Pharmacological Evaluation of Selective $\alpha_{2c}$-Adrenergic Agonists in Experimental Animal Models of Nasal Congestion

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ABSTRACT

Nasal congestion is one of the most troublesome symptoms of many upper airways diseases. We characterized the effect of selective $\alpha_{2c}$-adrenergic agonists in animal models of nasal congestion. In porcine mucosa tissue, compound A and compound B contracted nasal veins with only modest effects on arteries. In vivo experiments, we examined the nasal decongestant dose-response characteristics, pharmacokinetic/pharmacodynamic relationship, duration of action, potential development of tolerance, and topical efficacy of $\alpha_{2c}$-adrenergic agonists. Acoustic rhinometry was used to determine nasal cavity dimensions following intranasal compound 48/80 (1%, 75 μl). In feline experiments, compound 48/80 decreased nasal cavity volume and minimum cross-sectional areas by 77% and 40%, respectively. Oral administration of compound A (0.1–3.0 mg/kg), compound B (0.3–5.0 mg/kg), and $d$-pseudoephedrine (0.3 and 1.0 mg/kg) produced dose-dependent decongestion. Unlike $d$-pseudoephedrine, compounds A and B did not alter systolic blood pressure. The plasma exposure of compound A to produce a robust decongestion (EC80) was 500 nM, which related well to the duration of action of approximately 4.0 hours. No tolerance to the decongestant effect of compound A (1.0 mg/kg p.o.) was observed. To study the topical efficacies of compounds A and B, the drugs were given topically 30 minutes after compound 48/80 (a therapeutic paradigm) where both agents reversed nasal congestion. Finally, nasal-decongestive activity was confirmed in the dog. We demonstrate that $\alpha_{2c}$-adrenergic agonists behave as nasal decongestants without cardiovascular actions in animal models of upper airway congestion.

Introduction

Inflammatory diseases impacting and contributing to nasal cavity pathology continue to be prevalent in the general population. For example, allergic rhinitis is one of the most common global health issues in general medical practices, affecting upwards of 40% of the world population (Cobanoğlu et al., 2013; Izquierdo-Domínguez et al., 2013). The disease is characterized by Th2-mediated inflammation with several salient symptoms, including nasal and ocular pruritus, sneezing, rhinorrhea, and upper airway congestion (Uzzaman and Story, 2012). The most troublesome symptom reported by allergic rhinitis patients is nasal congestion (Nathan, 2008; Meltzer et al., 2009). In addition to being a breathing annoyance, nasal congestion is positively linked to disturbances in sleep and decreased work and school performance and productivity (Craig et al., 1998; Corey et al., 2000; Meltzer et al., 2009; Sardana and Craig, 2011). In humans and many mammalian species, basal nasal patency is governed by autonomic nervous system regulation of a highly complex network of resistance (arteries) and compliance (veins) blood vessels underlying the nasal mucosa (Widdicombe, 1986; Lung and Wang, 1989). Congestion occurs in part as a consequence of vasodilation of venous sinusoids that then become distended with blood, producing swelling and expansion of the mucosa into the nasal cavity (Corey et al., 2000; Wang and Lung, 2003). Central to this autonomic governance of nasal patency are $\alpha$-adrenergic receptors. Both postjunctional $\alpha_1$- and $\alpha_2$-adrenergic receptor subtypes are found prevalently distributed on capacitance and resistance blood vessels, where they mediate vascular constrictive responses (Andersson and Bende, 1984; Wang and Lung, 2003). Thus, it is not surprising that $\alpha$-adrenergic sympathomimetics, (e.g., oxymetazoline, phenylephrine, and $d$-pseudoephedrine) have found utility as nasal decongestants, because they directly or indirectly (i.e., through norepinephrine release from adrenergic nerve terminals) stimulate postjunctional $\alpha$-adrenergic receptors located on blood vessels of the nasal mucosa; this stimulation decreases blood flow through the mucosa, shrinks nasal erectile tissue, and improves cavity patency (Andersson and Bende, 1984). Although topical and oral $\alpha$-adrenergic sympathomimetics are effective nasal decongestants, they

ABBREVIATIONS: BHT-920, 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine dihydrochloride; JP 1302, acrindic-9-y1(4-(4-methylpiperazin-1-y1)phenyl)amine; PK, pharmacokinetics, PD, pharmacodynamics.
sometimes precipitate mechanism-based side effects that include restlessness, nervousness, insomnia, and hyperton-
sion. Moreover, topical agents (e.g., phenylephrine, oxymetazo-
line, naphazoline) can produce a condition of rebound nasal
congestion or medicamentosus (Corey et al., 2000; Nathan,
2008). Given the limitations of nonselective α-adrenergic sympto-
mimetics, there are unmet needs for the development
of novel decongestants, especially agents without the
systemic or central liabilities of currently available α-adrenergic
sympathomimetics.

In situ mRNA studies by Stafford-Smith et al., (2007) in
human nasal turbinates suggested that the α2c-receptor
subtype is the only α2 receptor localized to nasal vasculature,
specifically showing a high degree of expression in the
sinusoids and arteriovenous anastomoses. Consequently, se-
lectively targeting and stimulating α2c receptors may provide
a novel approach for the treatment of nasal decongestion
(Stafford-Smith et al., 2007; Corboz et al., 2011). As previously
described, nonselective α2 agonists can elicit constriction of
nasal vessels (Corboz et al., 2007). More importantly, non-
selective α2 activation has been demonstrated to have
significant impact on functional nasal responses and physi-
ology (i.e., nasal blood flows, nasal cavity pressures, and cross-
sectional areas) in man and animals (Andersson and Bende,
1984; Berridge and Roach, 1986; McLeod et al., 2001; Wang
and Lung, 2003). Unfortunately, nonselective α2 agonists,
particularly if they cross the blood-brain barrier and enter the
central nervous system, will likely precipitate unwanted side
effects. Clonidine, quanafacine, and guanabenz are prototypic
examples of centrally acting nonselective α2 agonists,
which produce centrally mediated hypotension and bradycardia
(Struthers and Dollery, 1985; Edwards et al., 2012). Additional
adverse effects associated with these drugs include sedation,
dry mouth, impaired alertness, and erectile dysfunction
(Edwards et al., 2012). The recent development and in vitro
pharmacological characterization of selective α2c agonists have
for the first time allowed the examination of the proposal that
these drugs can elicit nasal decongestion independently of
hypertensive or hypotensive actions in preclinical models.

The chemical structures and in vitro pharmacological
profiles for compound A (Corboz et al., 2011) and compound
B (Corboz et al., 2013) have been reported. In brief, both
compound A and compound B are potent α2c-adrenoceptor
agonists. The human binding affinity constant (Kᵢ) values for
compounds A and B are 12 and 18 nM, respectively. Both
drugs display greater than 100× selectivity over α2a and α2b
receptors, with Kᵢ activities on α1 adrenoceptors of ~10 nM.
In the current study, we evaluated the direct effects of these
two α2c-adrenergic agonists on porcine nasal mucosal blood
vessels in an ex vivo model. However, the main goal of the
current studies was to characterize the nasal decongestant
effect of compounds A and B in in vivo experimental models of
upper airway congestion.

Materials and Methods

Animal Care and Use

These studies were performed in accordance with the National
Institutes of Health Guide to the Care and Use of Laboratory Animals
and the Animal Welfare Act in association for the Assessment and
Accreditation of Laboratory Animal Care Program.

Differential Contractility Measurement in Arteries and Veins
in Porcine Nasal Mucosa Explants

The nasal mucosa explant technique was used to evaluate real-time
differential vessel constriction of arteries and veins in nasal mucosa
as described previously (Lieber et al., 2010). In brief, pig snouts from
male and female domestic pigs (110–230 kg) were provided by a local
abattoir, Animal Parts (Scotch Plains, NJ). Nasal mucosa was
removed from turbinates and cut into strips (0.5 × 1.5 cm). Mucosa
strips were fixed in 6% low-melt agarose in Krebs buffer at 37°C in
a 3-ml syringe and cooled on ice until the agarose became solid. The
fixed tissues were cut into 200–300-μm thick slices in Krebs buffer at
4°C using a Krumdieck Tissue Slicer (Alabama Research and
Development, Munford, AL). Tissue slices, free of agarose, were then
incubated in tissue culture dishes with Clonetics SmGM-2 culture
medium (BioWhittaker, Walkersville, MD) in the presence of 1%
penicillin/streptomycin (BioWhittaker) at 37°C in humidified air
containing 5% CO₂. The next day, nasal mucosa slices were
equilibrated for 15 minutes at 37°C in Krebs buffer before recording.
Images of nasal mucosa slices were recorded using the Zeiss Axiosvert
100 microscope (Carl Zeiss MicroImaging, Thornwood, NY) before and
20 minutes after the addition of each concentration of compounds
(0.01–100 μM) at 37°C. The cross-sectional area of vein or artery
lumen was measured using computer software NIH ImageJ. Vessel
constriction was expressed as percentage of vessel cross-sectional
area decrease from baseline in response to test compounds.

Acoustic Rhinometry and Blood Pressure Measurements in
the Anesthetized Cat

The methods used to evaluate nasal cavity patency in the cat have
been described previously (McLeod et al., 1999b,b). In brief, for feline
nasal decongestant studies male Harlan Sprague Dawley short-haired
cats (1.5–3.0 kg; Harlan, Madison, WI) were used. Methohexital sodium
(5 mg/kg i.v.) was used to anesthetize the animals while supplemental
doses of methohexital sodium (0.5–1 mg/kg i.v.) were given if required to
maintain an appropriate depth of anesthesia. We used an acoustic
rhinometer (NADAR, Aarhus, Denmark) to determine nasal cavity
volume and minimum cross-sectional area before and after nasal
provocation with compound 48/80, a mast cell mediator liberator. The
equipment consisted of a spark sound generator, a wave tube, a
microphone with an amplifier, and a computer for data acquisition.
To evaluate changes in nasal architecture, the spark generator was
triggered to produce an acoustic wave that was propagated from the
sound generator through the wave tube and into the nasal cavity.
Reflected acoustic waves from the left and right nasal cavities were
amplified and recorded, and the data obtained were converted to area-
distance curves. The acoustic rhinometer was calibrated to measure
a distance of 0–3 cm into the nasal cavity. The sampling frequency was
100 kHz. We also measured systolic blood pressure from the hind leg
using an ultrasonic Doppler flow detector (model S81-B; Park Medical
Electronics Inc., Aloha, OR). Heart rate was measured with a standard
pulse oximeter. After anesthetic recovery animals were returned to their
home cages.

Pharmacological Studies Conducted in a Feline
Experimental Model of Nasal Congestion

In all feline experiments, topical compound 48/80 (1%, 75 μl) was
given into the right naris to elicit nasal congestion. The left naris was
administered saline. Compounds A and B were profiled in a variety of
experimental protocols aimed at examining the drugs’ nasal de-
congestant dose-response characteristics, pharmacokinetic (PK)/
pharmacodynamics (PD) relationship, duration of action, potential
development of tolerance, and efficacy, by topical route of adminis-
tration. Finally, the decongestant effect of compound A was studied in
the presence of a selective α2c-adrenergic antagonist in this species.

Oral Dose Response Characteristics and PK Relationship in
the Cat. Oral doses of compound A (0.1–3.0 mg/kg), compound B

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(0.3–5 mg/kg), or d-pseudoephedrine (0.3–1.0 mg/kg) was given in a gelatin capsule (size #1; Torpac Inc., Fairfield, NJ) 1 hour before nasal provocation with compound 48/80 to the right nares. Acoustic measurements were performed immediately before compound 48/80 was given (baseline) and 60 minutes after baseline. Consequently, the timeframe for these efficacy measurements was 2 hours after oral treatment. PK samples were examined for drug plasma concentrations at 90 and 120 minutes after oral treatment (compound A). Oral Duration of Action. We examined the duration of action of compound A (1 mg/kg p.o.) by varying the time between administration and measurement of nasal efficacy (note that the time between compound 48/80 challenge and efficacy assessment remained the same as for the oral dose response studies: 60 minutes). The duration studies specifically studied the nasal decongestant effects of compound A at 1.5, 2.0, 3.5, 4.0, 5.5, and 6.0 hours after treatment. A terminal PK sample was collected at the end of each experiment for analysis of compound A concentrations in plasma. Compound A was measured by liquid chromatography-mass spectrometry/mass spectrometry as previously described (Corboz et al., 2011). PK/PD modeling was conducted using the Phoenix WinNonlin 5.3 program from Pharsight (Cary, NC).

**Decongestant Tolerance.** We conducted studies to determine whether the nasal decongestant effects of compound A (1.0 mg/kg p.o.) were diminished after a 5-day once-daily dosing paradigm (i.e., subacute dosing paradigm). In these experiments, compound A was given each day at 8:00 AM. On the 5th day the drug was administered 1 hour before the compound 48/80 challenge. Acoustic measurements were taken 60 minutes after administration of compound 48/80. Results from the subacute dosing paradigm were compared with the decongestant efficacy of compound A (1.0 mg/kg p.o.) given only once. We also investigated whether the decongestant efficacy of compound A (1.0 mg/kg p.o.) was attenuated when dosed 6 hours apart in a single day.

**Effect of Topical and Therapeutically Administered Compound A and Compound B.** Topical compound A (0.03–0.3%, 50 μl), compound B (0.1–1.0%, 50 μl), or phenylephrine (0.03–0.3%, 50 μl) was administered 30 minutes after nasal challenge with compound 48/80. Acoustic measurements were performed immediately before compound 48/80 was given (baseline) and 90 minutes after baseline.

**Effect of Compound A in the Absence and Presence of a Selective α2c-Adrenergic Antagonist.** JP 1302 [α-acridin-9-yl]-4-[4-methylpiperazin-1-yl]phenyl][amine] is a competitive selective α2c-antagonist (Sallinen et al., 2007; Tricklebank, 2007). We used this tool to shed light on the α-adrenergic subtype responsible for the decongestant actions of compound A. Topical compound A (3%, 50 μl), JP 1302 alone (0.1%, 50 μl), control (physiologic saline, 50 μl), or JP 1302 (0.1%, 50 μl) plus compound A (3%, 50 μl) was given 30 minutes before nasal provocation with compound 48/80 to the right nares. Acoustic measurements were performed immediately before compound 48/80 was given and at 60 minutes after baseline. The dose of JP 1302 was selected based on historical experience (Mingo et al., 2010). JP 1302 has been shown to affect nasal cavity volume changes after intranasal challenge with compound 48/80 (3%, 500 μl). Dogs were trained daily for several months to reliably accept the nosepiece of the acoustic rhinometer to the naris. Animals were gradually acclimated to the procedure with positive reinforcement (dog treats) offered in response to the desired behavior (Koss et al., 2002). Compound A (1.0–5.0 mg/kg p.o.), compound B (1.0–5.0 mg/kg p.o.), or control was administered 1 hour before compound 48/80. The generated data were expressed as the percent change from baseline nasal cavity volume values.

**Drugs.** Compound 48/80 and d-pseudoephedrine were purchased from Sigma-Aldrich (St. Louis MO). Phenylephrine hydrochloride was purchased from Research Biochemicals International (Natick, MA). Drug doses refer to their respective free bases. All drugs were dissolved in physiologic saline (0.9%) or delivered in a gelatin capsule.

**Acoustic Rhinometry in the Dog.** The decongestant activities of compounds A and B were also evaluated in conscious, adult male, purpose-bred beagle dogs (C & C Kennels, Wewoka, OK) weighing 9–11 kg. Similar to the feline studies, acoustic rhinometry was used to estimate nasal cavity volume changes after intranasal challenge with compound 48/80 (3%, 500 μl). Dogs were trained daily for several months to reliably accept the nosepiece of the acoustic rhinometer to the naris. Animals were gradually acclimated to the procedure with positive reinforcement (dog treats) offered in response to the desired behavior (Koss et al., 2002). Compound A (1.0–5.0 mg/kg p.o.), compound B (1.0–5.0 mg/kg p.o.), or control was administered 1 hour before compound 48/80. The generated data were expressed as the percent change from baseline nasal cavity volume values.

**Fig. 1.** Differential vessel constriction in porcine nasal mucosa. The effects of compound A and compound B on capacitance vessels (veins) and resistance vessels (arteries) were evaluated independently in porcine nasal mucosa explants. The compounds were added cumulatively and vessel constriction was recorded after each concentration. The α1α2-adrenoceptor agonist norepinephrine (NE) was added at the end of experiments for the maximal constriction in both arteries and veins. (A) Representative images of compound A–induced differential constriction in arteries and veins. (B) Summary results of compound A on nasal mucosa vessel constriction. (C) Summary results of compound B on nasal mucosa vessel constriction. Results are mean ± S.E.M.; n = 11–16 in vein recording and n = 6 in artery recording. EC50 of compound A on vein constriction is 210 nM; EC50 of compound B on vein constriction is 21 nM.
The selective α2-adrenergic agonist compounds A and B were synthesized by Merck Research Laboratories.

Statistics

The cat nasal cavity volume data were expressed as the ratio of the volume of left treated nares versus the right untreated nares (McLeod et al., 1999a,b). Values displayed in the tables and figures represent the mean ± S.E.M. of five to eight animals per group. Data were evaluated using a Kruskal-Wallis test in conjunction with a Mann–Whitney U test. Statistical significance was set at \( P < 0.05 \).

Results

Differential Contractility in Arteries and Veins in Porcine Nasal Mucosa. Nasal congestion is induced mainly by the dilation of capacitance vessels, which leads to engorgement of the nasal mucosa. The effects of compound A and compound B on capacitance vessels (veins) and resistance vessels (arteries) were evaluated independently in porcine nasal mucosa explants (Fig. 1). Compound A (10 nM–0.1 mM) induced vessel constrictions in a dose-dependent manner in nasal mucosa, with a greater effect on veins than arteries. Likewise, compound B induced concentration-dependent constriction in veins, with minimal effect on arteries (Fig. 1). These results indicate that compound A and compound B preferentially contract capacitance vessels in porcine nasal mucosa.

Oral Dose Response Characteristics, PK/PD Relationship, Duration of Action, and Decongestant Tolerance in the Cat. Baseline right/left nasal cavity volume ratios for all treatment groups ranged from 0.99 ± 0.05 to 1.10 ± 0.09, whereas baseline minimum cross-sectional areas ranged between 0.037 ± 0.002 and 0.042 ± 0.003 cm². These values were not different from baseline values of controls. Figure 2 shows that 60 minutes after topical application, compound 48/80 significantly decreased nasal volume ratios to 0.23 ± 0.03, representing a 77% decrease in cavity volume. Compound A (0.3–3.0 mg/kg p.o.) produced a dose-dependent attenuation of the nasal effects of compound 48/80 both on cavity volume and minimum cross-sectional area within the nose (Fig. 2; Table 1). The minimum dose of compound A required to produce a significant nasal decongestant effect was 0.3 mg/kg. At doses up to 3.0 mg/kg, the drug had no effect on systolic blood pressure. A similar dose-related decongestant effect was observed with compound B (Fig. 2; Table 1). As a positive comparator, d-pseudoephedrine (0.3 and 1.0 mg/kg p.o.), also inhibited the nasal effects of compound 48/80 compared with prospective controls (Fig. 2). However, in

![Compound A](image1)

![Compound B](image2)

![d-pseudoephedrine](image3)

**Fig. 2.** Nasal decongestant effect of orally administered compound A, compound B, and d-pseudoephedrine in the anesthetized cat. The figure displays the dose-dependent actions of compound A, compound B, and d-pseudoephedrine on nasal cavity volume ratio 60 minutes after nasal exposure to compound 48/80 (1%, 75 μl). All test drugs were administered 1 hour before compound 48/80 challenge. Each bar represents the mean ± S.E.M. of five or six animals. Mean heart rate values (beats per min) are in parentheses. \*\(P < 0.05\) compared with time 0; \**P < 0.05\) compared with compound 48/80 alone.
Nasal Decongestive Effect of α2c-Adrenergic Agonists

Effect of oral selective α2c-adrenergic agonists on heart rate in the cat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate (60 Minutes Postcompound 48/80)</th>
<th>bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>213 ± 12</td>
<td></td>
</tr>
<tr>
<td>Compound A</td>
<td>210 ± 6</td>
<td></td>
</tr>
<tr>
<td>0.1 %</td>
<td>208 ± 11</td>
<td></td>
</tr>
<tr>
<td>0.3 %</td>
<td>207 ± 12</td>
<td></td>
</tr>
<tr>
<td>1.0 %</td>
<td>211 ± 19</td>
<td></td>
</tr>
<tr>
<td>Compound B</td>
<td>219 ± 12</td>
<td></td>
</tr>
<tr>
<td>0.3 %</td>
<td>219 ± 12</td>
<td></td>
</tr>
<tr>
<td>1.0 %</td>
<td>203 ± 12</td>
<td></td>
</tr>
<tr>
<td>3.0 %</td>
<td>211 ± 8</td>
<td></td>
</tr>
<tr>
<td>5.0 %</td>
<td>214 ± 12</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>214 ± 12</td>
<td></td>
</tr>
<tr>
<td>d-Pseudoephedrine</td>
<td>214 ± 12</td>
<td></td>
</tr>
<tr>
<td>0.3 %</td>
<td>189 ± 16</td>
<td></td>
</tr>
<tr>
<td>1.0 %</td>
<td>189 ± 16</td>
<td></td>
</tr>
</tbody>
</table>

bpm, beats per minute.

Effect of topical selective α2c-adrenergic agonists on heart rate in the cat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate (60 Minutes Postcompound 48/80)</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>204 ± 11</td>
<td></td>
</tr>
<tr>
<td>Compound A</td>
<td>210 ± 7</td>
<td></td>
</tr>
<tr>
<td>0.03 mg/kg</td>
<td>202 ± 13</td>
<td></td>
</tr>
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<td>0.1 mg/kg</td>
<td>207 ± 10</td>
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<td>Compound B</td>
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<tr>
<td>0.3 mg/kg</td>
<td>212 ± 10</td>
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<td>1.0 mg/kg</td>
<td>205 ± 15</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>212 ± 10</td>
<td></td>
</tr>
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<td>0.03 mg/kg</td>
<td>205 ± 13</td>
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<td>0.3 mg/kg</td>
<td>200 ± 18</td>
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bpm, beats per minute.

TABLE 1
Effect of oral selective α2c-adrenergic agonists on minimum nasal cross-sectional areas in the cat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Minimum Cross-Sectional Area (60 Minutes Postcompound 48/80)</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
<td>0.016 ± 0.003</td>
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<tr>
<td>Compound A</td>
<td>0.023 ± 0.003*</td>
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<tr>
<td>0.1 mg/kg</td>
<td>0.021 ± 0.005</td>
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<tr>
<td>1.0 mg/kg</td>
<td>0.035 ± 0.002**</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>0.033 ± 0.005**</td>
</tr>
<tr>
<td>Compound B</td>
<td>0.025 ± 0.002*</td>
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<tr>
<td>0.3 mg/kg</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>0.036 ± 0.002**</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>0.042 ± 0.002**</td>
</tr>
<tr>
<td>Controls</td>
<td>0.011 ± 0.001*</td>
</tr>
<tr>
<td>d-Pseudoephedrine</td>
<td>0.030 ± 0.002**</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>0.029 ± 0.004**</td>
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<tr>
<td>1.0 mg/kg</td>
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*P < 0.05 compared with baseline (data not shown); **P < 0.05 versus control responses.

TABLE 2
Effect of oral selective α2c-adrenergic agonists on heart rate in the cat

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TABLE 3
Effect of topical selective α2c-adrenergic agonists on heart rate in the cat

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<td>200 ± 18</td>
</tr>
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bpm, beats per minute.

Effect of topical and therapeutically administered compound A and compound B. Nasal decongestants are often administered by the topical route. In separate experiments, we studied the topical nasal decongestant effects of compounds A and B using a therapeutic study design (Fig. 6). Specifically, the decongestant activities of compound A and compound B were determined after maximal congestion was elicited by topical compound 48/80 provocation. The mean nasal cavity volume ratio for control animals 30 minutes after administration of compound 48/80 (1%, 75 μl) was 0.23 ± 0.04. Figure 6 shows that compound A (0.03–0.3%, 50 μl), compound B (0.1–1.0%, 50 μl), and phenylephrine (0.03–0.3%, 50 μl) reversed the nasal effects of compound 48/80 60 minutes (90 minutes after compound 48/80 challenge) after delivery. The minimum effective concentrations of compound A and compound B required to produce a statistically significant effect were 0.1 and 0.3%, respectively. The maximum efficacies of the α2c-adrenergic agonists were equivalent to phenylephrine. There was a tendency for phenylephrine to increase systolic blood pressure, but these effects were not statistically relevant. There was no impact of compound A, compound B, or phenylephrine on heart rate (Table 3).
rhinometry was used to estimate nasal cavity volumes changes after intranasal compound 48/80 challenge. Compound A (1.0–5.0 mg/kg p.o.), compound B (1.0–5.0 mg/kg p.o.), or control was administered 1 hour before compound 48/80, and the generated data were expressed as the percentage change from baseline in nasal cavity volume values. Baseline nasal cavity volumes among treatment groups were not different. In control animals, compound 48/80 produced maximum nasal congestion between 120 and 150 minutes after topical intranasal delivery (Fig. 8). There was a tendency for compound A to produce nasal decongestion across all dose levels tested; however, this effect was not statistically significant. Compound B displayed efficacy at the 3 mg/kg dose level. Compound A and compound B at doses up to 5 mg/kg had no effects on blood pressure (data not shown). A PK/PD correction was attempted for both compounds (similar to efforts displayed for compound A in the cat); however, because of limited PK sampling and highly variable PD responses, an informative relationship could not be established.

Discussion

The present studies are the first to characterize the effect of selective a2c-adrenergic stimulation in experimental models of nasal congestion. The in vitro profiles of compound A and compound B have been described previously (Corboz et al., 2011, 2013). Notwithstanding, these compounds are adrenergic agonists that display significant preference for a2c over a2c.
maximal cross-sectional area of the nasal cavity. This can be directly assessed by changes in the amplitudes of reflective acoustic waveforms (Austin and Foreman, 1994). Our experiments provide evidence that selective α2c-agonists (compound A and compound B) behave as nasal decongestants in that they are able to diminish the nasal effects of the topically applied compound 48/80, a mast cell mediator liberator (Paton, 1951). It is important to note that, in this study, complete reversal of the effects of compound 48/80 on nasal cavity volume by selective α2c-agonists was not achieved. Specifically, the nasal cavity volume ratio after treatment was not restored to a baseline value of 1. With that being said, the maximum efficacies of compound A and compound B are equivalent to the maximum efficacies of a variety of decongestants that have previously been studied in this feline model (McLeod et al., 1999a; Erickson et al., 2001). The decongestant action of α2c-agonists is realized across the cavity, presumably as a consequence of nasal blood vessel constriction that lessens mucosal engorgement. Thus, it may not be surprising that all facets of nasal obstruction, for example, increases in mucus secretions and rhinorrhea, elicited by compound 48/80 are not completely attenuated by these drugs. In our experiments, we confirm that the nasal decongestant effects of compound A are completely blocked by JP 1302, indicating that these effects are mediated specifically by α2c-adrenergic receptors. Furthermore, these agents increase the minimum cross-sectional area within the nose, which is often referred to as the nasal valve. The valve region plays a major role in nasal breathing, is the location of highest resistance to airflow, and is germane to nasal physiology and pathology, including obstruction (Fattahi 2008; Nigro et al., 2009). Thus, demonstration of a drug’s action at the nasal valve area is an important aspect of its validation as a potential novel decongestant. For example, we found that α2c-adrenergic agents consistently improved

![Graph showing nasal decongestive effect of compound A](image)

**Fig. 5.** Potential nasal decongestant tolerance effect of compound A. Shown is the nasal effect of compound A administered using a subacute paradigm: 1 mg/kg p.o. daily for 5 days compared with responses generated after a single dose (A). Separate experiments (B) demonstrate that the efficacy of compound A is not diminished when compound A (1 mg/kg p.o.) is given twice daily 6 hours apart. In both (A) and (B), nasal cavity volume ratios 60 minutes after nasal exposure to compound 48/80 (%x, 75 μl) are presented. Each bar represents the mean ± S.E.M. of five or six animals. *P < 0.05 compared with time 0 (data not shown); **P < 0.05 compared with compound 48/80 alone.
minimum cross-sectional areas across a number of experimental paradigms.

We examined the PK/PD relationship of compound A. An $E_{\text{max}}$ model with a fixed $E_0$ of 0.22 (baseline compound 48/80 response) was used to relate drug plasma exposure to nasal decongestion effects (changes in nasal cavity dimensions). The model parameters for EC$_{50}$ and $E_{\text{max}}$ were found to be 0.13 ± 0.04 and 0.57 ± 0.03 μM, respectively. An estimated EC$_{80}$ value was 0.5 μM produced a near maximum decongestive response. This degree of target engagement appears to associate well with the duration of compound A (approximately 3.5–4.0 hours). Our topical compound A experiments indicate that maximum nasal decongestion can be achieved by this route. In addition, the topical estimated doses (0.025–0.05 mg/kg) are 20 to 40 times below the dose required to produce minimum decongestion by the oral route (1.0 mg/kg), confirming that the site of action of compound A is localized to the nose and that this action is not driven by undetermined systemic or central mechanisms. Taken together with the nasal explant results, these observations suggest that the decongestive action of compound A (at dose levels studied in the cat) involves local effects primarily on nasal veins. Efforts were undertaken to establish a PK/PD relationship of compound A in conscious dogs. Similar to the feline studies, acoustic rhinometry was used to estimate nasal cavity volume changes after intranasal compound 48/80 challenge. However, given the flat dose response (compound A) and the highly variable PK and PD in this model, a strong correlation between compound A exposure and decongestant efficacy could not be determined.

It is important to point out that our canine studies were performed in a fully awake and conscious state in which animals were trained and periodically retrained to accept an acoustic rhinometer to the nose (Koss et al., 2002). While this procedure is painless for the dog, it requires a high degree of collaboration between experimenter and subject to produce results. We recommend that acoustic studies with large conscious animals, such as dogs, be performed to ensure greater population sizes that will minimize excessive variance and improve PK/PD correlations.

Current nasal sympathomimetic decongestants are associated with mechanism-based adverse effects (Corey et al., 2000; Nathan, 2008; Greiner and Meltzer, 2011; Kushnir, 2011). For oral decongestants, these side effects include insomnia nervousness, anxiety, and tremors, as well as tachycardia, palpitations, and hypertension. For topical agents, side effects include nasal burning, stinging, dryness, mucosal

![Graphs showing nasal cavity volume, minimum cross-sectional area, and blood pressure and heart rate for Compound A, Compound B, and phenylephrine.](image-url)
ulceration, tolerance, and rebound congestion. Neither compound A nor compound B altered blood pressure in the cat at doses that produced nasal decongestion. Likewise, \( \alpha_2c \)-adrenergic agonists did not produce cardiovascular effects in our dog studies (data not shown) or previous rat experiments (Corboz et al., 2011). These observations are not surprising, given findings by Link et al. (1996) in knockout mice, suggesting that \( \alpha_2c \)-receptors appear not to play a role in modulating...
cardiovascular hemodynamic responses to adrenergic stimulation. In general, peripheral postsynaptic α2 receptors likely play a subordinate role (compared with α1 receptors) in regulating vascular resistance. Alexandre et al. (1995) demonstrated that the maximum pressor responses elicited by intravenous methoxamine (α1 agonist) and phenylephrine (predominately α1 agonist) were greater than those produced by BHT-920 (α2 agonist) in the pithed rat. This lack of a blood pressure effect with compounds A and B was in direct contrast to d-pseudoephedrine, which elicited hypertension in our model.

In summary, our studies demonstrate that α2c-adrenergic agonists constrict veins in porcine nasal mucosa explants and behave as decongestants in animal models of upper airway congestion. Furthermore, we show that α2c-adrenergic agonists appear to have little propensity to increase systemic blood pressure. Currently, there is a medical need for the development of nasal decongestants without hypertensive liabilities. α2c-Adrenergic subtype receptors may be a potential target for the treatment of nasal congestion with minimum impact on blood pressure.

Authorship Contributions

Participated in research design: Jia, Hunter, Koss, Hey, McLeod.
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Contributed new reagents or analytic tools: Boyce.
Performed data analysis: Mingo, Lieber, Palamanda, Me, McLeod.
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