Biochemical and Biophysical Research Communications 450 (2014) 906–911

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

μ–δ opioid receptor heteromer-specific signaling in the striatum and hippocampus

Noufissa Kabli [a,c], Theresa Fan [a], Brian F. O'Dowd [a,c], Susan R. George [a,b,c,*]

[a] Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario M5T1R8, Canada
[b] Department of Medicine, University of Toronto, Medical Sciences Building RM 4358, 1 King's College Circle, Toronto, Ontario M5S1A8, Canada
[c] Department of Pharmacology, University of Toronto, Medical Sciences Building RM 4358, 1 King's College Circle, Toronto, Ontario M5S1A8, Canada

ARTICLE INFO

Article history:
Received 2 June 2014
Available online 26 June 2014

Keywords:
μ opioid receptor
δ opioid receptor
$G\alpha_z$
Heterooligomer
GTPγS
Brain

ABSTRACT

The μ–δ opioid receptor heteromer activates the pertussis toxin-resistant $G\alpha_z$ GTP-binding protein following stimulation by the δ-agonist deltorphin-II whereas μ- and δ-receptors activate the pertussis toxin-sensitive $G\alpha_{i3}$ protein following stimulation by μ- and δ-agonists, respectively. Although the regulation of the μ–δ heteromer is being investigated extensively in vitro, its physiological relevance remains elusive owing to a lack of available molecular tools. We investigated μ–δ heteromer signaling under basal conditions and following prolonged morphine treatment in rodent brain regions highly co-expressing μ- and δ-receptors and $G\alpha_z$. Deltorphin-II induced $G\alpha_z$ activation in the striatum and hippocampus, demonstrating the presence of μ–δ heteromer signaling in these brain regions. Prolonged morphine treatment, which desensitizes μ- and δ-receptor function, had no effect on μ–δ heteromer signaling in the brain. Our data demonstrate that μ–δ heteromer signaling does not desensitize and is regulated differently from μ- and δ-receptor signaling following prolonged morphine treatment.

1. Introduction

μ- and δ-opioid receptors (OR) interact to form a receptor complex that acts as a novel signaling unit with characteristics distinct from those of its constituent μOR or δORs [1]. The μ–δ heteromer displays a novel pharmacological, internalization, desensitization and signaling profile in transfected cells [2,3] and *in vivo* [4]. μ- and δ-ORs co-localize extensively within the same neurons in several anatomic regions including the striatum (nucleus accumbens and caudate putamen), hippocampus, spinal cord and dorsal root ganglia [5,6], making these receptors poised for functional interactions in specific neuronal circuits. However, mere μOR and δOR co-localization cannot be considered indicative of heteromeric receptor–receptor interactions. Definitive evidence of heteromer formation in brain would require evidence such as could be provided by confocal FRET between the endogenous receptors *in situ*, evidence of binding of a heteromer-selective ligand to the receptor complex, or activation of a distinct heteromer-mediated signaling pathway not activated by ligands targeting the constituent receptors. Here, we focused on the distinct signaling pathway activated by an opioid ligand acting through the μ–δ heteromer.

Unlike μ- and δ-OR monomers/homomers, μ–δ heteromer function is resistant to pertussis toxin (PTX) [1]. The δ-agonist deltorphin-II acts as an agonist at the μ–δ heteromer whereby deltorphin-II binding to the receptor complex leads to selective activation of the PTX-resistant $G\alpha_z$ GTP-binding protein [2]. In contrast, agonist binding to the μ- or δ-OR monomers/homomers leads to activation of the PTX-sensitive $G\alpha_{i3}$ protein [2]. *In vivo* evidence suggesting existence of μ–δ heteromer signaling, namely deltorphin II-induced $G\alpha_z$ activation, is provided by several studies. Firstly, deltorphin-II's analgesic effects are not entirely abolished following central administration of PTX, suggesting that activation of PTX-resistant Gα-proteins is involved in transducing the antinociceptive effects of deltorphin-II [7]. Secondly, $G\alpha_z$, the G-protein to which the μ–δ heteromer couples selectively, has a predominant neuronal localization and is present in brain regions where μ- and δ-ORs are colocalized [8]. Further, deltorphin-II-induced analgesia is reduced in μOR gene-deleted animals, further supporting the involvement of both μOR and δOR in the actions of deltorphin-II [9].

Abbreviations: DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; Deltorphin-II, [D-Ala^2]deltorphin-II; δOR, δ-opioid receptor; DPDPE, [D-Pen^2, D-Pen^5]enkephalin; G-protein, GTP-binding protein; GTPγS, guanosine 5′-O-[gamma-thio]triphosphate; GPCR, G-protein coupled receptor; μOR, μ-opioid receptor; PTX, pertussis toxin.

* Corresponding author at: Department of Pharmacology, Faculty of Medicine, University of Toronto, Medical Sciences Building Rm 4358, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada. Fax: +1 (416) 971 2868.
E-mail address: s.george@utoronto.ca (S.R. George).

http://dx.doi.org/10.1016/j.bbrc.2014.06.099
0006-291X/

The μ–δOR heteromer may be intimately involved in the cellular mechanism of morphine tolerance. Prolonged morphine treatment potentiates deltorphin-II-induced antinociceptive effects and induces plasmalemmal targeting of δOR from intracellular stores in the CNS in a μOR-dependent manner [10,11]. Further, $G\alpha_z$ gene ablation in rodents leads to accentuated morphine tolerance, suggesting that signaling through $G\alpha_z$ may be protective under conditions of prolonged morphine treatment [12].

Owing to a lack of available selective pharmacological and molecular tools to study the μ–δ heteromer *in vivo*, we sought to leverage deltorphin II-induced $G\alpha_z$ activation as an index to investigate μ–δ heteromer-specific signaling in distinct brain regions co-expressing μOR, δOR and $G\alpha_z$ under basal conditions and following prolonged morphine treatment.

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (Charles River, Québec, Canada) were housed in pairs in a temperature-controlled room with corn chip bedding and free access to rodent chow and water. Rats were maintained under a standard 12-h/12-h light/dark cycle. Testing was performed during the light cycle. Animal protocols were approved by the University of Toronto Animal Care Committee and in accordance with the Canadian Council on Animal Care guidelines.

2.2. Drugs

Morphine sulfate was purchased from BDH Chemicals (Toronto, ON, Canada). Deltorphin II, DAMGO, and DPDPE were purchased from Sigma (Saint Louis, MO).

2.3. Prolonged morphine treatment

Rats were injected subcutaneously (s.c.) with ascending doses of morphine sulfate (5, 8, 10, and 15 mg/kg, s.c. every 12 h) for 48 h and sacrificed 12 h following the last morphine injection as described [10]. Control rats were injected with equal volumes of saline.

2.4. Tail immersion antinociception assay

Rats were restrained gently and the distal 5 cm of their tail dipped in a 52 °C-water bath. The latency to tail withdrawal from water was measured. Three pre-drug baseline (BL) measures were obtained and averaged. A cut-off of 4 × BL latency was imposed to minimize tissue damage. Rats were habituated to the tail immersion assay before testing. % Maximum Possible Effect was calculated according to the equation: % Maximum Possible Effect = (Latency − BL latency)/(Cut-off Latency − BL latency) × 100.

2.5. Brain membrane preparation

Following decapitation, rat brain was removed. The striatum (nucleus accumbens and caudate putamen) and hippocampus were isolated, placed in buffer (50 mM Tris, 250 mM sucrose, 25 mM KCl, 5 mM $MgCl_2$, pH 7.4), homogenized and centrifuged at 100*g*. The supernatant was centrifuged at 40,000*g* for 20 min at 4 °C to isolate the crude membrane fraction.

2.6. Total and Gα-specific [^{35}S]-GTPγS binding

Rat striatal and hippocampal membranes (200 μg/tube) were pre-incubated at 30 °C for 5 min, then treated with vehicle or 10 μM agonist in the presence of 2 nM [^{35}S]-GTPγS (1250 Ci/mmol, Perkin Elmer Life Sciences, Waltham, MA) and 10 μM GDP for 1 min. Binding was terminated by the addition of ice-cold assay buffer (10 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4) and centrifugation at 20,000*g* for 10 min at 4 °C. The pellet was dissolved in solubilization buffer (100 mM Tris, 200 mM NaCl, 1 mM EDTA, 1.25% (v/v) Igepal 630, pH 7.4) and 0.18% SDS for 1 h (hr) at 4 °C. Unsolubilized debris was pelleted at 20,000*g* for 20 min. To detect total G-protein activation, the pellet from the first centrifugation step was washed three times with assay buffer by centrifugation at 2500 rpm for 3 min each time. Scintillation cocktail (Ready Safe™, Beckman Coulter, Mississauga, ON) was added and radioactivity was counted (Beckman LS 6500 Counter, Beckman Coulter, Mississauga, ON). To detect activation of specific G-proteins, the supernatant was incubated with 5 μg of $G\alpha_z$ or $G\alpha_{i3}$ antibody (Sc-388 or Sc-262, respectively, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. Protein-G-agarose 50:50 was added and the tubes rotated at 4 °C for 4 h. Beads were washed 4 times with solubilization buffer, suspended in scintillation cocktail and radioactivity was counted.

2.7. Mammalian cells

HEK 293T human embryonic kidney cells (American Type Culture Collection, Manassas, VA, USA) were maintained as a monolayer at 37 °C with 5% CO_2 in advanced minimal essential medium containing 6% fetal bovine serum and antibiotics (Invitrogen, Burlington, ON, Canada). Cells were stably expressing μ- and δ-ORs as described previously [3].

2.8. SDS–polyacrylamide gel electrophoresis and immunoblotting

Membrane proteins were resolved on 10% Tris–Glycine gels (Novex, San Diego, CA, USA) under denaturing conditions by SDS–PAGE and then electroblotted onto polyvinylidene difluoride membranes as described [2]. Immunoreactivity was revealed by incubating in $G\alpha_z$ or $G\alpha_{i3}$ antibody diluted 1:250, HRP-conjugated goat-anti-rabbit secondary antibody diluted 1:1000 (Bio-Rad, Hercules, CA, USA), and enhanced chemiluminescent reagents, and then exposing to film.

2.9. Immunoprecipitation studies

Membrane fractions were re-suspended and stirred with protease inhibitors and the homogenate centrifuged. The solubilized portion was isolated and incubated with 5 μg anti-$G\alpha_z$ or 5 μg anti-$G\alpha_{i3}$ antibody and then protein-G-agarose beads. The immunoprecipitate was washed and solubilized in SDS sample for gel electrophoresis.

2.10. Data analysis

Statistical analysis and graph generation were performed using GraphPad Prism software 3.01 (San Diego, CA). The results are presented as a percentage over unstimulated (basal) controls, which were designated as 0%. The results were presented as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Dunnett's post hoc tests were utilized to analyze the statistical significance of agonist-induced effects on G-protein activation in comparison to unstimulated controls. The unpaired Student's *t*-test was used to analyze the statistical significance of the difference in agonist-induced G-protein activation in brain samples from animals treated with repeated morphine or saline injections. $P < 0.05$ was deemed significant.

3. Results and discussion

To interrogate the presence of μ–δ heteromer signaling in the striatum and hippocampus, we used the [^{35}S]-GTPγS incorporation assay followed by selective G-protein immunoprecipitation using a specific antibody to assess agonist-induced activation of $G\alpha_z$. Deltorphin-II-induced $G\alpha_z$ activation only occurs in cells co-expressing μ- and δ-ORs and is an index of functional μ–δ heteromers [2]. Deltorphin-II significantly enhanced [^{35}S]-GTPγS incorporation into $G\alpha_z$ over unstimulated levels (which were designated as 0% activation) in the striatum and hippocampus (Fig. 1A and B; hatched bars), suggestive of μ–δ heteromer expression in the striatum and hippocampus. Neither DAMGO (μ-agonist) nor DPDPE (δ-agonist) induced significant $G\alpha_z$ activation over unstimulated levels in these regions (Fig. 1A and B; hatched bars).

In separate experiments, brains were sectioned to obtain nucleus accumbens tissue, another region of high μOR and δOR co-expression, but of low protein yield. Conducting this assay using a sufficient number of samples and replicates would have necessitated pooling tissue from large numbers of animals, precluding us from analyzing the region.

Our findings align with those from Garzon's study demonstrating attenuation of the analgesic effects of deltorphin-II, but not DPDPE, following intra-periaqueductal gray administration of antisense oligodeoxynucleotides targeting $G\alpha_{x/z}$ [13]. We observed slight but non-significant activation of $G\alpha_z$ by DAMGO, suggesting that DAMGO may also activate the $G\alpha_z$-coupled μ–δ heteromer, although not as efficaciously as deltorphin-II. In Garzon's study, DAMGO-induced analgesic effects were also attenuated by diminishing $G\alpha_{x/z}$ expression in the periaqueductal gray, however, more potent inhibition of DAMGO's analgesic effects was achieved by inactivating PTX-sensitive Gα-proteins [13].

Our findings can be explained in the context of ligand-biased agonism whereby different ligands stabilize their corresponding G-protein coupled receptor (GPCR) in unique conformations resulting in selective recruitment of signaling molecules to the intracellular receptor domain [14]. Studies also demonstrate receptor pre-coupling with G-proteins and complements of signaling molecules in the endoplasmic reticulum and transportation as a signalosome to the plasma membrane where the receptor is available to bind ligand [14,15]. In our example, while DAMGO and DPDPE bind the μ–δ heteromer – along with the endogenous opioids Leu-enkephalin, Met-enkephalin, and Endomorphin-1 and 2 –, they do so with much lower affinity than deltorphin-II [1,2] and thus may not act as full agonists. Therefore these ligands may not be as efficient at stabilizing the active $G\alpha_z$-bound conformation of the μ–δ heteromer, which is likely responsible for the functional effects of the heteromer which are PTX-resistant [2]. The phenomenon of ligand-directed signaling has been documented for μ- and δ-ORs [14,16] and thus would be expected to govern ligand–heteromer interactions as well.

Following repeated morphine treatment, deltorphin-II significantly enhanced $G\alpha_z$ activation over unstimulated levels in the striatum and hippocampus whereas DAMGO and DPDPE did not (Fig. 1A and B; filled bars). The μ–δOR heteromer did not desensitize following repeated morphine treatment.

To confirm the presence of functional μ- and δ-OR monomers/homomers in the striatum and hippocampus, μ- and δ-agonist-induced $G\alpha_{i3}$ activation was examined. In cell lines expressing μ- or δ-ORs individually, selective agonists for each receptor activate the PTX-sensitive $G\alpha_{i3}$ G-protein [2]. Here, all opioid agonists tested significantly induced $G\alpha_{i3}$ activation over unstimulated levels in the striatum and hippocampus (Fig. 2A and B; hatched bars). As G-protein activation is a proximal step in the GPCR regulatory pathway and serves as a functional measure of receptor activity, these findings also confirm the presence of functional μ- and δ-OR monomers/homomers in these brain regions.

Interestingly, $G\alpha_{i3}$ activation in response to DAMGO, deltorphin-II, or DPDPE was abolished in striata and hippocampi from animals treated repeatedly with morphine (Fig. 2A and B; filled bars). The desensitization of $G\alpha_{i3}$ activation downstream of μ-and δ-OR homomers/monomers aligns with other studies demonstrating chronic morphine-induced desensitization of μ- and δ-OR homomer/monomer function [17].

All agonists induced comparable and significant [^{35}S]-GTPγS incorporation into Gα-proteins over unstimulated levels in the striatum and hippocampus (Fig. 3A and B; hatched bars), confirming the presence of functional ORs in brain regions assayed and verifying that the lack of $G\alpha_z$ activation by DAMGO and DPDPE was due to the lack of coupling to this particular subtype of G-protein, and not due to an inability to activate Gα-proteins in this assay.

Repeated morphine treatment abolished Gα-protein activation by all agonists in the striatum and hippocampus (Fig. 3A and B; filled bars). Thus, prolonged morphine leads to the desensitization

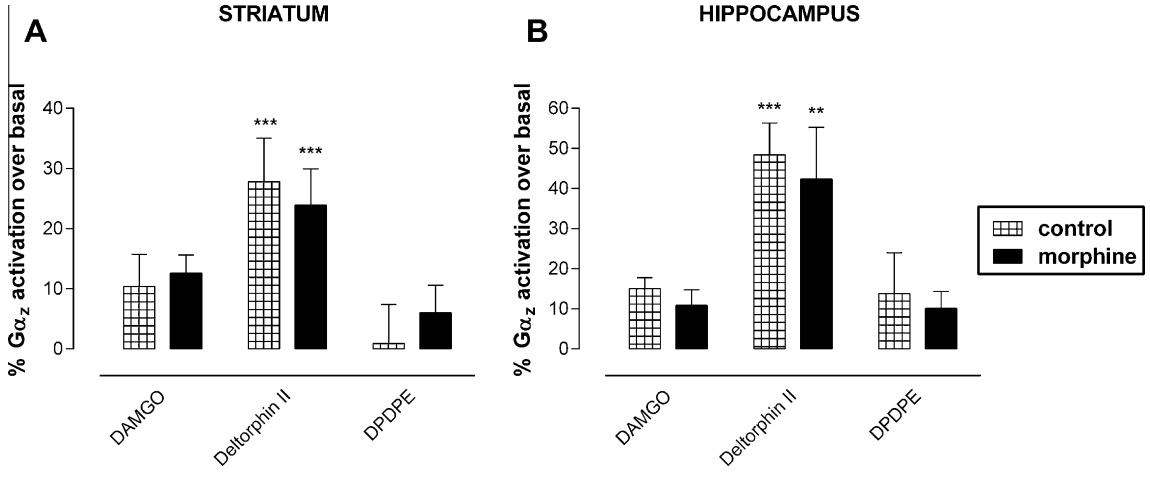

Fig. 1. Quantification of agonist-induced [^{35}S]-GTPγS incorporation into immunoprecipitated $G\alpha_z$ in the striatum and hippocampus of rats treated with saline or repeated morphine injections. Membrane preparations from striatum (A) or hippocampus (B) were treated with 10 μM DAMGO, deltorphin-II, or DPDPE. Data are presented as % activation over unstimulated basal (designated as 0%) and are shown as means ± S.E.M. for $n = 3$–4 separate experiments. Agonist-induced $G\alpha_z$ activation relative to basal in each group was assessed for statistical significance using one-way ANOVA followed by Dunnett's post hoc analysis. [$^{***}p < 0.001$, $^{**}p < 0.01$ relative to unstimulated basal]. Differences in % $G\alpha_z$ activation induced by the same agonist in striata and hippocampi from saline-treated versus morphine-treated animals were assessed for statistical significance using the unpaired Student's *t*-test. [$p > 0.05$ relative to saline-injected control animals].

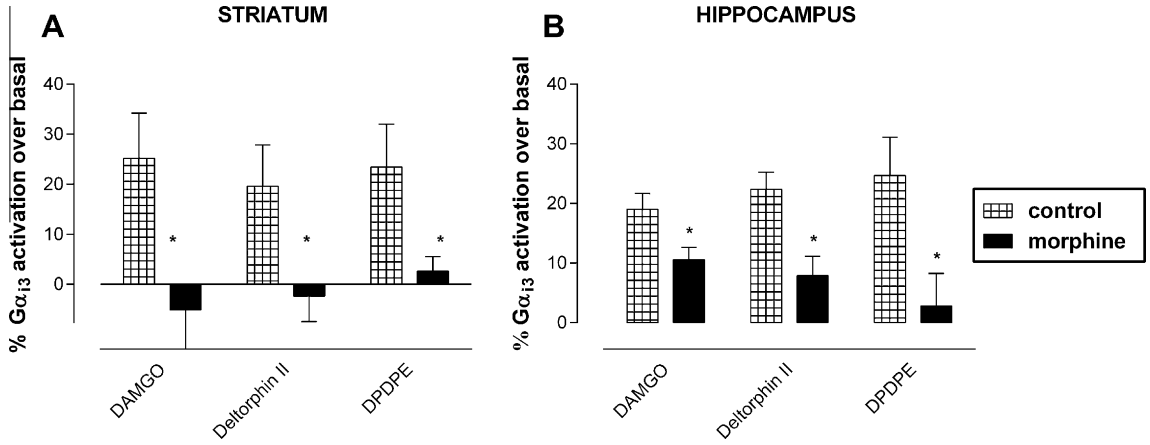

Fig. 2. Quantification of agonist-induced [^{35}S]-GTPγS incorporation into immunoprecipitated $G\alpha_{i3}$ in the striatum and hippocampus of rats treated with saline or repeated morphine injections. Membrane preparations from striatum (A) or hippocampus (B) were treated with 10 μM DAMGO, deltorphin-II, or DPDPE. Data are presented as % activation over unstimulated basal (designated as 0%) and are shown as means ± S.E.M. for $n = 3$ separate experiments. Differences in % $G\alpha_{i3}$ activation induced by the same agonist in striata and hippocampi from saline-treated versus morphine-treated animals were assessed for statistical significance using the unpaired Student's t-test. [$^{*}p < 0.05$ relative to saline-injected control animals].

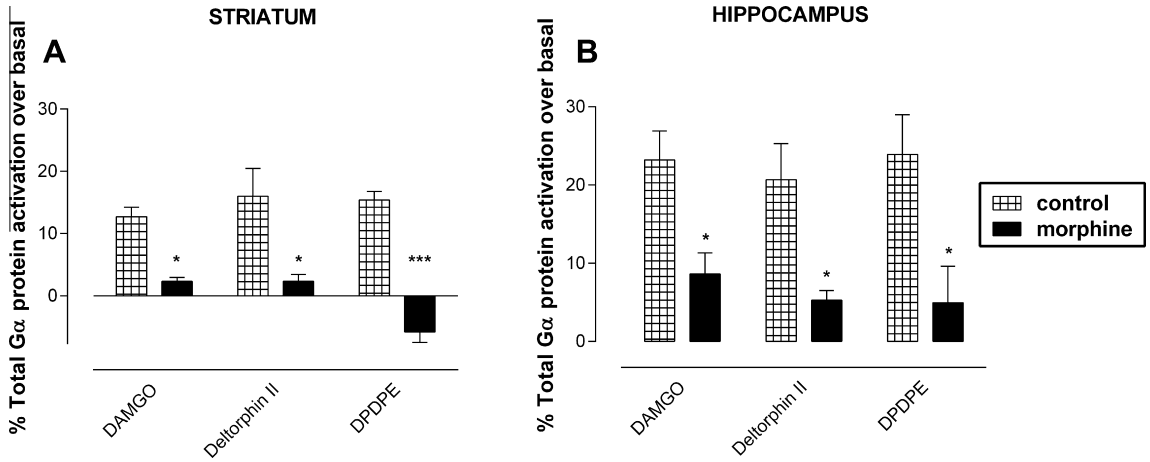

Fig. 3. Quantification of agonist-induced [^{35}S]-GTPγS incorporation into all Gα-protein subtypes in the striatum and hippocampus of rats treated with saline or repeated morphine injections. Membrane preparations from striatum (A) or hippocampus (B) were treated with 10 μM DAMGO, deltorphin-II, or DPDPE. Data are presented as % activation over unstimulated basal (designated as 0%) and are shown as means ± S.E.M. for $n = 3$–6 separate experiments. Differences in % Gα activation induced by the same agonist in striata and hippocampi from saline-treated control versus morphine-treated animals were assessed for statistical significance using the unpaired Student's t-test. [$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ relative to saline-injected animals].

of OR-mediated G-protein activation which may be due to the uncoupling of these receptors from select populations of G-proteins. Indeed, prolonged morphine treatment leads to cellular adaptations which act to dampen OR signaling. Reduced receptor-G-protein coupling is one of the desensitization mechanisms limiting the clinical utility of current clinically-used μ-agonists [18] and necessitating dose escalation to maintain therapeutic efficacy. Until now, the effect of repeated morphine treatment on agonist-induced activation of specific Gα-proteins had not been explored *in vivo*. Here, μ- and δ-agonist activation of $G\alpha_{i3}$ was dramatically reduced following prolonged morphine treatment, suggestive of μOR and δOR monomer/homomer uncoupling from G-proteins. This observation aligns with previous reports of marked attenuation in opioid receptor G-protein coupling following various chronic morphine regimens in cell lines and in the brain [19] and desensitization of individual μ- and δ-OR function [17]. Receptor density reductions are unlikely to account for this effect as reductions in μOR number are not consistently observed following chronic morphine treatment [20,21].

On the other hand, prolonged morphine exposure has been shown to enhance deltorphin-II-induced effects and to induce δOR targeting to the plasma membrane in a μOR-dependent manner [10,11]. Thus, it is likely that a proportion of the δORs undergoing plasmalemmal targeting may be complexed with μORs in a μ–δOR heteromer. Recent studies using a newly-synthesized μ–δ heteromer antibody revealed that chronic morphine treatment upregulated the μ–δ receptor complex in brain regions including the striatum and hippocampus [22]. Since our findings indicate that deltorphin-II-induced $G\alpha_z$ activation was maintained but not potentiated, we speculate that the efficiency of coupling to post-heteromer signaling cascades and effectors underlying the behavioral effects of deltorphin-II may be enhanced following prolonged morphine exposure.

A recent study advanced that an inhibitory peptide derived from the μOR TM1 domain abolished the interaction between μOR and δORs in the spinal cord and abolished morphine tolerance, leading the authors to conclude that μ–δ heteromer disruption would be effective in preventing tolerance [23]. However, modeling [24] and crystallization studies [25] demonstrate that μOR TM1 is part of the μOR homo-oligomerization interface. Further, the TM1 peptide had no effect on the analgesic effects of the δ-agonist deltorphin-I, which had been shown to activate the μ–δ

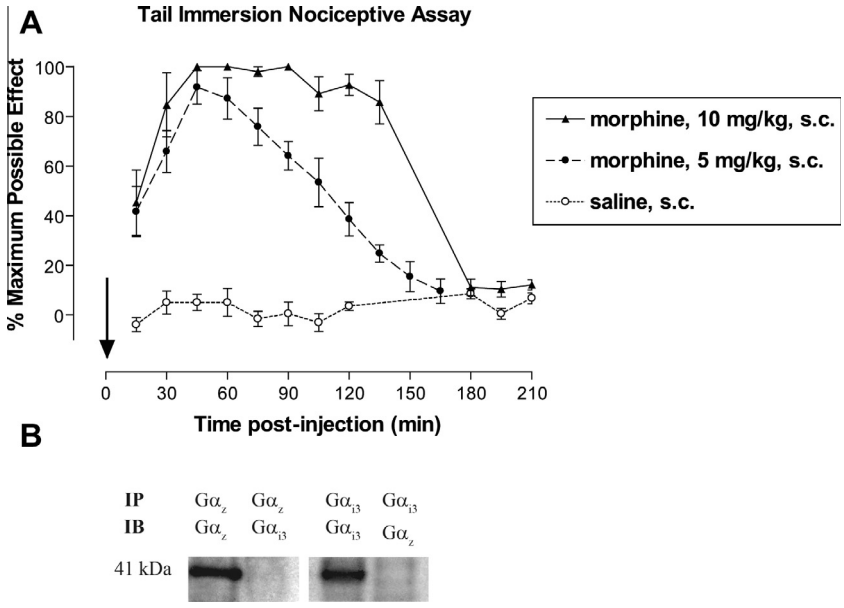

Fig. 4. The analgesic effects of morphine in the tail immersion nociceptive assay (A) and verification of $G\alpha_z$ and $G\alpha_{i3}$ antibody specificity (B). (A) Rats were injected subcutaneously (s.c.) with saline or morphine sulfate (5 mg/kg or 10 mg/kg). The latency to tail withdrawal from warm water (52 °C) was measured at baseline (BL) and following saline or morphine injection. The arrow denotes morphine or saline injection time. 4 × BL latency cut-off was imposed to minimize tissue damage. % Maximum Possible Effect was calculated as: **%** Maximum Possible Effect = (Latency − BL latency)/(Cut-off Latency − BL latency) × 100. (B) HEK 293T Cells co-expressing μOR, δOR, $G\alpha_{i3}$ and $G\alpha_z$ were immunoprecipitated with $G\alpha_z$ or $G\alpha_{i3}$ antiserum, and then probed with either $G\alpha_z$ or $G\alpha_{i3}$ antibody. Immunoblotting with $G\alpha_z$ or $G\alpha_{i3}$ antibody revealed an immunoreactive band with an approximate weight of 41 kDa as reported [27,28]. Molecular weights were estimated by comparing band migration with calibrated protein ladder.

heteromer in the same paper [23]. The TM1 peptide may have interfered with the integrity of the μOR, which would confound the interpretation of the observations presented. Thus, it is not possible to conclude that μ–δ heteromer loss would prevent the development of morphine tolerance.

Our findings point to unique regulation of μ–δ heteromer signaling by morphine exposure. The μ–δ receptor complex may still be activated and functional and could be recruited to elicit therapeutic effects even under prolonged morphine exposure conditions which desensitize μOR and δOR function and contribute to the diminished clinical efficacy of μ-opioid analgesics. The preferential coupling of the μ–δ heteromer to $G\alpha_z$ [2] together with the accentuated morphine tolerance in $G\alpha_z$ gene-deleted animals [12] further supports our interpretation of the findings.

To confirm the efficacy of morphine doses used in the repeated morphine treatment regimen, rats underwent behavioral testing in the tail immersion nociception assay. Morphine – but not saline – injections induced sustained analgesia (Fig. 4A), confirming that any lack of desensitization in G-protein activation following repeated morphine treatment is not due to morphine not reaching the nervous system. Further, the 10 mg/kg morphine dose produced comparable – not greater – analgesia as the 5 mg/kg dose at 30 min following administration (the timepoint frequently reported in the literature [26]), confirming that tolerance was present in this paradigm.

$G\alpha_z$ antiserum detected immunoprecipitated $G\alpha_z$ protein, the latter was not detected by the $G\alpha_{i3}$ antiserum, and vice versa (Fig. 4B), confirming that each antibody reacted selectively with its corresponding antigen.

In conclusion, this study demonstrates that the μ–δ heteromer is regulated differently from its constituent μ- and δ-receptors following prolonged morphine treatment and therefore may have a distinct role in modulating cellular adaptations to morphine tolerance in addition to other effects, which once elucidated, may reveal discrete and unique functions that may establish the μ–δ heteromer as a potential therapeutic target for treating pain, neurologic and neuropsychiatric disorders.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Funded by a grant from the Canadian Institutes of Health Research and an Ontario Mental Health Foundation Research Studentship (N.K.). Dr. Susan R. George holds a Tier I Canada Research Chair in Molecular Neuroscience.

References

[1] S.R. George, T. Fan, Z. Xie, R. Tse, V. Tam, G. Varghese, B.F. O'Dowd, Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties, J. Biol. Chem. 275 (34) (2000) 26128–26135.
[2] T. Fan, G. Varghese, T. Nguyen, R. Tse, B.F. O'Dowd, S.R. George, A role for the distal carboxyl tails in generating the novel pharmacology and G protein activation profile of mu and delta opioid receptor hetero-oligomers, J. Biol. Chem. 280 (46) (2005) 38478–38488.
[3] N. Kabli, N. Martin, T. Fan, T. Nguyen, A. Hasbi, G. Balboni, B.F. O'Dowd, S.R. George, Agonists at the delta-opioid receptor modify the binding of micro-receptor agonists to the micro-delta receptor hetero-oligomer, Br. J. Pharmacol. 161 (5) (2010) 1122–1136.
[4] I. Gomes, A. Gupta, J. Filipovska, H.H. Szeto, J.E. Pintar, L.A. Devi, A role for heterodimerization of mu and delta opiate receptors in enhancing morphine analgesia, Proc. Natl. Acad. Sci. U.S.A. 101 (14) (2004) 5135–5139.
[5] H.B. Wang, B. Zhao, Y.Q. Zhong, K.C. Li, Z.Y. Li, Q. Wang, Y.J. Lu, Z.N. Zhang, S.Q. He, H.C. Zheng, S.X. Wu, T.G. Hokfelt, L. Bao, X. Zhang, Coexpression of delta- and mu-opioid receptors in nociceptive sensory neurons, Proc. Natl. Acad. Sci. U.S.A. 107 (29) (2010) 13117–13122.
[6] A.C. Gray, I.M. Coupar, P.J. White, Comparison of opioid receptor distributions in the rat central nervous system, Life Sci. 79 (7) (2006) 674–685.
[7] L.F. Tseng, K.A. Collins, Pretreatment with pertussis toxin differentially modulates morphine- and beta-endorphin-induced antinociception in the mouse, J. Pharmacol. Exp. Ther. 279 (1) (1996) 39–46.

[8] I.K. Friberg, A.B. Young, D.G. Standaert, Differential localization of the mRNAs for the pertussis toxin insensitive G-protein alpha sub-units Gq, G11, and Gz in the rat brain, and regulation of their expression after striatal deafferentation, Brain Res. Mol. Brain Res. 54 (2) (1998) 298–310.
[9] H.W. Matthes, C. Smadja, O. Valverde, J.L. Vonesch, A.S. Foutz, E. Boudinot, M. Denavit-Saubie, C. Severini, L. Negri, B.P. Roques, R. Maldonado, B.L. Kieffer, Activity of the delta-opioid receptor is partially reduced, whereas activity of the kappa-receptor is maintained in mice lacking the mu-receptor, J. Neurosci. 18 (18) (1998) 7285–7295.
[10] C.M. Cahill, A. Morinville, M.C. Lee, J.P. Vincent, B. Collier, A. Beaudet, Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception, J. Neurosci. 21 (19) (2001) 7598–7607.
[11] A. Morinville, C.M. Cahill, M.J. Esdaile, H. Aibak, B. Collier, B.L. Kieffer, A. Beaudet, Regulation of delta-opioid receptor trafficking via mu-opioid receptor stimulation: evidence from mu-opioid receptor knock-out mice, J. Neurosci. 23 (12) (2003) 4888–4898.
[12] I.A. Hendry, K.L. Kelleher, S.E. Bartlett, K.J. Leck, A.J. Reynolds, K. Heydon, A. Mellick, D. Megirian, K.I. Matthaei, Hypertolerance to morphine in G(z alpha)-deficient mice, Brain Res. 870 (1–2) (2000) 10–19.
[13] J. Garzon, A. Garcia-Espana, P. Sanchez-Blazquez, Opioids binding mu and delta receptors exhibit diverse efficacy in the activation of Gi2 and G(x/z) transducer proteins in mouse periaqueductal gray matter, J. Pharmacol. Exp. Ther. 281 (1) (1997) 549–557.
[14] N. Audet, C. Gales, E. Archer-Lahlou, M. Vallieres, P.W. Schiller, M. Bouvier, G. Pineyro, Bioluminescence resonance energy transfer assays reveal ligand-specific conformational changes within preformed signaling complexes containing delta-opioid receptors and heterotrimeric G proteins, J. Biol. Chem. 283 (22) (2008) 15078–15088.
[15] D.J. Dupre, M. Robitaille, N. Ethier, L.R. Villeneuve, A.M. Mamarbachi, T.E. Hebert, Seven transmembrane receptor core signaling complexes are assembled prior to plasma membrane trafficking, J. Biol. Chem. 281 (45) (2006) 34561–34573.
[16] H. Zheng, H.H. Loh, P.Y. Law, Beta-arrestin-dependent mu-opioid receptor-activated extracellular signal-regulated kinases (ERKs) translocate to nucleus in contrast to G protein-dependent ERK activation, Mol. Pharmacol. 73 (1) (2008) 178–190.
[17] D.A. Eisinger, H. Ammer, R. Schulz, Chronic morphine treatment inhibits opioid receptor desensitization and internalization, J. Neurosci. 22 (23) (2002) 10192–10200.
[18] P.H. Tso, Y.H. Wong, Molecular basis of opioid dependence: role of signal regulation by G-proteins, Clin. Exp. Pharmacol. Physiol. 30 (5–6) (2003) 307–316.
[19] W.A. Klee, R.A. Streaty, Narcotic receptor sites in morphine-dependent rats, Nature 248 (443) (1974) 61–63.
[20] L.J. Sim-Selley, K.L. Scoggins, M.P. Cassidy, L.A. Smith, W.L. Dewey, F.L. Smith, D.E. Selley, Region-dependent attenuation of mu opioid receptor-mediated G-protein activation in mouse CNS as a function of morphine tolerance, Br. J. Pharmacol. 151 (8) (2007) 1324–1333.
[21] L.S. Brady, M. Herkenham, J.B. Long, R.B. Rothman, Chronic morphine increases mu-opiate receptor binding in rat brain: a quantitative autoradiographic study, Brain Res. 477 (1–2) (1989) 382–386.
[22] A. Gupta, J. Mulder, I. Gomes, R. Rozenfeld, I. Bushlin, E. Ong, M. Lim, E. Maillet, M. Junek, C.M. Cahill, T. Harkany, L.A. Devi, Increased abundance of opioid receptor heteromers after chronic morphine administration, Sci. Signal. 3 (131) (2010) 54.
[23] S.Q. He, Z.N. Zhang, J.S. Guan, H.R. Liu, B. Zhao, H.B. Wang, Q. Li, H. Yang, J. Luo, Z.Y. Li, Q. Wang, Y.J. Lu, L. Bao, X. Zhang, Facilitation of mu-opioid receptor activity by preventing delta-opioid receptor-mediated codegradation, Neuron 69 (1) (2011) 120–131.
[24] M. Filizola, H. Weinstein, Structural models for dimerization of G-protein coupled receptors: the opioid receptor homodimers, Biopolymers 66 (5) (2002) 317–325.
[25] A. Manglik, A.C. Kruse, T.S. Kobilka, F.S. Thian, J.M. Mathiesen, R.K. Sunahara, L. Pardo, W.I. Weis, B.K. Kobilka, S. Granier, Crystal structure of the micro-opioid receptor bound to a morphinan antagonist, Nature 485 (7398) (2012) 321–326.
[26] B. Milne, M. Sutak, C.M. Cahill, K. Jhamandas, Low doses of alpha 2-adrenoceptor antagonists augment spinal morphine analgesia and inhibit development of acute and chronic tolerance, Br. J. Pharmacol. 155 (8) (2008) 1264–1278.
[27] R.T. Premont, A. Buku, R. Iyengar, The G alpha z gene product in human erythrocytes. Identification as a 41-kilodalton protein, J. Biol. Chem. 264 (25) (1989) 14960–14964.
[28] G.G.t. Holz, R.M. Kream, A. Spiegel, K. Dunlap, G proteins couple alpha-adrenergic and GABAb receptors to inhibition of peptide secretion from peripheral sensory neurons, J. Neurosci. 9 (2) (1989) 657–666.