

Pharmacological Evaluation of Selective α_2 -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 α_2 -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 *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 α_2 -adrenergic agonists. Acoustic rhinometry was used to determine nasal cavity dimensions following intranasal compound 48/80 (1%, 75 μ l). 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 (EC_{80}) 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 α_2 -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 α -adrenergic receptors. Both postjunctional α_1 - and α_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 α -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 α -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 α -adrenergic sympathomimetics are effective nasal decongestants, they

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ABBREVIATIONS: BHT-920, 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine dihydrochloride; JP 1302, acridin-9-yl[4-(4-methylpiperazin-1-yl)phenyl]amine; PK, pharmacokinetics, PD, pharmacodynamics.

sometimes precipitate mechanism-based side effects that include restlessness, nervousness, insomnia, and hypertension. Moreover, topical agents (e.g., phenylephrine, oxymetazoline, naphazoline) can produce a condition of rebound nasal congestion or medicamentosa (Corey et al., 2000; Nathan, 2008). Given the limitations of nonselective α -adrenergic sympathomimetics, 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 α_2 c-receptor subtype is the only α_2 receptor localized to nasal vasculature, specifically showing a high degree of expression in the sinusoids and arteriovenous anastomoses. Consequently, selectively targeting and stimulating α_2 c 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 α_2 agonists can elicit constriction of nasal vessels (Corboz et al., 2007). More importantly, nonselective α_2 activation has been demonstrated to have significant impact on functional nasal responses and physiology (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 α_2 agonists, particularly if they cross the blood-brain barrier and enter the central nervous system, will likely precipitate unwanted side effects. Clonidine, guanafacine, and guanabenz are prototypic examples of centrally acting nonselective α_2 agonists that 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 α_2 c 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 α_2 c-adrenoceptor agonists. The human binding affinity constant (K_i) values for compounds A and B are 12 and 18 nM, respectively. Both drugs display greater than 100 $\times$ selectivity over α_2 a and α_2 b receptors, with K_i activities on α_1 adrenoceptors of $\sim$ 10 mM. In the current study, we evaluated the direct effects of these two α_2 c-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 $\times$ 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- μ m 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 Axiovert 100 microscope (Carl Zeiss MicroImaging, Thornwood, NY) before and 20 minutes after the addition of each concentration of compounds (0.01–100 μ M) 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., 1999a,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 811-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 μ l) 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 decongestant dose-response characteristics, pharmacokinetic (PK)/pharmacodynamics (PD) relationship, duration of action, potential development of tolerance, and efficacy, by topical route of administration. Finally, the decongestant effect of compound A was studied in the presence of a selective α_2 c-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

(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 decongestive 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 μ l), compound B (0.1–1.0%, 50 μ l), or phenylephrine (0.03–0.3%, 50 μ l) 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 α 2c-Antagonist. JP 1302 [acridin-9-yl[4-(4-methylpiperazin-1-yl)phenyl]amine] is a competitive selective α 2c-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 μ l), JP 1302 alone (0.1%, 50 μ l), control (physiologic saline, 50 μ l), or JP 1302 (0.1%, 50 μ l) plus compound A (3%, 50 μ l) 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 basal nasal patency (Mingo et al., 2010). However, we selected a dose that had no actions on basal nasal cavity dimensions.

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 μ l). 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

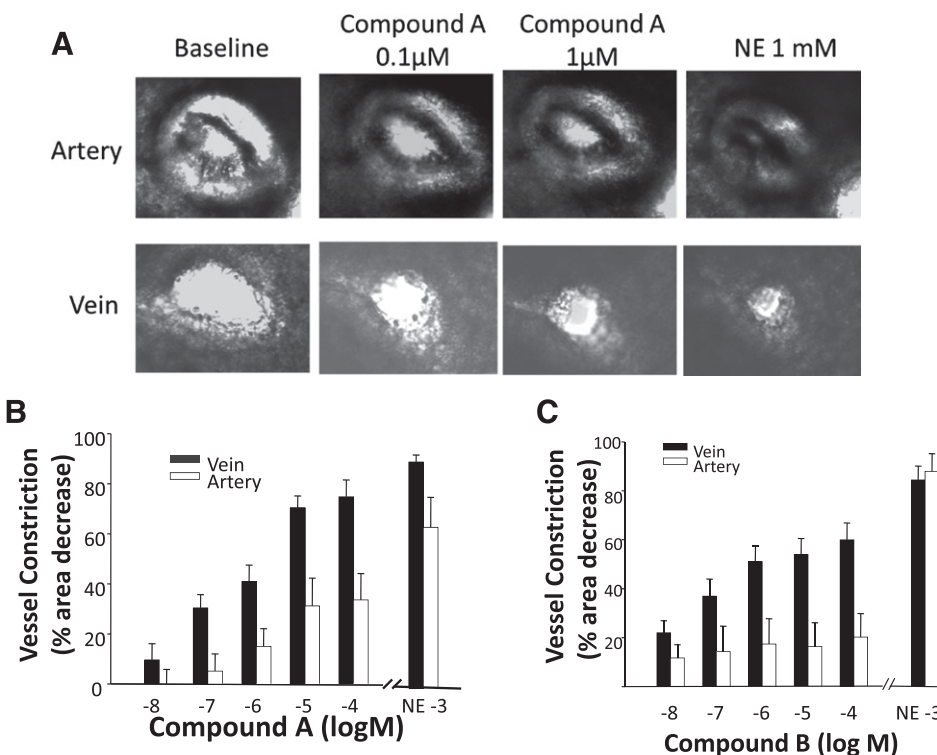


Fig. 1. Differential vessel constriction in porcine nasal mucosa. The effects of compound A and compound B on capacitance vessels (veins) and resistance vessels (arteries) were evaluated independently in porcine nasal mucosa explants. The compounds were added cumulatively and vessel constriction was recorded after each concentration. The α 1/ α 2-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 $\pm$ S.E.M.; n = 11–16 in vein recording and n = 6 in artery recording. EC_{50} of compound A on vein constriction is 210 nM; EC_{50} of compound B on vein constriction is 21 nM.

(size #1; Torpac Inc., Fairfield, NJ). The selective α_2 -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 $\pm$ 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

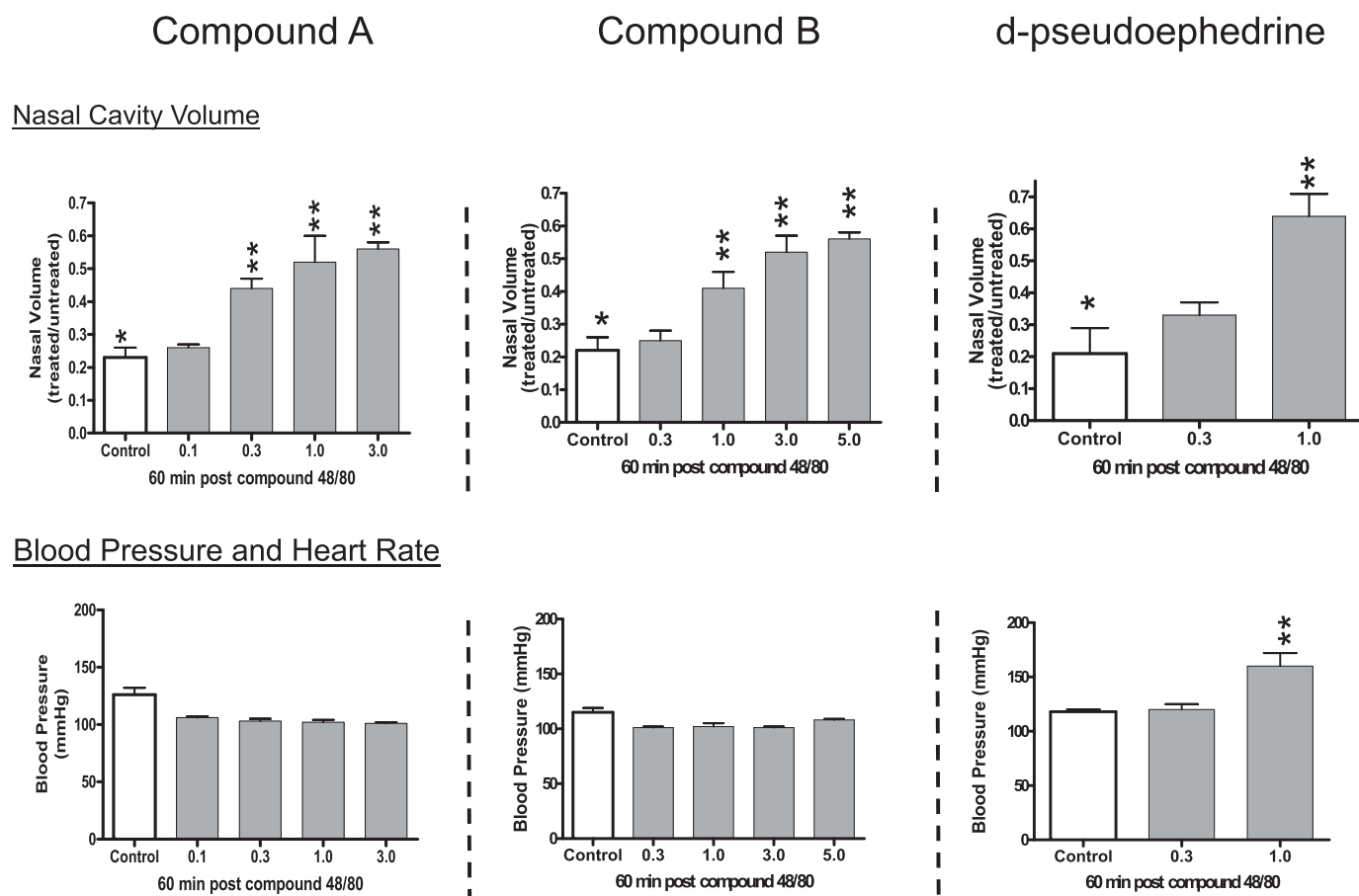


Fig. 2. Nasal decongestant effect of orally administered compound A, compound B, and *d*-pseudoephedrine in the anesthetized cat. The figure displays the dose-dependent actions of compound A, compound B, and *d*-pseudoephedrine on nasal cavity volume ratio 60 minutes after nasal exposure to compound 48/80 (1%, 75 μ l). All test drugs were administered 1 hour before compound 48/80 challenge. Each bar represents the mean $\pm$ 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.

TABLE 1

Effect of oral selective α 2c-adrenergic agonists on minimum nasal cross-sectional areas in the cat

Minimum nasal cross-sectional areas were measured in animals treated with compound A, compound B, or *d*-pseudoephedrine. Values represent mean $\pm$ S.E.M.

Treatment	Minimum Cross-Sectional Area (60 Minutes Postcompound 48/80)
	<i>cm</i> ²
Controls	0.016 $\pm$ 0.003
Compound A	
0.1 mg/kg	0.023 $\pm$ 0.003*
0.3 mg/kg	0.021 $\pm$ 0.005
1.0 mg/kg	0.035 $\pm$ 0.003**
3.0 mg/kg	0.033 $\pm$ 0.005**
Compound B	
0.3 mg/kg	0.025 $\pm$ 0.002*
1.0 mg/kg	0.028 $\pm$ 0.004
3.0 mg/kg	0.036 $\pm$ 0.003**
5.0 mg/kg	0.042 $\pm$ 0.002**
Controls	0.011 $\pm$ 0.001*
<i>d</i> -Pseudoephedrine	
0.3 mg/kg	0.030 $\pm$ 0.002**
1.0 mg/kg	0.029 $\pm$ 0.004**

P* < 0.05 compared with baseline (data not shown); *P* < 0.05 versus control responses.

contrast to compounds A and B, *d*-pseudoephedrine produced significant hypertensive effects at doses that elicited nasal decongestion (i.e., therapeutic index ≤ 1). Neither compound A, compound B, nor *d*-pseudoephedrine displayed a statistically relevant effect on heart rate at 60 minutes after compound 48/80 exposure (Tables 2 and 3). To estimate the plasma exposure of Compound A required for efficacy, blood samples were taken at 90 and 120 minutes after oral drug administration. The concentration required to produce a robust decongestant efficacy (EC₈₀) in the cat was approximately 0.5 μ M (Fig. 3). We evaluated the duration of action of compound A by varying the time between oral administration and nasal efficacy measurements. The duration of action of compound A at a 1 mg/kg (maximum efficacious dose) dose level was between 3.5 and 4.0 hours (Fig. 4), which was consistent with a plasma exposure above 0.5 μ M (Fig. 3). Figure 5 shows that compound A did not produce tolerance when given using a subacute dosing paradigm. In particular, the nasal decongestant effect of compound A (1.0 mg/kg p.o.) given once daily for 5 days was equivalent to efficacy of compound A (1.0 mg/kg p.o.) given only once. The decongestant action of compound A was also not attenuated when dosed 6 hours apart (Fig. 5).

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 μ l) was 0.23 $\pm$ 0.04. Figure 6 shows that compound A (0.03–0.3%, 50 μ l), compound B (0.1–1.0%, 50 μ l), and phenylephrine (0.03–0.3%, 50 μ l) 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

TABLE 2

Effect of oral selective α 2c-adrenergic agonists on heart rate in the cat
Heart rates were measured in animals treated orally with compound A, compound B, or *d*-pseudoephedrine. Values represent mean $\pm$ S.E.M.

Treatment	Heart Rate (60 Minutes Postcompound 48/80)
	<i>bpm</i>
Controls	213 $\pm$ 12
Compound A	
0.1 %	210 $\pm$ 6
0.3 %	208 $\pm$ 11
1.0 %	207 $\pm$ 12
3.0 %	211 $\pm$ 19
Compound B	
0.3 %	219 $\pm$ 12
1.0 %	219 $\pm$ 12
3.0 %	203 $\pm$ 12
5.0 %	211 $\pm$ 8
Controls	214 $\pm$ 12
<i>d</i> -Pseudoephedrine	
0.3 %	214 $\pm$ 12
1.0 %	189 $\pm$ 16

bpm, beats per minute.

compound B required to produce a statistically significant effect were 0.1 and 0.3%, respectively. The maximum efficacies of the α 2c-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).

Effect of Compound A in the Absence and Presence of a Selective α 2c Antagonist. Figure 7 shows that a high dose of compound A (3%) given topically 30 minutes before compound 48/80 produced a maximum degree of decongestion with effects on nasal cavity volumes and minimum cross-sectional areas. The selective α 2c-antagonist JP 1302 (alone) did not alter the congestive effect of compound 48/80 (Fig. 7). However, pretreatment with JP 1302 10 minutes before compound A fully blocked the nasal decongestion produced by the α 2c agonist.

Acoustic Rhinometry in the Dog. The decongestant activities of compounds A and B were also evaluated in conscious dogs (Fig. 8). Similar to the feline studies, acoustic

TABLE 3

Effect of topical selective α 2c-adrenergic agonists on heart rate in the cat

Heart rates were measured in animals treated topically with Compound A, Compound B, or phenylephrine. Values represent mean $\pm$ S.E.M.

Treatment	Heart Rate (60 Minutes Postcompound 48/80)
	<i>bpm</i>
Controls	204 $\pm$ 11
Compound A	
0.03 mg/kg	210 $\pm$ 7
0.1 mg/kg	202 $\pm$ 13
0.3 mg/kg	207 $\pm$ 10
Compound B	
0.1 mg/kg	210 $\pm$ 7
0.3 mg/kg	212 $\pm$ 10
1.0 mg/kg	208 $\pm$ 15
Phenylephrine	
0.03 mg/kg	212 $\pm$ 10
0.1 mg/kg	205 $\pm$ 13
0.3 mg/kg	200 $\pm$ 18

bpm, beats per minute.

Compound A

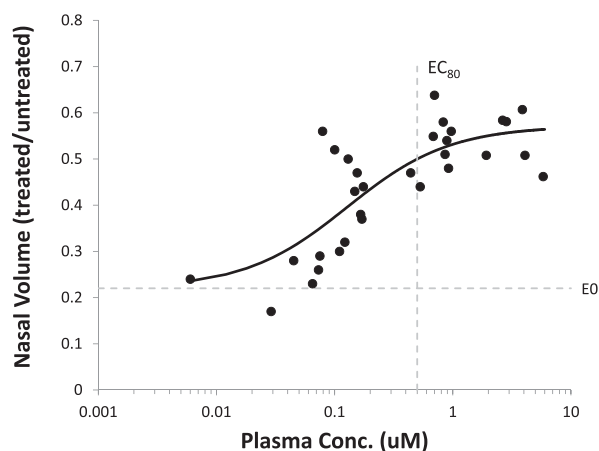


Fig. 3. Relationship between plasma PK and nasal decongestion in the cat. The figure displays an $E_{\max}$ model illustrating increasing nasal cavity volumes (nasal decongestion) associated with increasing plasma concentrations after a single oral dose of compound A (0.1–3.0 mg/kg). Note that blood samples were collected at 90 and 120 minutes post-treatment.

rhinometry was used to estimate nasal cavity volume 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 α_2c -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 adrenoceptor agonists that display significant preference for α_2c over

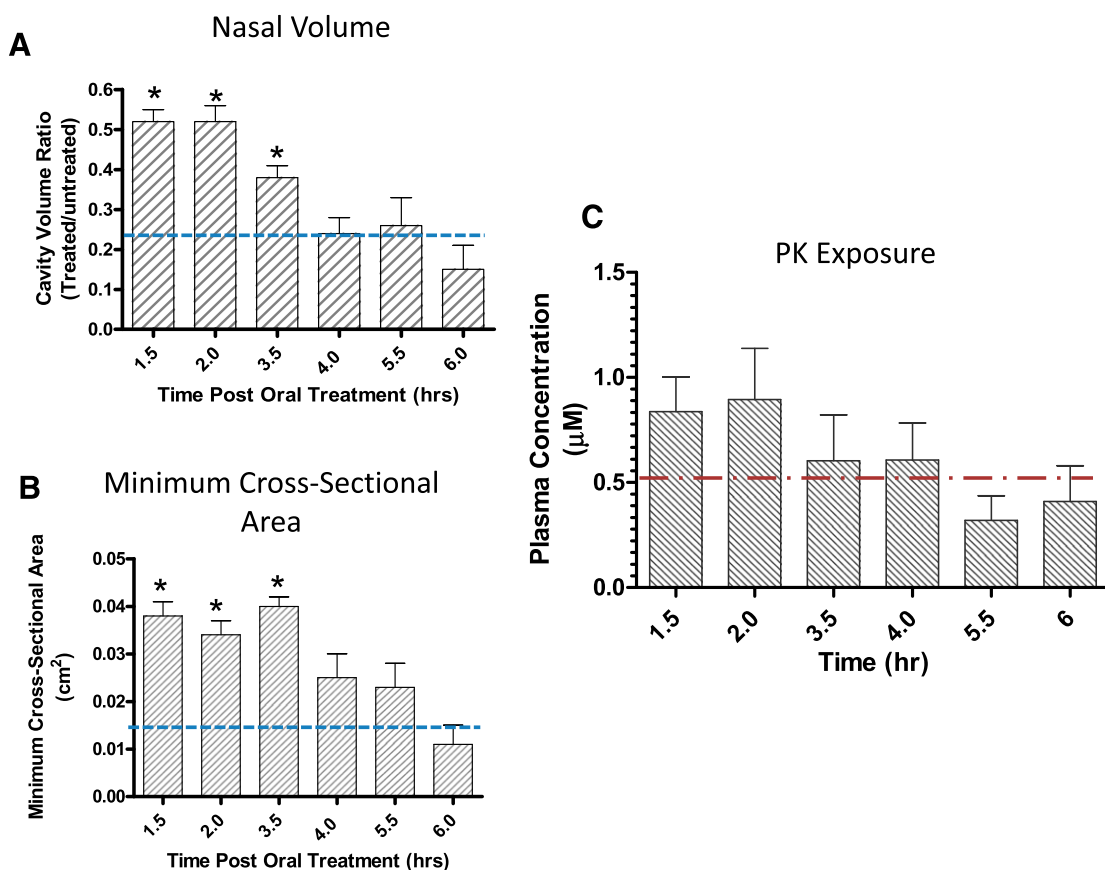


Fig. 4. Nasal duration of compound A in the cat. (A and B) The time-dependent actions of compound A (1.0 mg/kg p.o.) on nasal cavity volume ratio and minimum cross-sectional areas 60 minutes after nasal exposure to compound 48/80 (1%, 75 μ l). Also shown are the plasma exposures of compound A (1.0 mg/kg p.o.) between 1.5 and 6.0 hours (C). Each point represents the mean $\pm$ S.E.M. of four to six animals. Compound 48/80 response is denoted by the dashed line. The dash/dotted line denotes exposure based on an $E_{\max}$ model estimated to produce an 80% nasal decongestive effect. * $P < 0.05$ compared with control.

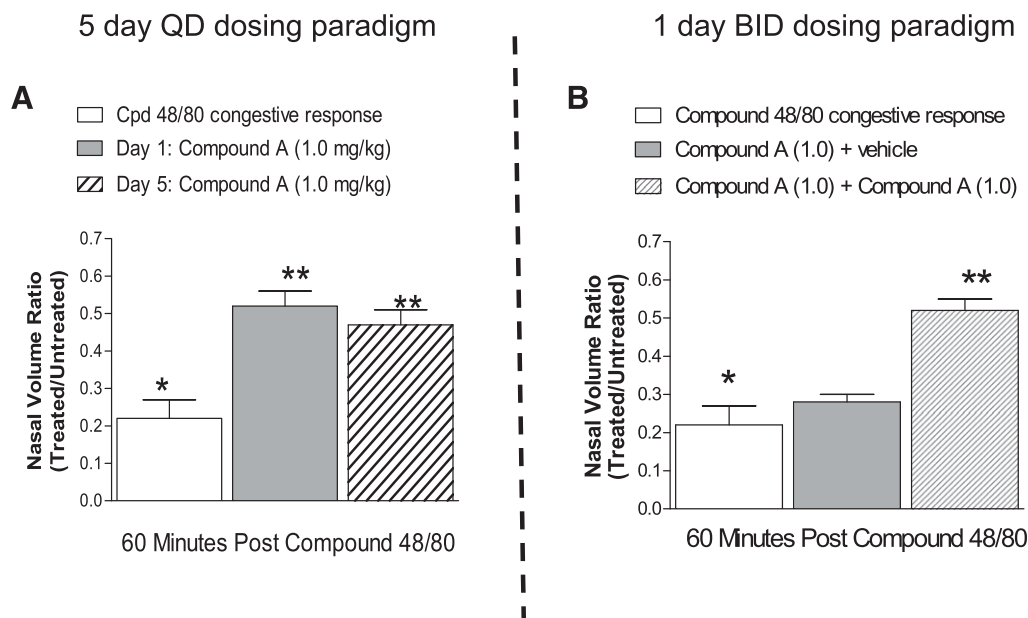


Fig. 5. Potential nasal decongestant tolerance effect of compound A. Shown is the nasal effect of compound A administered using a subacute paradigm: 1 mg/kg p.o. daily for 5 days compared with responses generated after a single dose (A). Separate experiments (B) demonstrate that the efficacy of compound 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 (1%, 75 μ l) are presented. Each bar represents the mean $\pm$ 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.

α 2 or α 1-adrenergic receptor subtypes (Corboz et al., 2011, 2013). In humans, α 2-receptors are prevalent in the nasal mucosa and are found distributed on both capacitance and resistance nasal blood vessels (Andersson and Bende, 1984). We used a pig nasal mucosa explant model to elucidate potential mechanisms of nasal decongestion elicited by compound A and compound B. Specifically, our porcine assay allowed the evaluation of real-time artery and vein activities independently in the nasal mucosa (Lieber et al., 2010). Selective activation of α 2c-receptors by both compound A and compound B produced vascular constrictions of arteries and veins; however, a superior pharmacological effect was noted in capacitance vessels.

The major focus of this article relates to the profiling of selective α 2c-adrenoceptor agonists in surrogate in vivo models of mast cell-mediated nasal congestion. Therefore, this report represents a natural extension of previous experiments where we demonstrated that nonselective α 2-adrenergic agonists such as BHT-920 [6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine dihydrochloride] produced nasal decongestion in our feline model of congestion (McLeod et al., 2001). We also previously demonstrated, using a selective α 2c-adrenergic antagonist, that α 2c subtypes appear to play a role in the regulation of basal patency in the cat (Mingo et al., 2010). Presently, we used acoustic rhinometry, which is a highly sensitive, noninvasive, and reliable technology increasingly used clinically to evaluate nasal patency (Hilberg et al., 1989; Grymer et al., 1991; Riechelmann et al., 1993; Austin and Foreman, 1994; Cingi et al., 2013). Our laboratory was the first to apply this technology to the assessment of preclinical drugs targets in large animals, such as cats and dogs (McLeod et al., 1999a,b; Erickson et al., 2001; Koss et al., 2002; Rudolph et al., 2003). The method allows evaluation of changes in the geometry (cross-sectional area) of nasal airways by means of sound reflection. With acoustic rhinometry,

congestion of the nasal airways results in a decrease in the minimal 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 α 2c-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 α 2c-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 α 2c-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 α 2c-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 α 2c-adrenergic agents consistently improved

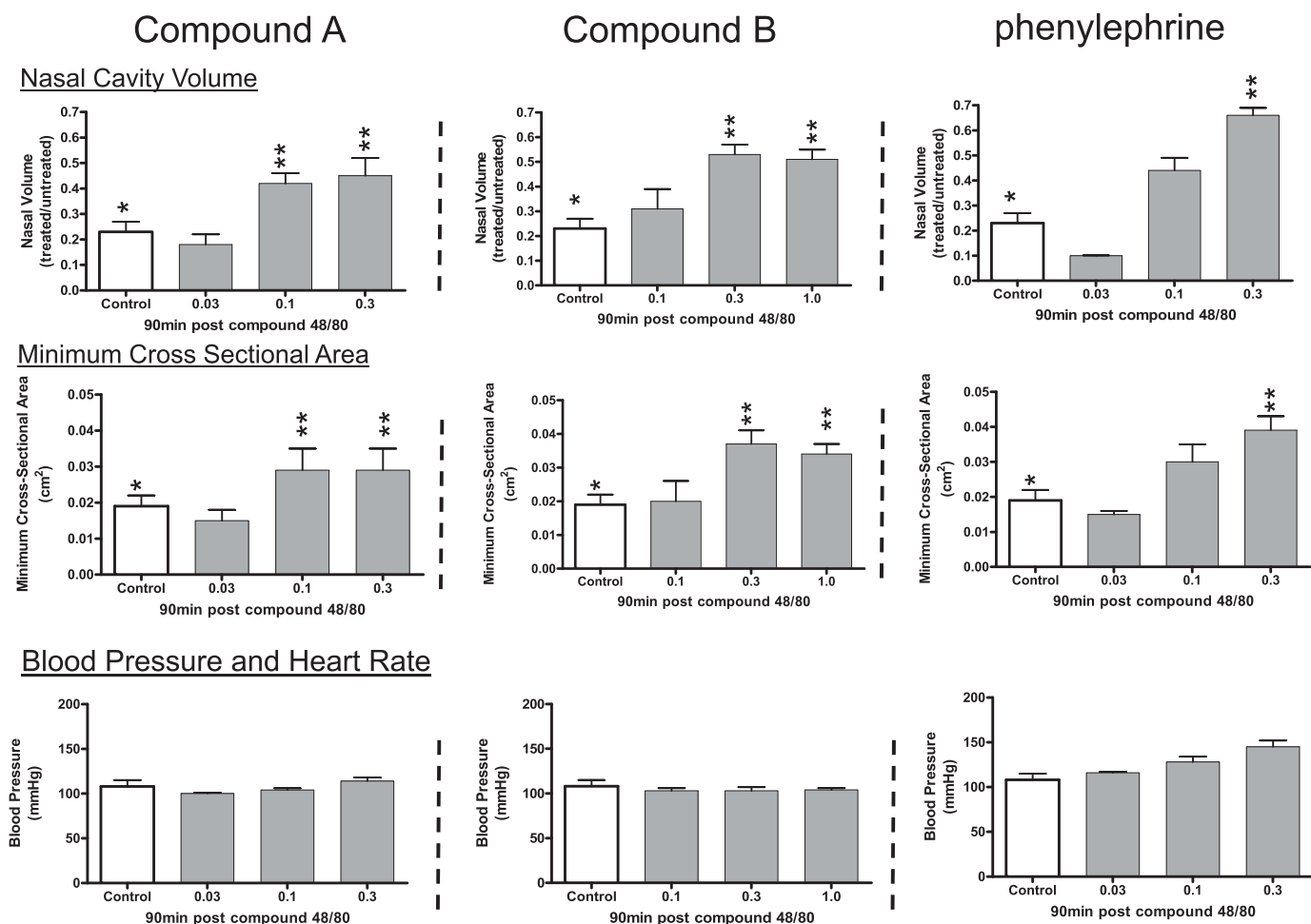


Fig. 6. Nasal decongestant effect of topically and therapeutically administered compound A, compound B, and phenylephrine in the anesthetized cat. The figure displays the dose-dependent actions of compound A (0.03–0.3%), compound B (0.1–1.0%), and phenylephrine (0.03–3.0%) on nasal cavity volume ratio, minimum cross-sectional area, and systolic blood pressure 90 minutes after nasal exposure to compound 48/80 (1%, 75 μ l). Note that test drugs were given by the intranasal route 30 minutes after compound 48/80. Each bar represents the mean $\pm$ 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 (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 $E_{\max}$ 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 $E_{\max}$ were found to be 0.13 ± 0.04 and 0.57 ± 0.03 μ M, respectively. An estimated EC_{80} value was 0.5 μ M 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 produced 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 wakeful 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

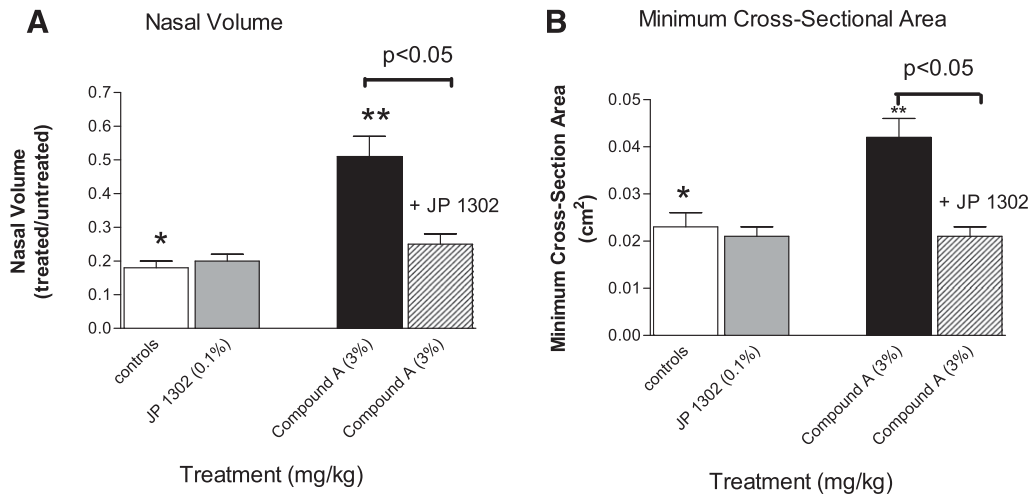


Fig. 7. Effect of compound A in the absence and presence of JP 1302. Depicted is the impact of the selective α 2c antagonist JP 1302 (0.1%, 50 μ l) on nasal volume (A) and minimum cross-sectional area (B) produced by topical compound A (0.3%). Each bar represents the mean $\pm$ S.E.M. of five or six animals. * $P < 0.05$ compared with time 0 (data not shown); ** $P < 0.05$ compared with compound 48/80 alone.

ulceration, tolerance, and rebound congestion. Neither compound A nor compound B altered blood pressure in the cat at doses that produced nasal decongestion. Likewise, α 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 α 2c-receptors appear not to play a role in modulating

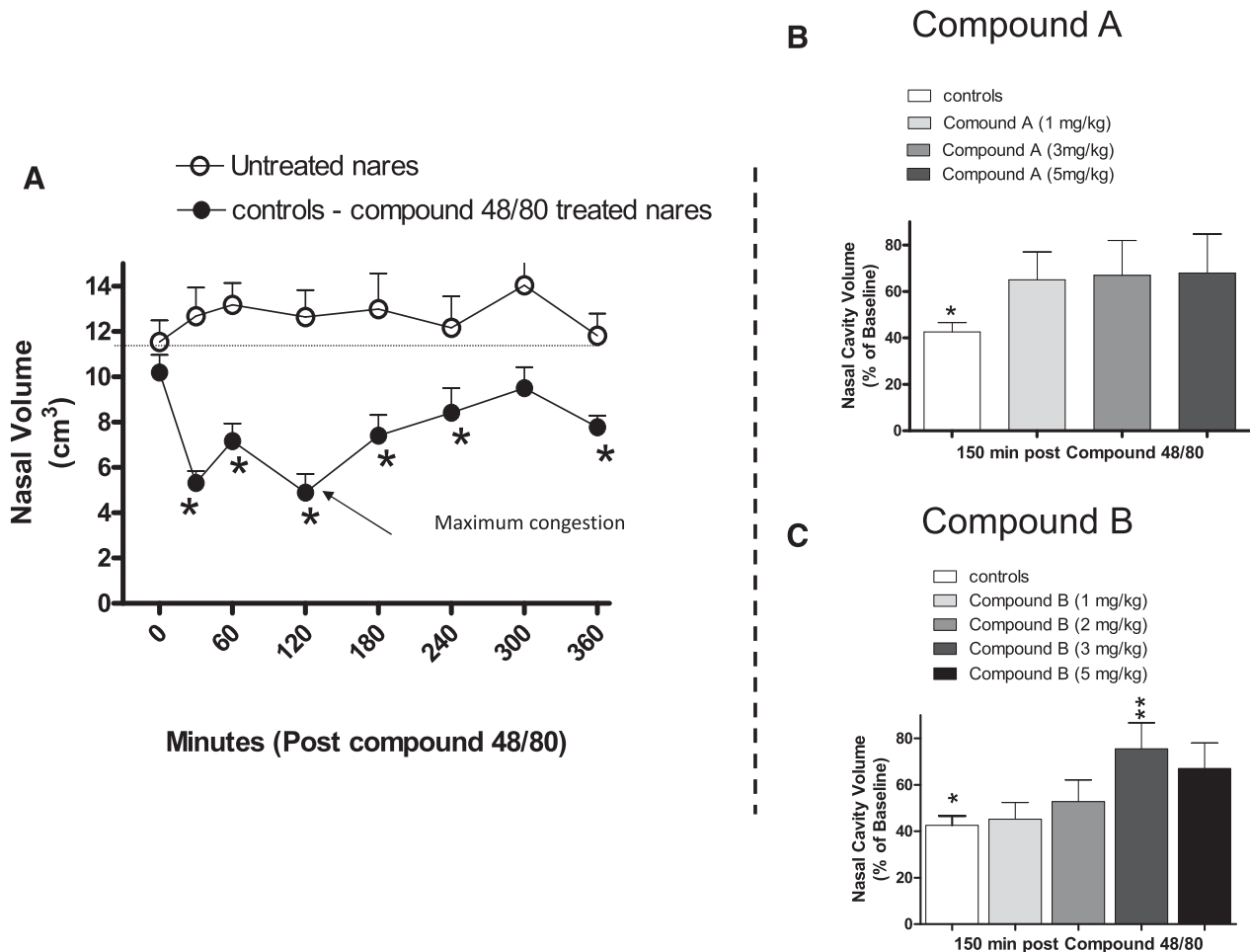


Fig. 8. Nasal decongestant effect of orally administered compound A and compound B in the conscious dog. (A) The time-related actions of compound 48/80 (3%, 500 μ l) on nasal cavity volume size. The maximum congestive action of compound 48/80 was observed between 120 and 150 minutes post-treatment. (B) The actions of compound A (1.0–5.0 mg/kg p.o.) and compound B (1.0–5.0 mg/kg p.o.) at 120 and 150 minutes after compound 48/80 treatment. Each bar represents the mean $\pm$ S.E.M. of six animals. * $P < 0.05$ compared with time 0; ** $P < 0.05$ compared with compound 48/80 alone.

cardiovascular hemodynamic responses to adrenergic stimulation. In general, peripheral postsynaptic α_2 receptors likely play a subordinate role (compared with α_1 receptors) in regulating vascular resistance. Aleixandre et al. (1995) demonstrated that the maximum pressor responses elicited by intravenous methoxamine (α_1 agonist) and phenylephrine (predominately α_1 agonist) were greater than those produced by BHT-920 (α_2 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 α_2 c-adrenergic agonists constrict veins in porcine nasal mucosa explants and behave as decongestants in animal models of upper airway congestion. Furthermore, we show that α_2 c-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. α_2 c-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.

Conducted experiments: Mingo, Lieber, Palamanda, Mei, Yu.

Contributed new reagents or analytic tools: Boyce.

Performed data analysis: Mingo, Lieber, Palamanda, Mei, McLeod.

Wrote or contributed to the writing of the manuscript: Jia, Cicmil, McLeod.

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