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

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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 β2-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 β2/β2-ARs, producing a unique blockade of β2-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 β2-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.
Evidence from human studies indicates that sequence variation in the gene encoding for the β2-AR (ADRB2) is associated with individual differences in the susceptibility to several chronic pain conditions. For instance, we have previously shown that haplotype variants within the ADRB2 gene locus are associated with the development of temporomandibular joint disorder (TMJD), a chronic musculoskeletal pain condition. Genetic variations in ADRB2 have also shown associations with chronic neck pain, irritable bowel syndrome, fibromyalgia, and mandibular disorders. Despite the promising clinical use of propranolol in the treatment of migraine,40 many studies have also shown associations with chronic pain, although propranolol is the prototypic β-blocker used for clinical pain management of migraine,50 many adverse effects are associated with this drug, including drowsiness, fatigue, depression, and cognitive changes (see Freitag for a review). Propranolol is typically administered as a racemic mixture to treat hypertension and acute pain conditions.61 However, there is emerging evidence that for propranolol and bupranolol, a structurally similar β-blocker, the S-enantiomers of both compounds show greater cardioselectivity and 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 β1-AR, β2-AR, or β3-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 and provided with food (Harlan Teklad 8604) and water ad libitum. All animals were housed under controlled lighting (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.

**Differences in Propranolol and Bupranolol Enantiomers**

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 (Accelor Rota-Rod 7650; UgoBasile, Gemonio, Varese, Italy) for mice. 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...
on a glass tabletop. Then, 20 μL of 5% formalin was injected subcutaneously into the plantar surface of the left hind paw using a 100-μL microsyringe with a 30-gauge needle. Mice were then returned to the cylinders and videotaped undisturbed for 60 minutes. Videos were later coded offline by a blinded observer, and the first 10 seconds of every minute was sampled for the presence of licking/biting (positive sample) of the left hind paw. The early phase was defined as the percentage of positive samples during the first 0 to 10 minutes after injection of formalin; the late phase was defined as the percentage of positive samples during the period 10 to 60 minutes after injection.

**Radiant Heat Paw Withdrawal Test**

Mice were placed on a glass floor within small Plexiglas cubicles as described earlier, and a focused high-intensity projector lamp beam was directed from below onto the midplantar surface of the hind paw.25 The commercial device (IITC Model 336; Harvard Apparatus, Holliston, MA) was set to 20% active intensity. Latency to withdraw from the stimulus was measured to the nearest 1 second. Baseline measurements consisted of testing both hind paws twice on 3 separate occasions separated by at least 30 minutes. After drug administration, both hind paws were tested only once at the indicated time point.

**von Frey Test**

The up-down method of Dixon was used.6 Mice were placed on a perforated metal floor (with 5-mm diameter holes placed 7 mm apart) within small Plexiglas cubicles (9 × 5 × 5 cm high), and a set of 8 calibrated von Frey fibers (Stoelting Touch Test Sensory Evaluator Kit 2 to 9; Stoelting Co, Wood Dale, IL; ranging from ≈0.015 g to ≈1.3 g of force) were applied to the plantar surface of the hind paw until the fibers bowed and then held for 3 seconds. The threshold force required to elicit withdrawal of the paw (median 50% withdrawal) was determined twice on each hind paw (and averaged) for all baseline measurements. (except for the spared nerve injury [SNI] experiments), with sequential measurements separated by at least 20 minutes. After drug administration, 1 measurement per hind paw was taken at the indicated time point.

**Carrageenan Hypersensitivity**

Carrageenan (2%; 20 mg/mL; Sigma, Oakville, ON, Canada) was suspended by sonication in saline and injected subcutaneously in a volume of 20 μL into the left plantar hind paw using a 100-μL microsyringe with a 30-gauge needle. Mice were tested for thermal and mechanical sensitivity of both hind paws using the radiant heat paw withdrawal and von Frey tests, respectively, before and 3 hours after carrageenan injection. All drugs were injected immediately after the postcarrageenan (3 hour) test, and postdrug measurements were taken 20, 40, and 60 minutes later.

**SNI**

SNI, an experimental nerve injury designed to produce neuropathic pain, was performed under isoflurane/oxygen anesthesia, as described previously.14 Mice were tested for mechanical sensitivity before and after surgery using the von Frey test, except that the spared sural region was targeted by applying the fibers to the lateral aspect of the hind paw. Drug administration immediately followed baseline measurements for SNI-induced mechanical allodynia 7 days postoperatively, and postdrug measurements were taken at 20, 40, and 60 minutes.

**In Silico Docking Calculations**

The structure of the engineered β2-AR at 2.8 Å resolution (PDB: 3NYB)6,62 and the structural models of β1-AR and β3-AR subtypes were used for the docking calculations. The homology models of β1-AR and β3-AR were derived from the template structure of β2-AR at 3.2 Å resolution49 using I-TASSER.51,69 The raw models obtained were optimized with Chiron46 (http://troll.med.unc.edu/chiron/index.php), a software for protein structure refinement developed in the Dokholyan Laboratory. The quality of the 3 final protein structures was comparable with high-resolution crystal structures, as assessed by Gaia35 (http://troll.med.unc.edu/chiron/index.php). Docking calculations were performed using MedusaDock17,18 a tool developed in house that simultaneously models the flexibility of both ligand and protein. For each system analyzed, we retrieved a reliable binding pose of the investigated molecules (Fig 1) by combining the values of the MedusaScore,67 a physical force field–based scoring function accounting for the protein-ligand interaction energy, with a hierarchical cluster analysis of the top-ranked ligand conformations. Specifically, in each single β-AR subtype, we performed 200 independent docking calculations for every compound and collected their top-scored conformations. We clustered the ensemble of docking solutions according to the root-mean-square deviation (RMSD) computed over the heavy atoms of the ligand. We identified the optimal number of highly populated clusters by applying the average linkage method and the Kelley penalty index31 to minimize the number of clusters and the spread of internal values in each cluster. The clustering level with the lowest Kelley penalty represents a condition in which the clusters are highly populated and concurrently maintain the smallest internal spread of RMSD values. The centroid of the most populated cluster was chosen as the representative conformation of the ligand bound to the protein. This procedure demonstrates high reliability because it reproduces the binding pose of the original cocrystallized molecule in β2-AR with an RMSD computed over the heavy atoms of the ligand of 1.4 Å (data not shown).

**Cell Culture and Transfection**

HEK293T (human embryonic kidney cells expressing large T antigen of SV40) cells were transfected with an expression vector containing β1-AR, β2-AR, or β3-ARs, using a calcium phosphate transfection protocol or Lipofectamine 2000 (Invitrogen, Waltham, MA). Cells were cotransfected with GloSensor-22F vector (GloSensor cAMP Assay kit;
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Differences in Propranolol and Bupranolol Enantiomers at various concentrations of isoproterenol or antagonists alone for 10 minutes. Luminescence was measured after 10 minutes of agonist treatment using the Victor3 multilabel reader (Perkin Elmer).

Statistical Analyses
A criterion level of \( \alpha = .05 \) was adopted for all experiments. Behavioral data were analyzed by analysis of variance followed by Dunnett and Tukey post hoc analyses where appropriate. Half-maximal antinociceptive doses (AD50s) and ataxia ED50s were calculated using the method of Tallarida and Murray as implemented by FlashCalc 40.1 software (M. Ossipov, University of Arizona).

Results

Differential Sedation Caused by Propranolol and Bupranolol Isomers

To identify the highest nonsedating dose of \( \beta \)-AR antagonists that could be used in our behavioral assessment of pain inhibition; the mouse Rota-Rod assay was used. We assessed the latency to fall from the Rota-Rod after administration of racemic propranolol, racemic bupranolol, and their respective enantiomers. We found that some of the \( \beta \)-AR antagonists caused sedation and ataxia, but only when high doses were administered (Fig 2A). Compared with saline-treated mice, racemic propranolol and both of its enantiomers impaired Rota-Rod performance (ie, time to ataxia) at 60 mg/kg, whereas none of the bupranolol compounds produced ataxia at this dose. Bupranolol and R-bupranolol produced ataxia at very high doses (>90 mg/kg); S-bupranolol produced no ataxia at any dose up to and including 120 mg/kg. S-Propranolol significantly impaired Rota-Rod latencies with doses as low as 30 mg/kg. ED50s and associated confidence intervals for maximal ataxia obtained 60 minutes after drug administration are shown in Table 1. In some cases, doses of R-bupranolol (90 and 120 mg/kg), racemic propranolol (60 mg/kg), and R-propranolol (30 and 60 mg/kg) resulted in mortality before Rota-Rod testing (Supplementary Table 1). Lethality after S-bupranolol administration was not observed, even when high doses (120 mg/kg) of S-bupranolol were used.

Formalin-Induced Pain Is Inhibited by the S-Enantiomers of Bupranolol and Propranolol

We next examined whether different enantiomers of propranolol and bupranolol were antinociceptive in the formalin test of tonic/inflammatory pain. Robust inhibition of formalin-induced licking was observed at varying doses of propranolol, bupranolol, and their enantiomers, R-propranolol, S-pupranol, R-bupranol, and S-bupranolol (Figs 2B and 2C). All drugs except R-bupranolol dose-dependently inhibited formalin-induced licking, with S-bupranolol showing the greatest effect in both the early and late phases. AD50s and associated
Figure 2. The antinociceptive effects of propranolol and bupranolol enantiomers. (A) The sedation or ataxia produced by racemic or enantiomeric versions of the nonselective β-AR antagonists propranolol or bupranolol (propranolol [PRO], F3,14 = 4.0, P < .05; R-propranolol [R-PRO], F3,15 = 7.1, P < .01; S-propranolol [S-PRO], F3,15 = 14.5, P < .001; bupranolol [BUP], F3,14 = 15.5, P < .001; R-bupranolol [R-BUP], F2,7 = 4.8, P < .05). The only antagonist that was found not to be sedating, even at a very high dose, was S-bupranolol (S-bupranolol, F3,13 = .92, nonsignificant [n.s.]). Bars represent the percentage of maximal ataxia when measured against baseline ± standard error of the mean. n.t. = no trial. (B) Antagonists reduce the amount of licking in the formalin test during the early phase (0–10 minutes) but only when high (and in most cases, sedating) doses are used (propranolol, F4,39 = 7.2, P < .001; R-propranolol, F4,33 = 2.2, n.s.; S-propranolol, F4,33 = 6.9, P < .001; bupranolol, F4,46 = 3.5, P < .001; R-bupranolol, F4,43 = 2.0, n.s.; S-bupranolol, F4,50 = 9.6, P < .001). (C) Antagonists reduce the amount of licking during the late phase (10–60 minutes) of the formalin test (propranolol, F4,39 = 10.2, P < .001; R-propranolol, F4,33 = 2.2, n.s.; S-propranolol, F4,33 = 10.1, P < .001; bupranolol, F4,46 = 6.1, P < .001; R-bupranolol, F4,43 = 3.0, n.s.; S-bupranolol, F4,50 = 9.8, P < .001). S-Bupranolol was equal to, if not better than, racemic and S-propranolol in blocking formalin-induced nociception. Sample sizes in all groups for Rota-Rod testing range from n = 5 or 6 and for formalin testing range from n = 8–10 mice/dose/drug. *P < .05, **P < .01, ***P < .001 compared with vehicle (VEH); all P-values corrected for multiple comparisons.
confidence intervals for maximal analgesia observed in the early and late phases are shown in Table 1.

S-Bupranolol Produces Antiallodynic Effects in Inflammatory and Chronic Neuropathic Pain Assays

Because S-bupranolol was the most effective compound against formalin-induced pain without affecting sedation or causing mortality, we tested its antinociceptive properties in other pain assays. Mice injected with a high dose (60 mg/kg) of S-bupranolol displayed increased thermal thresholds to noxious radiant heat test at 20 minutes after injection, but no effect was observed on mechanical thresholds (Figs 3A and 3B). S-Bupranolol (60 mg/kg) produced a complete and sustained (>60 minute) reversal of both thermal and mechanical hypersensitivity produced by the inflammatory agent 3-carrageenan; lower doses produced a transient reversal of both thermal and mechanical hypersensitivity (Figs 3C and 3D). Mechanical allodynia after SNI was also reversed by a high dose (60 mg/kg) of S-bupranolol, but not after a lower dose (30 mg/kg) (Fig 3E).

In Silico Docking

The β2-AR binding site has a narrow cleft of nonpolar residues and few polar amino acids (Fig 4A), and has similar structural features as those identified for both β1-ARs and β3-ARs. The representative binding conformations for propranolol and bupranolol enantiomers for the β2-ARs exhibit similar binding modes as shown for several β2-AR ligands. The β-hydroxyethylamine moiety establishes a network of hydrogen bonds with D133, N312, and Y316, and the aromatic portion of the molecule is engaged in nonpolar interactions with F193, F289, F290, V114, V117, N312, and N363 (Supplementary Figs 2A and 2B). Moreover, in the binding site of β2-AR, S-bupranolol presents a flipped orientation of the aromatic ring with respect to the R-enantiomer that does not affect the hydrophobic interactions with F309. Thus, there are subtle differences in the docking of R-enantiomers and S-enantiomers within β-AR binding sites.

**Table 1.** Half-Maximal Doses to Achieve Ataxia and Analgesia (AD50s; 95% Confidence Intervals in Parentheses) of Propranolol, R-Propranolol, S-Propranolol, Bupranolol, R-Bupranolol, and S-Bupranolol

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ATAXIA</th>
<th>EARLY PHASE</th>
<th>LATE PHASE</th>
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<tr>
<td>Propranolol</td>
<td>4.8</td>
<td>45.0</td>
<td>62.0</td>
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<tr>
<td></td>
<td>(1.8–12.7)</td>
<td>(25.8–78.0)</td>
<td>(15.7–242.0)</td>
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<tr>
<td>R-Propranolol</td>
<td>13.3</td>
<td>&gt;1000</td>
<td>121.0</td>
</tr>
<tr>
<td></td>
<td>(9.8–18.3)</td>
<td></td>
<td>(39–380)</td>
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<tr>
<td>S-Propranolol</td>
<td>22.2</td>
<td>35.0</td>
<td>25.5</td>
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<tr>
<td></td>
<td>(18.3–29.4)</td>
<td>(18.3–67)</td>
<td>(7.8–83.0)</td>
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<tr>
<td>Bupranolol</td>
<td>88.0</td>
<td>&gt;1000</td>
<td>99.0</td>
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<td></td>
<td>(74–105)</td>
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<td>(46–212)</td>
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<tr>
<td>R-Bupranolol</td>
<td>74.0</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(62–87)</td>
<td></td>
<td>&gt;1000</td>
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<tr>
<td>S-Bupranolol</td>
<td>166.0</td>
<td>51.0</td>
<td>26.6</td>
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<td></td>
<td>(93–294)</td>
<td>(30.2–85.0)</td>
<td>(14.1–50)</td>
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NOTE. Mice were tested in the Rota-Rod test to assess ataxia and the formalin assay to quantify analgesia induced by drug treatment (based on changes from saline-treated means). All doses are in mg/kg.

Inhibition of Isoproterenol-Induced CAMP Production

The inhibition of isoproterenol-evoked CAMP production by racemic propranolol, R-bupranolol, or S-bupranolol was examined for all 3 β-ARs. In these studies, we first verified the agonistic effects of isoproterenol on all 3 β-ARs and determined the concentrations giving half-maximal responses (ie, EC50 values) (Fig 5A). These values were generally very low for all 3 receptors, within the picomolar to nanomolar range (see Supplementary Table 3A).

Next, dose-response curves were determined for the antagonists in the presence of isoproterenol, namely racemic propranolol, R-bupranolol, or S-bupranolol. We used a specific concentration close to the EC50 value of isoproterenol for the stimulation of each receptor type: 0.22 nM for β1-AR, 15 pM for β2-AR, and 2.2 nM for β3-AR. All 3 compounds behaved as antagonists for β1-AR (Fig 5B and Supplementary Table 3B). Comparison of the concentrations that produced a 50% inhibition (ie, IC50 values) showed that racemic propranolol and S-bupranolol are the most potent of the test agents. However, closer inspection of the unnormalized data values revealed that the maximum antagonistic effect
of racemic propranolol is about 80% of that of S-bupranolol (see Supplementary Fig 3A). For β2-AR, the IC50 values (half-maximal inhibitory concentrations) of all antagonists were similar (Fig 5C and Supplementary Table 3C), ranging from 6.7 nM for S-bupranolol to 29 nM for R-bupranolol. For β3-AR, all compounds showed antagonistic properties; however, S-bupranolol gave nearly a full sigmoidal inhibition curve for β3-AR (Fig 5D, Supplementary Fig 3C and Supplementary Table 3D) but did not fully block the formation of cAMP even at the highest concentration tested. Partial (~20%) inhibition was observed for propranolol. Very high concentrations of R-bupranolol were required to produce a steep (ie, within 1 log unit) inhibition of cAMP production, suggesting a possible nonselective inhibition. Therefore, the β3-AR-associated IC50 values obtained for R-bupranolol and racemic propranolol may not be reliable. Also, the data point at 10 μM for racemic propranolol was discarded, because it seemed to increase cAMP production (Supplementary Fig 3C), providing additional evidence for a nonspecific effect of propranolol at β3-ARs.

pA2 Values of Antagonists

Next, to determine the relative potencies and receptor selectivity of the β-AR antagonists, Schild plots were generated (Fig 6A, Supplementary Figure 4-6 and
Supplementary Table 4) to obtain $pA_2$ values. According to the $pA_2$ plots, racemic propranolol and S-bupranolol have similar effects on $\beta_1$-ARs and are more potent than R-bupranolol (Fig 6A). All of the compounds have $pA_2$ slopes equivalent to $-1$ (ie, 95% confidence intervals overlap with $-1$), suggesting that the antagonism is competitive, selective, and does not show partial agonist or intrinsic sympathomimetic activity (ISA) for all compounds tested. This can be concluded from the lack of cAMP signal at the beginning of the curve, when the cells are under the effect of the antagonists, and the added isoproterenol (10$^{-14}$ M final concentration) does not trigger significant cAMP production. The lack of ISA at $\beta_1$-ARs was also evident by examining individual dose-response curves in the assay in which cells were treated with antagonists alone (Supplementary Figs 7A and 7B).

However, for $\beta_2$-ARs, reliable Schild analyses were not obtained. Although the $pA_2$ values of the antagonists are almost equal, slopes differ significantly from $-1$ (except for R-bupranolol; Fig 6B). A $pA_2$ slope that deviates significantly from $-1$ indicates that the antagonism is not competitive, that the test antagonist is acting at more than 1 receptor, or that some other factor (eg, a partial agonist) is obscuring the effect. It is also evident from the Schild plots and dose-response curves of antagonists alone (Supplementary Figs 5, 7C and 7D) that all compounds show partial agonistic effects on $\beta_2$-ARs.

The presence of ISA activity and the differences in the $pA_2$ slopes suggest that racemic propranolol and the R-enantiomers and S-enantiomers of bupranolol are able to stimulate $\beta_2$-ARs receptors at high concentrations, with propranolol showing the greatest ISA at $\beta_2$-ARs.

Similar to $\beta_1$-AR, racemic propranolol and S-bupranolol were the most potent antagonists for $\beta_3$-ARs according to their $pA_2$ values (Fig 6C). The slopes of the $pA_2$ plots were equivalent to $-1$ (eg, within the 95% confidence intervals) for racemic propranolol and the enantiomers of bupranolol. However, as with $\beta_2$-AR, comparing the $pA_2$ values may not be the most suitable way to rank the antagonists, because they have different properties: R-bupranolol showed weak antagonism for $\beta_3$-ARs, and propranolol displayed mixed agonist-antagonist characteristics (Supplementary Figs 7E and 7F). S-Bupranolol was the only compound that can be reliably classified as a competitive antagonist, with potent antagonistic effects on $\beta_3$-AR and with a negligible ISA effect.

**Discussion**

In the present study, we demonstrated that enantiomers of 2 nonselective $\beta$-AR antagonists, bupranolol and propranolol, display different antinociceptive efficacies in mice and have different affinities and properties for $\beta$-AR subtypes. In the formalin test, the S-enantiomers were substantially more potent than the R-enantiomers.
In contrast to R-bupranolol, S-bupranolol was equally as effective in blocking nociceptive behaviors as propranolol and S-propranolol in the late phase of the formalin test. However, S-propranolol and propranolol were found to be more sedative (or ataxia producing) on the Rota-Rod, and likely confounded the licking behavior scored in the formalin test. Thus, it is difficult to determine whether formalin-evoked behaviors decreased because of antinociceptive effectiveness or sedation.

The antinociceptive effects of S-bupranolol are generalizable to other pain assays, as we also demonstrated efficacy in tests of inflammatory and neuropathic pain. S-Bupranolol was not effective against acute thermal (except transiently at a high dose) or mechanical pain. This is consistent with previously published experimental pain work showing that intravenous propranolol has a minimal effect on decreasing heat pain in healthy men. In the clinic, analgesia is not typically observed in patients treated with propranolol for hypertension at a dosage that typically ranges from 40 to 80 mg per day. However, pain resulting from angina in cardiac patients is significantly reduced with higher propranolol doses, and nonselective β-blockers either reduce pain sensitivity to noxious stimuli or pain scores in clinical studies. To a certain extent, our data are analogous to findings seen in the clinical setting such that β-AR blockade is most effective in alleviating hypersensitivity without raising experimentally assessed pain thresholds. This is important in light of recent research demonstrating that blocking β-ARs reduces

Figure 5. Dose-response curves (DRC) of isoproterenol (iso) and the β-AR antagonists used. (A) Dose-response curves of isoproterenol for each β-AR. ADR = adrenergic receptor. (B–D) Inhibition dose-response curves of antagonists at EC50 of isoproterenol for β1-AR (B; n = 9), β2-AR (C; n = 11), and β3-AR (D; n = 7), respectively.

Figure 6. pA2 plots for the antagonists of β1-AR (A; n = 6), β2-AR (B; n = 9), and β3-AR (C; n = 9). Dose-response curves for isoproterenol were determined in the presence of various concentrations of antagonists, and the EC50 values obtained (A) were compared with the EC50 values without antagonist (A). Log((A0/A)−1) was plotted against the negative logarithm of antagonist concentration (−log [antagonist]). The x-intercept determines the estimated pA2 value, assuming also that −1 is within the 95% confidence interval of the slope. Higher pA2 values indicate higher affinity of the antagonist. Refer to Supplementary Table 3 for a more detailed summary of the findings.
pain associated with a variety of conditions, including chronic TMD,59 irritable bowel syndrome,60 and peripheral nerve damage.62 The hypersensitivity resulting from tissue or nerve injury leads to a hyperadrenergic state67 and it is possible that the antinociceptive properties of β-AR blockade are revealed only when adrenergic activity is increased. In this regard, β-AR blockade may act to downregulate β-AR17 or reduce epinephrine-induced sensitization of sensory terminals.32 Of course, this is not the only explanation because hypersensitivity resulting from the sensitization of P2X2/3 receptors is also reversed by β-AR blockade.63

Although propranolol and bupranolol are sympatholytic drugs, we do not believe that these drugs are important only for sympathetically maintained pain syndromes such as complex regional pain syndrome. The R-enantiomers of both compounds were associated with greater side effects and lethality. Relative to propranolol, both the R-enantiomers and S-enantiomers of bupranolol produced less sedation/ataxia, with the R-enantiomer of bupranolol producing greater Rota-Rod deficits than the corresponding S-enantiomer. These findings demonstrate that S-bupranolol is superior to the other enantiomers tested based on both antinociceptive response and side effects, and we believe that β-AR blockade with bupranolol will provide an efficient analgesic strategy for a wide range of pain disorders.

Furthermore, the cellular assays revealed different pharmacodynamic properties of propranolol, bupranolol, and associated enantiomers toward β1/β2/β3-ARs. We found that R-bupranolol is a relatively potent β2-AR antagonist and it has weak or essentially little antagonist properties at β1/β3-ARs, respectively, and weak antinociceptive properties. This is striking considering that β2-ARs are considered to be important for the analgesia mediated by β-AR blockade.15 In contrast, S-bupranolol and S-propranolol displayed the strongest antinociceptive properties in the formalin assay and showed high protein-ligand interactions for the representative binding poses for β1/β2/β3-ARs. At the cellular level, the inhibitory effects of S-bupranolol were similar to propranolol at blocking β1/β2/β3-ARs. Our data along with previously published data suggest that β1-ARs and β3-ARs may be just as important, if not more important than β2-ARs in producing antinociception in mice. In rats, esmolol, a selective β1-AR blocker, can suppress nociception induced by formalin12 and is effective as an adjunct for perioperative pain management in human patients by reducing postoperative opioid requirement.12 Increased catecholamine levels resulting from reduced enzymatic activity of catechol-O-methyltransferase increase pain sensitivity,16 which can be blocked by antagonists of β2-ARs and β3-ARs. Furthermore, the stimulation of β3-ARs on dorsal root ganglion neurons evokes the release of the pronociceptive molecule ATP after peripheral nerve injury.30 The current data do not indicate whether β2-ARs are exhibiting their effects via peripheral or central mechanisms; however, these receptors are expressed at very low levels in the central nervous system.30 Stimulation of peripheral β2/β3-ARs produces hyperalgesia and the activation of β-AR in the spinal cord produces analgesia (see Segall et al54 for a review), supporting a bias toward a peripheral site of action for β2-antagonists. However, we cannot exclude a central site of action, which is consistent with the anxiolytic effects of β-blocking drugs.64 When coupled with our finding that S-bupranolol is a full β2-AR antagonist, its superior effects in producing antinociception are consistent with earlier findings that combined treatment with selective β2/β3-ARs produces greater antinociception than blocking β2/β2-AR separately.45

Although bupranolol and propranolol are clearly nonselective β-AR antagonists, they are not believed to exhibit ISA.66 In contrast to previous findings, we observed that propranolol increased cAMP production, indicative of clear ISA at all β-AR receptor subtypes reaching up to 15%, 37%, and 45% of responses to isoproterenol at β1/β2/β3-ARs, respectively. In contrast to propranolol, the R-enantiomers and S-enantiomers of bupranolol produced weak ISA at β1/β2-ARs (S-enantiomer>R-enantiomer) and exhibited very weak or no β3-AR ISA. S-Bupranolol was the only compound examined that can be classified as a relatively potent, nonselective competitive antagonist without notable ISA for β3-ARS. Thus, compared with propranolol, the receptor mechanisms mediating the antinociceptive effects of S-bupranolol are less confounded because of the weak or no ISA exhibited at β1/β2/β3-ARs. ISA is typically regarded as a beneficial feature of β-blockers because it minimizes the bradycardia found in elderly patients,22 and patients tend to tolerate β-blockers with ISA better than those without.10 However, our results suggest that the absence of ISA activity (particularly at β2-ARs) by S-bupranolol may be an important feature for antinociception without significant side effects.

In silico docking calculations were performed to explore the binding properties of propranolol and bupranolol enantiomers with respect to the 3 β-AR subtypes. The binding mode of propranolol and bupranolol enantiomers discloses minimal structural differences, especially in the interaction with N3.39 or D3.32 residues of β1/β2/β3-AR. The estimated binding energies of the top-ranked protein-ligand conformations indicate that all stereoisomers can effectively bind the 3 receptors. Although characterized by a flipped orientation, the aromatic moiety of S-bupranolol is engaged in hydrophobic interactions with the same protein residues (ie, F341, V139) as all the other molecules. Consequently, the estimated energy of binding for S-bupranolol (ie, −38.4 ± .6 kcal/mol) is not significantly different from R-bupranolol (ie, −37.8 ± .1 kcal/mol) or propranolol enantiomers (ie, −38.7 ± .1 and −39.2 ± .6 for S-enantiomers and R-enantiomers, respectively). These data suggest that all the stereoisomers under investigation can equally bind the targeted receptor, confirming the common limitation of current docking scoring functions in quantifying fine differences in the affinity of potent ligands to a given target.15 These findings also suggest that the differences in the potencies observed for the different enantiomers in the cellular and behavioral assays do not result from differences in ligand binding affinities for a given β-AR.
receptor but instead result from different capacities to engage downstream signaling pathways. Binding assays alone are not able to differentiate the relative potencies of these agents at β-AR and emphasize the importance of conducting in vitro cellular and in vivo assessments.

These findings suggest that a nonselective antagonist of β1/β2/β3-ARs (which expresses little ISA, produces little ataxia, and expresses a high therapeutic index) would be more effective as a strategy to produce analgesia compared with blocking individual β-ARs. S-Bupranolol fits this unique profile compared with racemic propranolol, R-propranolol, S-propranolol, and R-bupranolol.

References


S-Bupranolol is effective in producing antinociceptive behaviors across several algesiometric assays in mice and in blocking β1/β2/β3-β1-ARs without producing significant sedation or lethality. S-Bupranolol has a superior preclinical safety profile and shows greater antinociceptive efficacy compared with the other test agents and should be considered as a unique β-AR compound to advance future clinical pain studies.

Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpain.2015.09.004.


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