American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 16, No 12 (December), 2015: pp 1321-1333
Available online at www.jpain.org and www.sciencedirect.com

Differences in the Antinociceptive Effects and Binding Properties of Propranolol and Bupranolol Enantiomers

Loren J. Martin,[*,†,‡] Marjo H. Piltonen,[†,§] Josee Gauthier,[¶] Marino Convertino,[‖] Erinn L. Acland,[*,†,‡] Nikolay V. Dokholyan,[‖] Jeffrey S. Mogil,[*,†] Luda Diatchenko,[†,§,¶] and William Maixner[¶]

**Department of Psychology, †Alan Edwards Centre for Research on Pain, and §Department of Anesthesiology, McGill University, Montreal, Quebec, Canada.*
‡Department of Psychology, University of Toronto Mississauga, Mississauga, Ontario, Canada.
¶Center for Pain Research and Innovation and ‖Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, North Carolina.

Abstract: **Recent efforts have suggested that the β-adrenergic receptor (β-AR) system may be a novel and viable therapeutic target for pain reduction; however, most of the work to date has focused on the β_2-adrenergic receptor (AR). Here, we compared the antinociceptive effects of enantiomeric configurations of propranolol and bupranolol, two structurally similar nonselective β-blocking drugs, against mouse models of inflammatory and chronic pain. In addition, we calculated in silico docking and measured the binding properties of propranolol and bupranolol for all 3 β-ARs. Of the agents examined, S-bupranolol is superior in terms of its antinociceptive effect and exhibited fewer side effects than propranolol or its associated enantiomers. In contrast to propranolol, S-bupranolol exhibited negligible β-AR intrinsic agonist activity and displayed a full competitive antagonist profile at $\beta_1/\beta_2/\beta_3$-ARs, producing a unique blockade of β_3-ARs. We have shown that S-bupranolol is an effective antinociceptive agent in mice without negative side effects. The distinctive profile of S-bupranolol is most likely mediated by its negligible β-AR intrinsic agonist activity and unique blockade of β_3-AR. These findings suggest that S-bupranolol instead of propranolol may represent a new and effective treatment for a variety of painful conditions.**

***Perspective:** The S enantiomer of bupranolol, a β-receptor antagonist, shows greater antinociceptive efficacy and a superior preclinical safety profile and it should be considered as a unique β-adrenergic receptor compound to advance future clinical pain studies.*

Key words: *Pain, propranolol, bupranolol, β-adrenergic receptors, antinociception.*

Current treatment options for chronic pain display limited efficacy and are associated with problematic side effects. Thus, novel treatment strategies and therapeutic targets are needed to address a profound unmet medical need that affects the lives of hundreds of millions worldwide.[43] Within recent years, the β-adrenergic receptor (β-AR) system has emerged as a promising therapeutic target for drug development and pain management. The endogenous ligands for β-ARs are catecholamines such as epinephrine ($\beta_2 > \beta_1 \ggg \beta_3$) and norepinephrine ($\beta_3 \approx \beta_1 > \beta_2$),[24,60] with the functional roles of β-ARs best characterized as affecting cardiovascular, airway, uterine, and metabolic functions. However, β-ARs are also densely distributed in the nervous system, making them prime targets for altering memory, mood, and pain (see Molinoff[44] for a review). Among the β-AR subtypes, β_2-ARs are believed

Received May 25, 2015; Revised August 7, 2015; Accepted September 21, 2015.
This research was supported by the Louise and Alan Edwards Foundation (J.S.M.). L.J.M. was supported by postdoctoral fellowships from the Canadian Institutes of Health Research (CIHR) and the Canadian Pain Society. J.S.M. is a consultant and equity stock holder, and L.D. and W.M. are co-founders and equity stock holders in Algynomics, Inc, a company providing research services in personalized pain medication and diagnostics.
L.J.M. and J.S.M. designed the behavioral studies; M.C. and N.V.D. designed the in silico modeling simulations; L.D. and W.M. designed the in vitro cellular assays. L.J.M., J.G., M.C., and E.L.A. collected and analyzed data. L.J.M., M.H.P., and W.M. wrote the article. All authors read, approved, and commented on the article.
Supplementary data accompanying this article are available online at www.jpain.org and www.sciencedirect.com.
Address reprint requests to Loren J. Martin, PhD, Department of Psychology, University of Tornoto Mississauga, 3359 Mississauga Rd., CCT Bldg. Room 4061, Mississauga, ON L5L 1C6. E-mail: lj.martin@utoronto.ca
1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2015.09.004

to be the most relevant for pain. β_2-ARs are 1) located on peripheral nociceptors,[1] 2) located in discrete spinal cord regions that directly participate in nociceptive transmission,[46] and 3) essential for the antiallodynic action of antidepressant drugs.[9] Furthermore, stimulation of β_2-ARs on peripheral afferents sensitizes nociceptors[1] and enhances pain signaling through the release of proinflammatory cytokines from the central nervous system[27,28] as well as from peripherally located adipocytes and macrophages.[58,60]

Evidence from human studies indicates that sequence variation in the gene encoding for the β_2-AR (*ADRB2*) is associated with individual differences in the susceptibility to several chronic pain conditions. For instance, we have previously shown[15] that haplotypic variants within the *ADRB2* gene locus are associated with the development of temporomandibular joint disorder (TMD), a chronic musculoskeletal pain condition. Genetic variations in *ADRB2* have also shown associations with chronic neck pain,[55] irritable bowel syndrome,[36] and fibromyalgia.[61]

Clinically, propranolol, a lipophilic nonselective β-AR antagonist (ie, β-blocker), has shown promise with respect to pain management, especially in the treatment of migraine headaches,[52] fibromyalgia,[38] and temporomandibular disorders.[59] Despite the promising clinical usefulness of β-blockers, including propranolol, there is still a paucity of experimental research demonstrating the effectiveness of β-blockers in pain reduction. Even although propranolol is the prototypic β-blocker used for clinical pain management of migraine,[40] many adverse effects are associated with this drug, including drowsiness, fatigue, depression, and cognitive changes (see Freitag[21] for a review). Propranolol is typically administered as a racemic mixture to treat hypertension and normalize tachycardia responses.[19] However, there is emerging evidence that for propranolol and bupranolol, a structurally similar β-blocker, the S-enantiomers of both compounds show greater cardiosympatholytic activity.[3,39,56]

Here, we investigated whether enantiomers of 2 nonselective β-blocking drugs, propranolol and bupranolol, were effective in multiple algesiometric assays in mice. In addition, we explored at the cellular and structural level whether racemic mixtures and optically pure enantiomers of propranolol and bupranolol produced β_1-, β_2-, or β_3-AR blockade in a manner that matches the effects on both sensory and motoric behaviors in mice.

Methods

Mice

All behavioral experiments were performed on naive, adult (6–12 weeks of age), CD-1 (ICR:Crl) mice of both sexes, bred in house from breeders obtained from Charles River (Boucherville, Quebec, Canada). All mice were housed with their same-sex littermates (2–4 animals per cage) in standard shoebox cages, maintained in a temperature-controlled (20°C ± 1°C) environment (14/10 hour light/dark cycle), where they had access to food (Harlan Teklad 8604) and water ad libitum. All animal experiments were approved by McGill University and were in accordance with the Canadian Council on Animal Care and with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting experiments involving animals.[33,41]

Drugs

The HCl salts of racemic bupranolol, S-bupranolol, and R-bupranolol were provided by Algynomics Inc (Chapel Hill, NC), a company specializing in personalized pain medicine, of which 3 authors [J.S.M., L.D., and W.M.] are either equity stock holders or cofounders (as stated in the disclosure). Fig 1 shows the chemical structures of propranolol and bupranolol and indicates the position of their stereocenters. Racemic propranolol and the R-enantiomers and S-enantiomers of propranolol were purchased from Sigma Aldrich (St. Louis, MO) and dissolved in saline.

Behavioral Assays

All mice were habituated to the testing environment for at least 20 minutes before testing commenced. In all experiments, mice were assigned randomly to drug and dose, and experimenters were blinded to drug and dose. Sample sizes in all pain assays were n = 6–12 mice/dose/drug.

Rota-Rod Test

Drug effects on motor coordination were tested using an accelerating Rota-Rod treadmill (Acceler Rota-Rod 7650; UgoBasile, Gemonio, Varese, Italy) for mice.[29] Mice were placed on the Rota-Rod, which accelerated from 4 to 40 revolutions/min over a period of 5 minutes, and the time spent on the rotating drum was recorded for each mouse. On the test day, 1 drug-free baseline trial was performed and then the mice were treated with drugs and retested 3 times at 20-minute intervals. Performance was quantified by calculating the percentage of maximal ataxia 60 minutes after drug administration compared with the baseline scores: ((Baseline – Post-drug/Baseline) × 100).

Formalin Test

Mice were injected with drugs (see later discussion) and then allowed to habituate for 20 minutes within Plexiglas cylinders (30 cm high, 15 cm diameter) placed

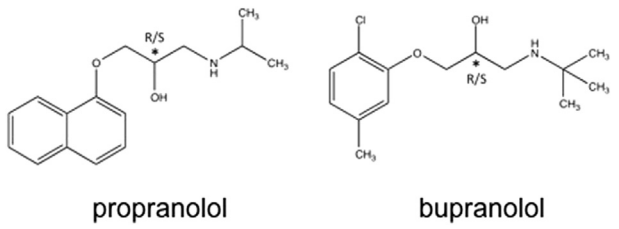

Figure 1. Chemical structures of R/S propanolol (left) and bupranolol (right). The position of the stereocenter is indicated by the star.