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Agonist-selective effects of opioid receptor ligands on cytosolic calcium concentration in rat striatal neurons

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Abstract

Background—Buprenorphine is an opioid receptor ligand whose mechanism of action is incompletely understood.

Methods—Using Ca^{2+} imaging, we assessed the effects of buprenorphine, β -endorphin, and morphine on cytosolic Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ in rat striatal neurons.

Results—Buprenorphine (0.01–1 μM) increased $[\text{Ca}^{2+}]_i$ in a dose-dependent manner in a subpopulation of rat striatal neurons. The effect of buprenorphine was largely reduced by naloxone, a non-selective opioid receptor antagonist, but not by μ , κ , δ or NOP-selective antagonists. β -endorphin (0.1 μM) increased $[\text{Ca}^{2+}]_i$ with a lower amplitude and slower time course than buprenorphine. Similar to buprenorphine, the effect of β -endorphin was markedly decreased by naloxone, but not by opioid-selective antagonists. Morphine (0.1–10 μM), did not affect $[\text{Ca}^{2+}]_i$ in striatal neurons.

Conclusions—Our results suggest that buprenorphine and β -endorphin act on a distinct type/subtype of plasmalemmal opioid receptors or activate intracellular opioid-like receptor(s) in rat striatal neurons.

Keywords

calcium imaging; opioid receptors; striatum

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Contributors

E Brailoiu and K Benamar designed the study. GC Brailoiu, E Deliu, R Hooper, and E Brailoiu collected the data, prepared the figures and wrote the manuscript. AS Undieh, NJ Dun, MW Adler and K Benamar wrote and revised the draft. All the authors have been contributed to and approved the final version of the manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

1. Introduction

Buprenorphine is a unique and potent opioid whose pharmacology is complex and incompletely elucidated (Lufty and Cowan, 2004). Unlike morphine, which is a full μ opioid receptor agonist, buprenorphine acts as a partial agonist at μ (Toll et al., 1998), antagonist at κ (Zhu et al., 1997) or δ opioid receptors (Negus et al., 2002) and partial or full agonist of NOP receptors (Lufty and Cowan, 2004).

Opioid agonists are the mainstay of analgesic and opiate dependence therapy (Amass et al., 2011; Kahan et al., 2011). Buprenorphine, an analgesic 30 times more potent than morphine, is beneficial in acute, chronic, neuropathic, musculoskeletal, and cancer-related pain (Pergolizzi et al., 2008; Trescot et al., 2008). Due to its slow dissociation from the μ opioid receptor, the risk of the development of drug dependence and analgesic tolerance is lower with buprenorphine than with full μ opioid agonists (Walsh and Eissenberg, 2003).

Despite its proven clinical benefits, there is limited and contradictory evidence regarding the cellular effects of buprenorphine. In the present work, we examined the effects of buprenorphine on cytosolic Ca^{2+} concentration in rat striatal neurons, which express opioid receptors (Mansour et al., 1987; Ding et al., 1996). For comparison, we also examined the effects on cytosolic Ca^{2+} of morphine and an endogenous opioid peptide, β -endorphin.

2. Materials and Methods

2.1 Neuronal Culture

Striatal neurons were dissociated from 1-2day-old Sprague-Dawley rats as previously described (Brailoiu et al., 2005). In brief, newborn rats were euthanized by decapitation and the brains removed and immersed in ice-cold Hanks balanced salt solution (HBSS; Mediatech, Manassas, VA). Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee. The striatum was identified, removed, and minced; the cells were subjected to enzymatic and mechanical dissociation. Cells were plated on 25 mm glass coverslips in Neurobasal-A medium (Invitrogen, Carlsbad, CA) containing 1% GlutaMax (Invitrogen), 2% antibiotic-antimycotic (Mediatech) and 10% fetal bovine serum. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 . Cytosine β -arabino furanoside (1 μM) was added to inhibit glial cell proliferation (Schoniger et al., 2001). Cultures were used for calcium imaging after 3 days.

2.2. Calcium Imaging

Measurements of intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$ were performed as previously described (Brailoiu et al., 2005, 2011; Deliu, 2011). Briefly, cells were incubated with 5 μM fura-2 AM (Invitrogen) in HBSS at room temperature for 45 min, washed three times with dye-free HBSS, and then incubated for another 45 min. Coverslips were mounted in an open bath chamber (RP-40LP, Warner Instruments, Hamden, CT) on the stage of an inverted microscope Nikon Eclipse TiE (Optical Apparatus Co., Ardmore, PA). The microscope was equipped with a Perfect Focus System and a Photometrics CoolSnap HQ2 CCD camera (Roper Scientific, Optical Apparatus Co.). Fura-2 AM fluorescence (emission = 510 nm), following alternate excitation at 340 and 380 nm, was acquired at a frequency of 0.25 Hz. Images were acquired and analyzed using NIS-Elements AR 3.1 software (Nikon/Optical Apparatus Co.). The ratio of the fluorescence signals (340/380 nm) was converted to Ca^{2+} concentration (Grynkiewicz et al., 1985). Cultured cells with neurites at least three times longer than the cell bodies, which responded to 20 mM KCl solution by an increase in $[\text{Ca}^{2+}]_i$ were considered neurons (Brailoiu et al., 2005).

2.3. Drugs

Buprenorphine, morphine and norbinaltorphimine were obtained from the National Institute on Drug Abuse and were dissolved in sterile pyrogen-free saline. CTAP, naltrindole, JTC-801, and β -endorphin were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in saline and 10% DMSO respectively.

3. Results

3.1 Buprenorphine increased $[Ca^{2+}]_i$ in rat striatal neurons

Administration of buprenorphine (0.1 μ M) induced a fast and transient $[Ca^{2+}]_i$ elevation by 355 ± 3.4 nM in 53 of 311 striatal neurons tested (17 %). A lower concentration of buprenorphine (0.01 μ M) increased $[Ca^{2+}]_i$ by 112 ± 1.8 nM ($n = 21$). Buprenorphine (1 μ M) induced a Ca^{2+} peak with an amplitude of 481 ± 4.2 nM followed by a short plateau ($n = 23$) (Fig. 1A, C, D). Pretreatment with selective opioid receptor antagonists (1 μ M, 15 min): CTAP (μ antagonist), naltrindole (δ antagonist), norbinaltorphimine (κ antagonist) or JTC-801 (NOP antagonist) did not significantly affect $[Ca^{2+}]_i$ elevation induced by buprenorphine (0.1 μ M). The increase in $[Ca^{2+}]_i$ produced by buprenorphine in the presence of CTAP (356 ± 3.7 nM, $n = 31$), naltrindole (337 ± 4.6 nM, $n = 54$), norbinaltorphimine (341 ± 3.7 nM, $n = 29$) or JTC-801 (372 ± 4.3 nM, $n = 16$) was not significantly different ($P > 0.05$) from that produced in the absence of the antagonists (355 ± 3.5 nM) (Fig 1E). In contrast, naloxone (1 μ M, 15 min), a non-selective opioid receptor antagonist, largely reduced buprenorphine-induced $[Ca^{2+}]_i$ elevation to 51 ± 1.6 nM ($n = 26$).

3.2 β -endorphin increased $[Ca^{2+}]_i$ in rat striatal neurons

β -endorphin (0.1 μ M) increased $[Ca^{2+}]_i$ by 228 ± 3.4 nM ($n = 38$); with onset 1-2 min after drug addition and peaked at 4-5 min (Fig. 1F). β -endorphin-induced $[Ca^{2+}]_i$ increase in the presence of CTAP (246 ± 3.7 nM, $n = 37$), naltrindole (217 ± 3.7 , $n = 43$), norbinaltorphimine (233 ± 4.2 nM, $n = 51$) or JTC-801 (244 ± 5.1 nM, $n = 46$) was not significantly different ($P > 0.05$) from that produced by β -endorphin alone (Fig. 1G). Naloxone (1 μ M), markedly decreased β -endorphin-induced $[Ca^{2+}]_i$ elevation to 37 ± 1.6 nM ($n = 62$).

3.3. Morphine did not increase cytosolic Ca^{2+} concentration in striatal neurons

In contrast with buprenorphine and β -endorphin, morphine (0.1, 1 and 10 μ M) did not produce a significant change in $[Ca^{2+}]_i$ in rat striatal neurons ($n = 214$, 182 and 263 neurons tested, respectively; Fig. 2).

4. Discussion

Buprenorphine is an opioid with mixed agonist and antagonist properties at different types of opioid receptors. The members of opioid receptor family, μ (MOP), κ (KOP), and δ (DOP) receptors, are G protein-coupled receptors (GPCR) primarily coupled with G_i/G_o ; their activation is associated with inhibition of adenylyl cyclase and inhibition of Ca^{2+} entry (Dhawan et al., 1996; Standifer and Pasternak, 1997). In addition, G_{16} signaling and phospholipase C activation has been reported for μ and κ opioid receptors (Lee et al., 1998; Ueda et al., 1995). Nociceptin/orphanin FQ peptide (NOP) receptor, despite evolutionary and functional homology with μ , κ , and δ receptors, is not considered an opioid receptor from a pharmacological perspective (Cox et al., 2009).

In vivo studies indicate significant differences in spinal and supraspinal analgesic mechanisms of buprenorphine and morphine (Ding and Raffa, 2009). At the supraspinal level, buprenorphine-, but not morphine- or fentanyl-induced antinociception involves

naloxone-, PTX- and NOP-insensitive, G_z -sensitive pathways (Ding and Raffa, 2009). Intriguingly, buprenorphine antinociception is abolished in μ opioid knockout mice (Ide et al., 2004), but not by blockade of μ opioid receptors in wild type animals; however the effect is largely attenuated in $G_{q/11}$ knockout mice (Sanchez-Blazquez et al., 2001). Thus, antinociception induced by heroin and buprenorphine, but not by morphine or methadone, required intact G_o or G_q proteins.

Our results indicate that buprenorphine increased $[Ca^{2+}]_i$ in striatal neurons, supporting the involvement of $G_{q/11}$ pathway. However, molecular studies did not clearly indicate $G_{q/11}$ -coupling of opioid receptors. Ca^{2+} elevation induced by buprenorphine is not sensitive to selective μ , δ , κ or NOP-receptor antagonists, but only to naloxone. To reconcile these findings we propose that buprenorphine acts on a distinct opioid receptor type/subtype coupled with $G_{q/11}$, which is consistent with the observation that several splice variants of the μ opioid receptor having different affinities for agonists were identified in human and rodents (Bolan et al., 2004; Xu et al., 2011).

We also examined the effects on Ca^{2+} of an endogenous opioid, β -endorphin, an agonist of μ and δ receptors and partial agonist of κ receptors (Raynor et al., 1994; Toll et al., 1998). Similar to buprenorphine, β -endorphin increased $[Ca^{2+}]_i$ via a naloxone-sensitive mechanism. Naloxone at 1 μ M concentration inhibited Ca^{2+} response to β -endorphin in an epithelial cell line model of the cortical collecting duct (A6 cells), which express μ opioid receptors (Di Sole et al., 2001). β -endorphin also increased Ca^{2+} uptake by striatal synaptosomes (Barr and Leslie, 1985).

GPCR agonists acting at the same receptor may selectively and differentially activate specific downstream signaling pathways (Kenakin, 1995). An “agonist-directed receptor trafficking” phenomenon has been reported for serotonin, dopamine, vasopressin, adrenergic and opioid receptors (Urban et al., 2007). A similar mechanism may be responsible for the differences between buprenorphine and morphine reported here. Buprenorphine is more lipophilic than morphine and, as a result, may elicit a receptor conformation distinct from that adopted upon morphine binding.

Accumulating evidence indicates that peptides, such as angiotensin II, act in autocrine fashion activating intracellular receptors in various cell types (Haller et al., 1996; Re and Cook, 2007; Deliu et al., 2011). We recently reported activation of intracellular cannabinoid CB_1 receptors by anandamide through pathways distinct from plasmalemmal receptors (Brailoiu et al., 2011). Distinct coupling mechanisms were reported for plasmalemmal D_1 receptors and the D_5 receptors which are localized mostly intracellularly (Undieh, 2010). In contrast to morphine, buprenorphine can penetrate membrane bilayers (Reig et al., 1992). Also, naloxone can interact with phosphatidylinositol, a plasmalemmal component (Reig et al., 1988). Thus, both compounds may act on intracellular opioid-like receptor(s) coupled to $G_{q/11}$ pathway.

In conclusion, buprenorphine, but not morphine, elevates $[Ca^{2+}]_i$ in a concentration-dependent manner in a subpopulation of striatal neurons; the effect is similar to that observed for β -endorphin, largely reduced by naloxone but not by subtype-selective opioid receptor antagonists. Our results suggest that buprenorphine acts on a distinct type/subtype of plasmalemmal opioid receptors or activates intracellular opioid-like receptor(s).

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Fig. 1. Buprenorphine increases $[Ca^{2+}]_i$ in rat striatal neurons

A-B, Fura-2 AM fluorescence ratio (340/380 nm) before and after buprenorphine (0.1 μ M) (A) or β -endorphin (0.1 μ M) (B). C, Examples of buprenorphine-induced $[Ca^{2+}]_i$ increases. D, Buprenorphine (0.01-1 μ M) produced a dose-dependent increase in $[Ca^{2+}]_i$. E, Comparison of $[Ca^{2+}]_i$ increase produced by buprenorphine (0.1 μ M) in the absence (first column) and presence of the opioid receptor antagonists CTAP, naltrindole, norbinaltorphimine, JTC-801 and naloxone. F, Example of β -endorphin-induced increase in $[Ca^{2+}]_i$. G, Comparison of $[Ca^{2+}]_i$ increases produced by β -endorphin (0.1 μ M) in the absence (first column) and presence of the opioid receptor antagonists (* $P < 0.05$).

Fig. 2. Morphine does not increase $[Ca^{2+}]_i$ in rat striatal neurons

A, Fura-2 fluorescence ratio (340/380 nm) before and after morphine (10 μ M). B, Recordings of $[Ca^{2+}]_i$ in the presence of morphine. C, None of the concentrations of morphine (0.1-10 μ M) tested produced a significant increase in $[Ca^{2+}]_i$.