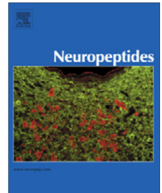
Neuropeptides 46 (2012) 383–394

Contents lists available at SciVerse ScienceDirect

Neuropeptides

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



The endogenous opioid dynorphin is required for normal bone homeostasis in mice

Paul A. Baldock [a,c,f,1], Frank Driessler [a,1], Shu Lin [a], Iris P.L. Wong [a], Yanchuan Shi [a], Ernie Yulyaningsih [a], Lesley Castillo [a], Sonia Janmaat [a], Ronaldo F. Enriquez [a], Ayse Zengin [a], Brigitte L. Kieffer [d], Christoph Schwarzer [e], John A. Eisman [b,c], Amanda Sainsbury [a,g], Herbert Herzog [a,c,f,*]

[a] Neuroscience Research Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, Sydney, NSW 2010, Australia
[b] Osteoporosis and Bone Biology Research Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, Sydney, NSW 2010, Australia
[c] Faculty of Medicine, University of NSW, Sydney, NSW, Australia
[d] Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université de Strasbourg, Illkirch, France
[e] Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria
[f] St. Vincent's Hospital Clinical School, University of New South Wales, Kensington, NSW, Australia
[g] School of Medical Sciences, University of New South Wales, Kensington, NSW, Australia

ARTICLE INFO

Article history:
Received 26 July 2012
Accepted 16 August 2012
Available online 9 October 2012

Keywords:
Animal models
Neural factors
Bone histomorphometry
Osteoblasts
Molecular pathways

ABSTRACT

Chronic opiate usage, whether prescribed or illicit, has been associated with changes in bone mass and is a recognized risk factor for the development of osteoporosis; however, the mechanism behind this effect is unknown. Here we show that lack of dynorphin, an endogenous opioid, in mice (Dyn−/−), resulted in a significantly elevated cancellous bone volume associated with greater mineral apposition rate and increased resorption indices. A similar anabolic phenotype was evident in bone of mice lacking dynorphin's cognate receptor, the kappa opioid receptor. Lack of opioid receptor expression in primary osteoblastic cultures and no change in bone cell function after dynorphin agonist treatment *in vitro* indicates an indirect mode of action. Consistent with a hypothalamic action, central dynorphin signaling induces extracellular signal-regulated kinase (ERK) phosphorylation and c-fos activation of neurons in the arcuate nucleus of the hypothalamus (Arc). Importantly, this signaling also leads to an increase in Arc NPY mRNA expression, a change known to decrease bone formation. Further implicating NPY in the skeletal effects of dynorphin, Dyn−/−/NPY−/− double mutant mice showed comparable increases in bone formation to single mutant mice, suggesting that dynorphin acts upstream of NPY signaling to control bone formation. Thus the dynorphin system, acting via NPY, may represent a pathway by which higher processes including stress, reward/addiction and depression influence skeletal metabolism. Moreover, understanding of these unique interactions may enable modulation of the adverse effects of exogenous opioid treatment without directly affecting analgesic responses.

Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.

1. Introduction

The opioid family is complex, consisting of more than 20 peptides derived from three independent genes, proopiomelanocortin (POMC), proenkephalin (PENK), and prodynorphin (PDyn) (Boer and Van Minnen, 1985). These genes produce inactive polypeptide precursors that are activated following tissue-specific processing. In the case of the prodynorphin precursor, processing produces six dynorphins: dynorphin A, dynorphin B, alpha- and beta- neoendorphin, leumorphine and big dynorphin (Han and xie, 1982). Dynorphin is produced almost exclusively in the CNS, including the hypothalamus (Lin et al., 2006). Pharmacologically, dynorphins preferentially bind to the kappa-opioid receptor (KOR), but they also bind with reduced affinity to mu-opioid

* Corresponding author at: Neuroscience Research Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, Sydney, NSW 2010, Australia. Tel.: +61 2 9295 8296; fax: +61 2 9295 8281.

E-mail addresses: p.baldock@garvan.org.au (P.A. Baldock), frank.driessler@web.de (F. Driessler), s.lin@garvan.org.au (S. Lin), i.wong@garvan.org.au (I.P.L. Wong), y.shi@garvan.org.au (Y. Shi), e.yulyaningsih@garvan.org.au (E. Yulyaningsih), l.castillo@garvan.org.au (L. Castillo), s.janmaat@garvan.org.au (S. Janmaat), r.enriquez@garvan.org.au (R.F. Enriquez), a.zengin@garvan.org.au (A. Zengin), briki@esbs.u-strasbg.fr (B.L. Kieffer), schwarzer.christoph@i-med.ac.at (C. Schwarzer), schwarzer.christoph@i-med.ac.at (J.A. Schwarzer), schwarzer.christoph@i-med.ac.at (J.A. Schwarzer), schwarzer.christoph@i-med.ac.at (J.A. Schwarzer), schwarzer.christoph@i-med.ac.at (J.A. Schwarzer), j.eisman@garvan.org.au (J.A. Eisman), a.sainsbury-salis@garvan.org.au (A. Sainsbury), h.herzog@garvan.org.au (H. Herzog).

[1] These authors have contributed equally to this manuscript.

0143-4179/$ - see front matter Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.npep.2012.08.010

receptors (MOR) and delta-opioid receptors (DOR) (Mansour et al., 1995). While opiate analgesia is the result of activation of MOR signaling (Matthes et al., 1996), both dynorphin and KOR expression are elevated by chronic opiate exposure (Wang et al., 1999) indicating that chronic opiate use may involve responses from multiple opioid receptors, including the dynorphin/KOR system.

Opioids are critical agents for pain management but chronic use is associated with adverse side effects including an increased risk for the development of osteoporosis, affecting people of all ages, not just the elderly (Daniell et al., 2006; Shorr et al., 1992). Several mechanisms have been proposed to explain the increase in fracture evident following opiate exposure. In men, opiates may induce androgen deficiency, due to inhibition of gonoadotrophin release from the hypothalamus and subsequent reduction of testosterone synthesis (Daniell et al., 2006). A recent study in people with an average duration of 2.5 years opiate treatment for non-malignant pain found clinically significant hypogonadism in over 25% of men. Of these hypogonadal men, 50% had bone mass in the osteopenic or osteoporotic range (Fortin et al., 2008). However, central opioid effects on gonadal steroid production is not the only factor contributing to bone loss, as the same study found 42% of men had a testosterone level that was in the normal range but were osteopenic or osteoporotic. In women, a large cohort study of elderly individuals using opiates for pain management reported the age-adjusted risk of fracture was elevated for any non-spine (HR 1.76) and hip (HR 2.12) fracture (Ensrud et al., 2003). These associations were still evident following adjustment for potential confounders including dizziness, gait speed, cognitive function and femoral neck bone mineral density (BMD), further indicating a unique opiate effect on bone. These findings are also consistent with a case-control study (Shorr et al., 1992), which reported a 1.6-fold increase in the risk of hip fracture among current users of opioid analgesics, and a prospective cohort study which found a 2-fold risk of hip fracture in users of opioid analgesics (Guo et al., 1998). Indeed, opiate-associated osteoporosis is now sufficiently recognized that routine bone density screening is being proposed for those on chronic opiate treatment for pain (Fortin et al., 2008). Despite this growing concern, the mechanism behind opiate-induced osteoporosis remains poorly defined.

Interestingly, mouse models have shown that loss of dynorphin signaling reduces neuropeptide Y (NPY) expression in the arcuate nucleus of the hypothalamus (Arc) (Sainsbury et al., 2007; Wittmann et al., 2009). Importantly, alterations in hypothalamic NPY, acting through the Arc, have previously been demonstrated to exert powerful effects upon bone mass and osteoblast activity. Arcuate-specific elevation of NPY expression induces marked reduction in osteoblast activity (Baldock et al., 2005) and acted to correct the increased bone mass following germline NPY ablation (Baldock et al., 2009). The relationship between opioid signaling and NPY was clearly demonstrated in feeding studies with NPY-induced feeding being dose-dependently reduced by pretreatment with kappa opioid receptor antagonist or kappa antisense probes (Israel et al., 2005), demonstrating the downstream actions of dynorphin signaling in mediating NPY effects on feeding. A similar interaction between dynorphin and NPY has been demonstrated in other brain regions, with over 50% of NPY-ergic neurons in some hippocampal regions expressing the dynorphin-specific kappa opioid receptor (Racz and halasy, 2002). In light of these findings, we hypothesized that the opiate system, acting through dynorphin, may mediate its effect on bone through a central circuit involving the NPY pathway. To investigate this we generated a series of transgenic mouse models and studied their effects on bone homeostasis in vivo and in vivo.

2. Materials and methods

2.1. Animals

Dynorphin knockout mice (Dyn−/−) and neuropeptide Y knockout mice (NPY−/−) were generated and maintained as previously published (Loacker et al., 2007; Karl et al., 2008). The dynorphin knockout leads to deletion of the entire prodynorphin gene including the initiation start codon, thereby eliminating all six potential dynorphin peptides. Double (Dyn−/−NPY−/−) knockout mice were generated by crossing Dyn and NPY single knockout mice and subsequently crossing of the double heterozygous mice. Bone tissue from KOR−/− mice (Simonin et al., 1998) were kindly provided by Prof. Kieffer, University of Strasbourg. Littermate controls were used where possible. Mice were fed a normal chow diet *ad libitum* (6% calories from fat, 21% calories from protein, 71% calories from carbohydrate, 2.6 kcal/g; Gordon's Specialty Stock Feeds, Yanderra, New South Wales, Australia). All research and animal care procedures were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee and were in agreement with the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose. Mice were housed under conditions of controlled temperature (22 °C) and illumination (12-h light, 12-h dark cycle, lights on at 0700 h).

2.2. Statistical analyses

Values are presented as mean ± standard error (SEM), and statistical comparisons were made using a two-tailed student's *t*-test, or one-way ANOVA and Bonferroni post hoc tests when an overall significant difference was detected. p values <0.05 were considered statistically significant.

2.3. Analysis of body composition

Mice were anesthesised with 100 mg/kg ketamine and 20 mg/kg xylazine (Parke Davis-Pfizer, Sydney, Australia; and Bayer, Leverkusen, Germany) and then scanned for whole body bone mineral density (BMD) and bone mineral content (BMC), lean mass, fat mass and percentage of fat using dual energy X-ray absorptiometry (DXA), (Lunar PIXImus2 mouse densitometer; GE Healthcare, WI). Following cull by cervical dislocation and decapitation, isolated femoral scans were conducted on defleshed femurs.

2.4. Bone histomorphometry

Mice were injected with the fluorophor calcein (Sigma Chemical Company, St. Louis, USA) at 20 mg/kg (s.c.), 10 and 3 days prior to collection. Mice were killed by cervical dislocation between 10.00–14.00 h for tissue collection. Both femora and the lumbar spine were excised and fixed in 4% paraformaldehyde for 16 h at 4 °C. The right femur was bisected transversely at the midpoint of the long axis and the distal half embedded undecalcified in ethacrylate resin (Medim-Medizinische Diagnostik, Giessen, Germany). Sagittal sections with 5 μm thickness were analysed for osteoblastic and osteoclastic parameters, as previously described (Allison et al., 2006; Lundberg et al., 2007a,b.

2.5. Micro-computed tomography

Femurs and third lumbar vertebra (L3) were assessed using micro-computed tomography (micro-CT) as described before (Baldock et al., 2009). Briefly, following fixation, left femora were cleaned of muscle and placed in a Skyscan 1174 (Skyscan, Aartselaar, Belgium). The X-ray source was set at 50 kV and

Download English Version:

https://daneshyari.com/en/article/5904317

Download Persian Version:

https://daneshyari.com/article/5904317

Daneshyari.com