limb compression withdrawal reflex. Rats were implanted with guide cannula in the left lateral ventricle and NAc shell region at the same time and were not subjected to multiple surgeries. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Uludag University and were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Intracerebroventricular injections

A 21-gauge stainless steel guide cannula was implanted in the left lateral ventricle of sevoflurane-anesthetized rats 1.6 mm lateral and 1.0 mm caudal to bregma (Paxinos and Watson, 1986). The guide cannula was lowered through a burr hole through the skull to a depth of 4 mm, fixed to the skull with acrylic cement and sealed with a stylet. For i.c.v. injections, the stylet was removed and a 25 gauge injection cannula was lowered through the guide cannula to a depth 1 mm below the end of the guide cannula. The injection cannula was connected to a 25-μl Hamilton syringe with polyethylene tubing and 5 μl saline or saline containing Gly–Gln (100 nmol/5 μl; Sigma-Aldrich, Inc., St. Louis, MO) was infused at a constant rate over a 60 s period. The injection volume was monitored by observing the movement of an air bubble in the tubing and the cannula was left in place for 60 s after the injection was complete. At the end of each experiment the i.c.v. cannula placement was verified by injecting 5 μl of India ink through the injection cannula.

2.3. Microdialysis

Microdialysis was conducted in awake, freely-moving rats, maintained in individual glass cages during sample collection as described previously (Basaran et al., 2010). For microdialysis probe placement, a guide cannula was lowered through a burr hole placed in the skull 0.9 mm lateral and 1.8 mm anterior to bregma to a depth of 6.5 mm below the skull surface and fixed to the skull with acrylic cement (Paxinos and Watson, 1986). A stylet was placed in the guide cannula and rats were housed in individual cages and allowed to recover from anesthesia. Twenty-four hours after the surgical procedure a custom made microdialysis probe was lowered through the guide cannula into the NAc shell with its tip positioned at a depth of 7.5 mm below the skull surface. The microdialysis probe was equipped with a 2 mm long microdialysis membrane with a molecular weight cutoff of 13,000 Da. After a 1 h habituation period, the dialysis probe was connected to microdialysis pump and perfused at 1 μl/min with artificial Krebs Ringer solution (pH = 7.4) of the following composition: 147 mM NaCl, 4.0 mM KCl and 1.4 mM $CaCl_2$. Samples were collected on ice at 20 min intervals during a 2 h equilibration period.

At the end of the equilibration period three 20 min samples were collected to determine baseline GABA or serotonin concentrations. Subsequently, Gly–Gln (100 nmol/5 μl; Sigma-Aldrich, Inc., St. Louis, MO) or saline was injected i.c.v., morphine HCl (2.5 mg/kg; Galen Drug Company, Istanbul, Turkey) or saline (1 ml/kg) was administered i.p. 2 min later and microdialysis samples were collected at 20 min intervals for 2 h. The Gly–Gln dose was selected from previous dose–response experiments (Basaran et al., 2010; Cavun et al., 2005). Recovery of GABA and serotonin were 10.2% and 10.7%, respectively, consistent with recovery data reported previously (Buck et al., 2009; de Boer and Sutanto, 1997). Extracellular serotonin and GABA concentrations were analyzed in separate experiments using entirely different groups of animals. Time points for drug injection and sample collection were based on data from previous microdialysis (Basaran et al., 2010), behavioral (Cavun et al., 2005) and physiological (Owen et al., 2000) studies. A schematic diagram of the experimental protocol is presented in Fig. 1.

2.4. HPLC analysis

For analysis of GABA, microdialysis samples (20 μl) were derivatized with o-Phthalaldehyde, separated on a C18 Bio-Rad column (Bio-Rad Laboratories, Hercules, CA) and detected with an ESA Model 5041 electrochemical cell equipped with a glassy carbon electrode and an ESA Model 5020 guard cell interfaced with an ESA Coulochem III electrochemical detector (ESA, Inc., Bedford, MA, USA). The voltage applied to the two cells was 400 mV and 700 mV respectively. The mobile phase consisted of 100 mM $NaH_2PO_4(H_2O)_2$, 20% (*v*/*v*) methanol and 3.5% (*v*/*v*) acetonitrile, adjusted to pH 6.7 with phosphoric acid (Acworth and Cunningham, 1999).

For serotonin analyses, dialysate samples (20 μl) were acidified with $HClO_4$ (final concentration 0.4 M) to retard oxidation of monoamines and applied immediately to an isocratic HPLC system. Serotonin was separated on a C18 MD-150 narrow bore (2 × 150 mm, 3 μm) column and detected with an ESA Model 5041 electrochemical cell with glassy carbon electrode and an ESA Model 5020 guard cell interfaced with an ESA Coulochem III detector (ESA, Inc., Bedford, MA, USA). The voltage applied to the cells was 200 mV and 400 mV, respectively. The mobile phase consisted of 75 mM $NaH_2PO_4(H_2O)_2$, 1.1 mM 1-octanesulfonic acid sodium salt, 50 μM EDTA, 100 μl triethylamine and 11% (*v*/*v*) acetonitrile, adjusted to pH 3.7 with concentrated phosphoric acid.