Published in final edited form as:

Brain Res. 2013 December 2; 1540: . doi:10.1016/j.brainres.2013.09.050.

Involvement of spinal cord opioid mechanisms in the acute antinociceptive effect of hyperbaric oxygen in mice¹

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Abstract

Earlier research has demonstrated that treatment with hyperbaric oxygen (HBO₂) can elicit an antinociceptive response in models of acute pain. We have demonstrated that this antinociceptive effect is centrally-mediated and is dependent on opioid receptors. The purpose of the present study was to examine the role of endogenous opioid peptides and opioid receptors specifically in the spinal cord in the acute antinociceptive effect of HBO₂ in mice. Male NIH Swiss mice were exposed to HBO₂ (100% oxygen @ 3.5 atmospheres absolute) for 11 min and their antinociceptive responsiveness was determined using the glacial acetic acid-induced abdominal constriction test. HBO₂-induced antinociception was sensitive to antagonism by intrathecal (i.t.) pretreatment with the κ - and μ -selective opioid antagonists norbinaltorphimine and β -funalrexamine, respectively, but not the δ -selective antagonist naltrindole. The antinociceptive effect of HBO₂ was also significantly attenuated by i.t. pretreatment with a rabbit antiserum against rat dynorphin₁₋₁₃ but not antisera against β -endorphin or methionine-enkephalin. Based on these experimental findings, the acute antinociceptive effect of HBO₂ appears to involve neuronal release of dynorphin and activation of κ and μ opioid receptors in the spinal cord.

Keywords

Hyperbaric oxygen; antinociception; opioid receptors; endogenous opioid peptides; spinal cord; mouse

¹This work was presented in part at Experimental Biology 2013, Boston, MA, April 20-24, 2013.

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1. Introduction

Hyperbaric oxygen (HBO₂) treatment is the use of 100% oxygen at a greater-than-normal atmospheric pressure controlled by a hyperbaric chamber for limited periods of time. The Food and Drug Administration (FDA) has approved HBO₂ therapy for a limited number of clinical indications (Gesell, 2008). However, it has yet to be approved for treating neuropathic pain despite evidence that it can produce relief of chronic pain in experimental animals (Thompson et al., 2010; Li et al., 2011; Gu et al., 2012; Zhang et al., 2012) and human subjects (Lukich et al., 1991; Rui-Chang, 1994; Peach, 1995; Wilson et al., 1998; Kiralp et al., 2004; Yildiz et al., 2004; Handschel et al., 2007; Gu et al., 2012). Based on the results of these studies, the mechanism of action of HBO₂-induced antinociception is not well understood but has been suggested to involve suppression of inflammation (Wilson et al., 2006, 2007).

Previously, we demonstrated that the antinociceptive effect of HBO₂ was sensitive to antagonism by supraspinally-administered opioid receptor blocker naltrexone (Chung et al., 2010) as well as a rabbit antiserum against rat dynorphin (Zelinski et al., 2009). These findings implicate supraspinal opioid mechanisms in the antinociceptive action of HBO₂. The purpose of the present investigation was to examine the role of opioid mechanisms in the spinal cord in HBO₂-induced acute antinociception in mice.

2. Results

An i.p. injection of 0.6% glacial acetic acid typically induced abdominal constrictions that were counted for a 6-min period commencing 5 min after the injection. On average, the control reference group (room air) exhibited 15.6 ± 1.1 abdominal constrictions (N=19). Exposure of mice to HBO₂ @ 3.5 atmospheres absolute (ATA) during that 11-min period evoked a robust antinociceptive effect, causing a significant reduction in the number of abdominal constrictions.

In control animals that were pretreated i.t. with saline vehicle in room air, abdominal constrictions were suppressed by about 30%. Mice treated with norbinaltorphimine (norBNI), naltrindole (NTI) or β -funaltrexamine (β FNA) alone also exhibited a level of antinociception that was comparable to that of the saline-treated control group and were not statistically different from one other.

Fig. 1 shows the effects of i.t.-administered opioid antagonist pretreatment on HBO₂-induced antinociception. Testing of saline-pretreated mice under HBO₂ @ 3.5 ATA resulted in roughly a 50% reduction in the number of abdominal constrictions. The decrease in abdominal constrictions was essentially reversed in mice that were pretreated with norBNI or β FNA prior to the 11-min HBO₂ treatment. Pretreatment with NTI failed to have an appreciable influence on the HBO₂-induced reduction in abdominal constrictions.

I.t. pretreatment of control animals with normal rabbit serum (NRS) alone suppressed abdominal constrictions by about 10%. Pretreatment with rabbit antisera against rat dynorphin₁₋₁₃ (DYN-AS), β -endorphin (β EP-AS) and methionine-enkephalin (ME-AS) alone produced levels of antinociception comparable to the NRS-treated control group. Fig. 2 shows the results of i.t. administration of rabbit antiserum against each rat endogenous opioid peptide. Testing of NRS-pretreated mice under HBO₂ @ 3.5 ATA resulted in a 60% reduction in the number of abdominal constrictions. Pretreatment with DYN-AS reversed the HBO₂-induced inhibitory effect, whereas pretreatment with ME-AS and β EP-AS prior to the 11-min HBO₂ treatment did not reverse the HBO₂-induced decrease in abdominal constrictions.

3. Discussion

In earlier research, the exposure of mice to HBO₂ at 3.5 ATA has been shown to reduce the number of glacial acetic acid-induced abdominal constrictions (Zelinski et al., 2009; Ohgami et al., 2009; Chung et al., 2010; Quock et al., 2011). More recent studies in our laboratory has also demonstrated that HBO₂ treatment can provide pain relief in rats with peripheral nerve injury due to sciatic nerve crush (Gibbons et al., 2012; Gibbons et al., in press) and injections of paclitaxel (Zhang et al., 2012, 2013). These studies were the first to propose that HBO₂ activates a nitric oxide-initiated, opioid-mediated pain-modulating system in the central nervous system.

Intracerebroventricular (i.c.v.) pretreatment with 1.0 µg/mouse of the non-selective nitric oxide synthase (NOS)-inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) or the neuronal-selective NOS inhibitor *S*-methyl-L-thiocitrulline (SMTC) significantly inhibited the early-phase antinociceptive response following a 60-min HBO₂ treatment (Zelinski et al., 2009). The HBO₂-induced antinociception was also antagonized by i.c.v. pretreatment with DYN-AS but not ME-AS or βEP-AS. Subsequently, it was also shown that the immediate acute antinociceptive effect of HBO₂ was also sensitive to antagonism by both i.c.v. as well as intrathecal (i.t.) pretreatment with L-NAME or an antisense oligodeoxynucleotide directed against neuronal NOS (Ohgami et al., 2009). The acute antinociceptive effect of HBO₂ appears to be mediated by a NO–cyclic GMP–protein kinase G (PKG) brain pathway. I.c.v. pretreatment with the PKG-inhibitor Rp-8-(4-chlorophenylthio)-guanosine-3',5'-cyclic monophosphorothioate interfered with the expression of the acute antinociceptive effect of HBO₂ (Quock et al., 2011). Following a 60-min/day, 4-day HBO₂ regimen, the early-phase antinociceptive effect of HBO₂ was attenuated by i.c.v. pretreatment with either L-NAME or naltrexone (Chung et al., 2010). Antagonism of the HBO₂ response by these i.c.v. drug pretreatments strongly indicates that HBO₂ works supraspinally to produce its antinociceptive effect.

In the present study, it can be seen from the results that HBO₂-induced acute antinociception was sensitive to antagonism by i.t. pretreatment with the N opioid antagonist norBNI (Portoghese et al., 1987) or DYN-AS. These findings are both consistent with the hypothesis that HBO₂-induced antinociception in the mouse abdominal constriction test might be mediated by stimulated neuronal release of the endogenous N opioid ligand dynorphin (Chavkin et al., 1982) with subsequent activation of κ opioid receptors in the spinal cord.

Another finding of the present study was that HBO₂-induced acute antinociception was significantly antagonized by i.t. pretreatment with βFNA, which is an irreversible antagonist selective for the µ-opioid receptor at lower concentrations (Liu-Chen et al., 1991). There is evidence that dynorphin is capable of activating both κ-µ-opioid receptor (Young et al., 1983, 1986). This would explain the susceptibility of HBO₂-induced acute antinociception to antagonism by both norBNI and βFNA.

βEP can be localized in the spinal cord (Marvizón et al., 2009) and has been implicated in spinally-mediated antinociception (Crisp et al., 1989). Furthermore, there is evidence that opioid peptides in the thoracic and lumbar spinal cord, including βEP, can be released by microinjection of glutamate into the hypothalamic paraventricular nucleus (PVN) (Yang et al., 2009).

However, βEP-AS failed to antagonize HBO₂-induced antinociception, which seems inconsistent with the ability of βFNA to antagonize HBO₂. The presumption is that βEP activates µ opioid receptors, so one would have expected a similar pattern of interaction with the receptor antagonist and the opioid peptide AS. One possible explanation of this

discrepancy is that the β EP-AS is less potent when compared to DYN-AS and ME-AS. An alternative reason might be that HBO₂ stimulates release of an endogenous μ peptide other than β EP, possibly endomorphin (Ide et al., 2000), which does not react with β EP-AS. There is evidence that asynchronous 2/100 Hz electroacupuncture can stimulate the release of endomorphin and dynorphin in the rat spinal cord to cause antinociception (Wang et al., 2005).

Results from the present study is that neither NTI (Portoghese et al., 1988) nor ME-AS had any effect on HBO₂-induced acute antinociception. This suggests that ME and δ opioid receptors in the spinal cord do not play a role in the acute antinociceptive effect of HBO₂.

A final point worth mentioning in the findings of the present study is the unexpected antinociceptive effect that ensued microinjection of saline and opioid antagonists into the spinal cord. Previous studies have shown i.t. administration of saline or artificial cerebrospinal fluid (aCSF) was capable of producing antinociception in thermal pain tests (Leiphart et al., 2002). This is consistent with clinical observations in which patients reported reduction in pain following i.t. administration of cold saline (Collins et al., 1969) or room-temperature hypertonic saline (King et al., 1972; Squire et al., 1974). Leiphart et al. (2002) have posited that the antinociceptive effect of saline and aCSF may be attributed to minute perturbation of the ion concentrations in the spinal cord microenvironment or microinjection volume dilution of excitatory amino acids responsible for maintenance of the pain state.

While our research findings have consistently implicated a central supraspinal mechanism for HBO₂-induced suppression of pain, it must be acknowledged that others have attributed HBO₂-induced pain relief analgesia to a peripheral anti-inflammatory action (Warren *et al.*, 1979; Sümen *et al.*, 2001; Wilson *et al.*, 2006, 2007). It has been shown that HBO₂ treatment reduced levels of the inflammatory cytokine, tumor necrosis factor- α (TNF- α), supporting the argument for an anti-inflammatory effect of HBO₂ (Yang et al., 2006; Li et al., 2011). We do not preclude the contribution of a peripheral anti-inflammatory action to HBO₂-induced pain relief but suggest that HBO₂ is also capable of activating a supraspinal pathway that modulates neuropathic pain.

4. Experimental procedures

4.1. Animals

Male NIH Swiss mice, weighing 18-22 g, were purchased from Harlan Laboratories (Indianapolis, IN) and used in this study. Experiments were approved by the Washington State University Institutional Animal Care and Use Committee (IACUC) with post-approval review and carried out in accordance with The Guide for the Care and Use of Laboratory Animals, 8th Edition (National Academies Press, Washington, DC, 2010).

Mice were housed five per cage in the Animal Resource Unit at Washington State University with access to food and water ad libitum. The facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), was maintained on a regular 12-h light:dark cycle (lights on 07:00-19:00 h) under standard conditions of temperature ($22 \pm 1^\circ\text{C}$) and humidity (33%). Mice were kept in the holding room for at least three days following arrival in the facility and prior to experimentation.

4.2. Treatment with Hyperbaric Oxygen

Mice were placed in a B-11 research hyperbaric chamber (Reimers Systems, Inc., Lorton, VA). The chamber was ventilated with 100% O₂, U.S.P. (A-L Compressed Gases Inc., Spokane, WA) at a flow rate of 20 L/min to minimize nitrogen and carbon dioxide

accumulation. The pressure within the cylindrical clear acrylic chamber (27.9 cm diameter × 55.9 cm L) was increased from 1.0 to 3.5 ATA over 2 min. The mice breathed spontaneously during HBO₂ treatment. After completion of the HBO₂ treatment, mice were then decompressed over 2-3 min. The 3.5 ATA pressure was determined from determination of the pressure-response relationship between HBO₂ and antinociceptive response (Zylstra et al., 2008).

4.3. Antinociceptive Testing

Antinociceptive responsiveness was assessed using the abdominal constriction test. Mice were treated i.p. with 0.1 mL per 10 g body weight of 0.6% glacial acetic acid and placed into the hyperbaric chamber. Exactly 5 min later, the number of abdominal constrictions—lengthwise stretches of the torso with concave arching of the back—in each animal was counted for 6 min while under HBO₂. The 5 min period was coincident with the increase in ATA. Multiple raters were used for some but not all experiments; at least one of the raters was blinded to the drug treatment. All experiments were consistently conducted between 1300 and 1700 h. The control groups were exposed to room air.

4.4. Drugs

The following drugs were used in this research: medical grade oxygen (from A-L Compressed Gases, Inc. (Spokane, WA); norbinaltorphimine, naltrindole and β -funaltrexamine from Tocris Bioscience (Ellisville, MO); rabbit antisera against dynorphin₁₋₁₃, β -endorphin and methionine-enkephalin from Bachem/Peninsula Laboratories (San Carlos, CA); and normal rabbit serum from Cell Signaling Technology (Danvers, MA). The norBNI, NTI and β FNA were prepared in sterile 0.9% physiological saline solution and administered in intrathecal (i.t.) doses of 10 μ g norBNI, 10 μ g NTI and 2.0 μ g β FNA. Drugs doses were based on previous experiments conducted in our laboratory or taken from the scientific literature. All antisera and NRS were prepared in 0.1 M phosphate-buffered saline and administered in an i.t. dose of 30 μ g.

4.5. Intrathecal Microinjection Procedures

I.t. pretreatments were made using the microinjection technique of Hylden and Wilcox (1980). Briefly, mice were anesthetized with 2% isoflurane in oxygen in an anesthesia chamber or with a nosecone during injection. The mouse was held by the pelvic girdle and the microinjection was made through the skin into the spinal cord. A ½-inch, 30-gauge disposable needle attached to a 10- μ l luer-tipped microsyringe (Hamilton, Reno, NV) was inserted between the lumbar vertebrae below the L6 level (at the start of the cauda equina). A volume of 5.0 μ l of drug solution or vehicle was delivered directly into the spinal cord over 30 sec. Mice typically emerged from anesthesia within several minutes of the microinjection and appeared fully recovered by the time of the HBO₂ treatment and antinociceptive testing. Occasionally, there was a mouse with a transient paralysis of the hindlegs but no fatalities.

4.6. Statistical Analysis of Data

The percent antinociceptive responses of control and experimental groups were compared using a one-way analysis of variance (ANOVA) with a post-hoc Bonferroni's multiple comparison test.

5. Conclusion

I.t. pretreatments with a rabbit antibody against the endogenous κ opioid ligand, κ opioid antagonist and μ opioid antagonist prevented the reduction in glacial acetic acid-induced

abdominal constrictions in HBO₂-treated mice. These findings implicate spinal cord opioid (notably κ and μ) mechanisms in the acute antinociceptive effect of HBO₂.

Acknowledgments

This research was supported by NIH Grant AT-007222 and the Allen I. White Distinguished Professorship at Washington State University and an institutional Summer Undergraduate Research Fellowship (SURF) Award from the American Society for Pharmacology and Experimental Therapeutics (ASPET).

References

- Branda EM, Ramza JT, Cahill FJ, Tseng LF, Quock RM. Role of brain dynorphin in nitrous oxide antinociception in mice. *Pharmacol Biochem Behav.* 2000; 65:217–222. [PubMed: 10672972]
- Cahill FJ, Ellenberger EA, Mueller JL, Tseng LF, Quock RM. Antagonism of nitrous oxide antinociception in mice by intrathecally administered opioid peptide antisera. *J Biomed Sci.* 2000; 7:299–303. [PubMed: 10895052]
- Chavkin C, James IF, Goldstein A. Dynorphin is a specific endogenous ligand of the κ -opiate receptor. *Science.* 1982; 215:413–415. [PubMed: 6120570]
- Chung E, Zielinski LM, Ohgami Y, Shirachi DY, Quock RM. Hyperbaric oxygen treatment induces a two-phase antinociceptive response of unusually long duration in mice. *J. Pain.* 2010; 11:847–853. [PubMed: 20418186]
- Collins JR, Juras EP, Van Houten RJ. Intrathecal cold saline solution: a new approach to pain. *Anesth. Analg.* 1969; 48:813–823.
- Crisp T, Stafinsky JL, Hess JE, Uram M. Spinal beta-endorphin analgesia involves an interaction with local monoaminergic systems. *Eur. J. Pharmacol.* 1989; 160:211–217. [PubMed: 2569405]
- Gesell, LB., editor. *The Hyperbaric Oxygen Therapy Committee Report: Indications and Results.* 12th Ed.. Undersea and Hyperbaric Medical Society; Durham: 2008.
- Gibbons C, Liu S, Sayre CL, Levitch B, Moehlmann S, Zhang Y, Shirachi DY, Quock RM. Possible involvement of opioid receptors in the antiallodynic effect of hyperbaric oxygen (HBO₂) in rats with sciatic nerve crush-induced neuropathic pain. *Soc. Neurosci. Abst.* 2012; 38:575.30.
- Gibbons CR, Liu S, Zhang Y, Sayre CL, Levitch B, Moehlmann S, Shirachi DY, Quock RM. Involvement of brain opioid receptors in the antiallodynic effect of hyperbaric oxygen (HBO₂) in rats with sciatic nerve crush-induced neuropathic pain. *Brain Research.* (in press).
- Gu N, Niu JY, Liu WT, Sun YY, Liu S, Lv Y, Dong HL, Song XJ, Xiong LZ. Hyperbaric oxygen therapy attenuates neuropathic hyperalgesia in rats and idiopathic trigeminal neuralgia in patients. *Eur. J. Pain.* 2012; 16:1094–1105. [PubMed: 22354664]
- Handscheil J, Brüssermann S, Depprich R, Ommerborn M, Naujoks C, Kübler NR, Meyer U. Evaluation of hyperbaric oxygen therapy in treatment of patients with osteomyelitis of the mandible. *Mund. Kiefer Gesichtschir.* 2007; 11:285–290. [PubMed: 17786490]
- Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. *Eur. J. Pharmacol.* 1980; 167:313–316. [PubMed: 6893963]
- Ide S, Sakano K, Seki T, Awamura S, Minami M, Satoh M. Endomorphin-1 discriminates the mu-opioid receptor from the delta- and kappa-opioid receptors by recognizing the difference in multiple regions. *Jpn. J. Pharmacol.* 2000; 83:306–311. [PubMed: 11001176]
- King JS, Jewelt DL, Sundberg HR. Differential blockade of cat dorsal root C fibers by various chloride solutions. *J. Neurosurg.* 1972; 36:569–593. [PubMed: 5067506]
- Kiralp MZ, Yildiz S, Vural D, Keskin I, Ay H, Dursun H. Effectiveness of hyperbaric oxygen therapy in the treatment of complex regional pain syndrome. *J. Int. Med. Res.* 2004; 32:258–262. [PubMed: 15174218]
- Leiphart JW, Dills CV, Levy RM. The analgesic effects of intrathecally pumped saline and artificial cerebrospinal fluid in a rat model of neuropathic pain. *Neuromodulation.* 2002; 5:214–220. [PubMed: 22150849]

- Li F, Fang L, Huang S, Yang Z, Nandi J, Thomas S, Chen C, Camporesi E. Hyperbaric oxygenation therapy alleviates chronic constrictive injury-induced neuropathic pain and reduces tumor necrosis factor- α production. *Anesth. Analg.* 2011; 113:626–633. [PubMed: 21596875]
- Liu-Chen LY, Li SX, Lewis ME. Autoradiographic study of irreversible binding of [^3H] β -funaltrexamine to opioid receptors in the rat forebrain: comparison with μ and δ receptor distribution. *Brain Res.* 1991; 544:235–242. [PubMed: 1645609]
- Lukich VL, Poliakova LV, Sotnikova TI, Belokrinskii DV. Hyperbaric oxygenation in the comprehensive therapy of patients with rheumatoid arthritis (clinico-immunologic study). *Fiziol. Zh.* 1991; 37:55–60. [PubMed: 1838726]
- Marvizon JC, Chen W, Murphy N. Enkephalins, dynorphins, and beta-endorphin in the rat dorsal horn: an immunofluorescence colocalization study. *J. Comp. Neurol.* 2009; 517:51–68. [PubMed: 19711397]
- Ohgami Y, Zylstra CC, Quock LP, Chung E, Shirachi DY, Quock RM. Nitric oxide in hyperbaric oxygen-induced acute antinociception in mice. *NeuroReport.* 2009; 20:1325–1329. [PubMed: 19730405]
- Peach G. Hyperbaric oxygen and the reflex sympathetic dystrophy syndrome: a case report. *Undersea Hyperb. Med.* 1995; 22:407–408. [PubMed: 8574129]
- Portoghese PS, Lipkowski AW, Takemori AE. Binaltorphimine and norbinaltorphimine, potent and selective kappa-opioid receptor antagonists. *Life Sci.* 1987; 40:1287–1292. [PubMed: 2882399]
- Portoghese PS, Sultana M, Takemori AE. Naltrindole, a highly selective and potent non-peptide delta opioid receptor antagonist. *Eur. J. Pharmacol.* 1988; 146:185–186. [PubMed: 2832195]
- Quock LP, Zhang Y, Chung E, Ohgami Y, Shirachi DY, Quock RM. The acute antinociceptive effect of HBO_2 is mediated by a NO-cyclic GMP-PKG- K_{ATP} channel pathway in mice. *Brain Res.* 2011; 1368:102–107. [PubMed: 20977894]
- Quock RM, Best JA, Chen DC, Vaughn LK, Portoghese PS, Takemori AE. Mediation of nitrous oxide analgesia in mice by spinal and supraspinal N-opioid receptors. *Eur J Pharmacol.* 1990; 175:97–100. corrigendum 187, 564. [PubMed: 2157601]
- Rui-Chang, Y. Proceedings of the XI International Congress of Hyperbaric Medicine. Best Publishing; San Pedro, CA: 1994. Rheumatoid arthritis treated with HBO..
- Squire AW, Carvillo O, Bromage PR. Painless intrathecal hypertonic saline. *Can Anaesth. Soc. J.* 1974; 21:308–314. [PubMed: 4406724]
- Sümen G, Çim it M, Ero lu L. Hyperbaric oxygen treatment reduces carrageenan-induced acute inflammation in rats. *Eur. J. Pharmacol.* 2001; 431:265–268. [PubMed: 11728435]
- Thompson CD, Uhelski ML, Wilson JR, Fuchs PN. Hyperbaric oxy-gen treatment decreases pain in two nerve injury models. *Neurosci. Res.* 2010; 66:279–283. [PubMed: 19951724]
- Wang Y, Zhang Y, Wang W, Cao Y, Han JS. Effects of synchronous or asynchronous electroacupuncture stimulation with low versus high frequency on spinal opioid release and tail flick nociception. *Exp. Neurol.* 2005; 192:156–162. [PubMed: 15698629]
- Warren J, Sacksteder MR, Thuning CA. Therapeutic effect of prolonged hyperbaric oxygen in adjuvant arthritis of the rat. *Arthritis Rheum.* 1979; 22:334–339. [PubMed: 426880]
- Wilson HD, Toepfer VE, Senapati AK, Wilson JR, Fuchs PN. Hyperbaric oxygen treatment is comparable to acetylsalicylic acid treatment in an animal model of arthritis. *J. Pain.* 2007; 8:924–930. [PubMed: 17690013]
- Wilson HD, Wilson JR, Fuchs PN. Hyperbaric oxygen treatment de-creases inflammation and mechanical hypersensitivity in an animal model of inflammatory pain. *Brain Res.* 2006; 1098:126–128. [PubMed: 16750177]
- Wilson JR, Foresman BH, Gamber RG, Wright T. Hyperbaric oxygen in the treatment of migraine with aura. *Headache.* 1998; 38:112–115. [PubMed: 9529766]
- Yang J, Yang Y, Chu J, Wang G, Xu H, Liu WY, Wang CH, Lin BC. Endogenous opiate peptides in the spinal cord are involved in the analgesia of hypothalamic paraventricular nucleus in the rat. *Peptides.* 2009; 30:740–744. [PubMed: 19452637]
- Yang Z, Nandi J, Wang J, Bosco G, Gregory M, Chung C, Xie Y, Yang X, Camporesi EM. Hyperbaric oxygenation ameliorates indomethacin-induced enteropathy in rats by modulating TNF- α and IL-1 β production. *Dig. Dis. Sci.* 2006; 51:1426–1433. [PubMed: 16838118]

- Yildiz S, Kiralp MZ, Akin A, Keskin I, Ay H, Dursun H, Çim it M. A new treatment modality for fibromyalgia syndrome: hyperbaric oxygen therapy. *J. Int. Med. Res.* 2004; 32:263–267. [PubMed: 15174219]
- Young EA, Walker JM, Lewis ME, Houghten RA, Woods JH, Akil H. [³H]dynorphin A binding and kappa selectivity of prodynorphin peptides in rat, guinea-pig and monkey brain. *Eur. J. Pharmacol.* 1986; 121:355–365. [PubMed: 2870933]
- Young E, Walker JM, Houghten R, Akil H. [³H] dynorphin binding to guinea pig and rat brain. *Life Sci.* 1983; 33(Suppl. 1):287–290. [PubMed: 6141490]
- Zelinski LM, Ohgami Y, Chung E, Shirachi DY, Quock RM. A prolonged NO-dependent, opioid-mediated antinociceptive effect of hyperbaric oxygen in mice. *J. Pain.* 2009; 10:167–172. [PubMed: 18976963]
- Zhang, Y.; Brewer, AL.; Shirachi, DY.; Quock, RM. Hyperbaric oxygen (HBO₂) antinociception in chronic pain is blocked by neuronal nitric oxide synthase (nNOS) inhibitor.. 4th International Congress on Neuropathic Pain; Toronto, ON, Canada. May 23-26, 2013; 2013.
- Zhang Y, Gibbons CR, Howlader S, Son MC, Yeon J, Shirachi DY, Quock RM. Suppression of paclitaxel-induced neuropathic pain by hyperbaric oxygen. *FASEB J.* 2012; 26:662.9.136.
- Zylstra CC, Ohgami Y, Chung E, Shirachi DY, Quock RM. Comparison of the antinociceptive effect of two pharmacological gases, nitrous oxide (N₂O) and hyperbaric oxygen (HBO₂). *FASEB J.* 2008; 22:711.16.

Highlights

- HBO₂ produces acute antinociception in the mouse abdominal constriction test.
- HBO₂ antinociception is antagonized by norbinaltorphimine and β -funaltrexamine.
- HBO₂ antinociception is antagonized by rabbit antiserum against rat dynorphin.
- HBO₂ antinociception involves activation of opioid mechanisms in the spinal cord.

Fig. 1.

Influence of opioid antagonists on the antinociceptive effect of HBO₂ in the glacial acetic acid-induced abdominal constriction test. Abbreviations: SAL, saline; norBNI, norbinaltorphimine (10 μg); NTI, naltrindole (10 μg); FNA, -funaltrexamine (2.0 μg); RA, room air; HBO₂, hyperbaric oxygen (3.5 ATA). Each column represents the mean number (#) of abdominal constrictions ± S.E.M. of at least 10 mice per group. Significance of difference: ****, $P < 0.0001$, and n.s., not significant, compared to the corresponding drug-pretreated, RA-exposed group.

Fig. 2.

Influence of opioid peptide antisera on the antinociceptive effect of HBO₂ in the glacial acetic acid-induced abdominal constriction test. Abbreviations: NRS, normal rabbit serum; DYN-AS, dynorphin₁₋₁₃ antiserum (30 μg); ME-AS, methionine-enkephalin antiserum (30 μg); βEP-AS, β-endorphin antiserum (30 μg); RA, room air; HBO₂, hyperbaric oxygen (3.5 ATA). Each column represents the mean number (#) of abdominal constrictions ± S.E.M. of at least 10 mice per group. Significance of difference: ****, $P < 0.0001$, and n.s., not significant, compared to the corresponding antiserum-pretreated, RA-exposed group.