Pharmacological Evaluation of Selective α2c Adrenergic Agonists in Experimental Animal Models of Nasal Congestion

Yanlin Jia, Garfield G Mingo, John C Hunter, Gissela B Lieber, Jairam R Palamanda, Hong Mei, Christopher W. Boyce, Michael C Koss, Yongxin Yu, Milenko Cicmil, John A Hey, and Robbie L McLeod

In Vivo Pharmacology: GGM, JCH, GBL, MC, JAH, RLM

Immunology: YJ

Merck Research Laboratories, Boston, MA, USA

Departments of Pharmacokinetics: JRP, HM

Departments of Chemistry: CWB

Merck Research Laboratories, Rahway NJ, USA

Department of Cell Biology: MCK, YY

University of Oklahoma College of Medicine, Oklahoma City, OK, USA

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Corresponding Author: Robbie L. McLeod, Ph.D.

Merck Research Laboratories

In Vivo Pharmacology - Respiratory and Immunology

BMB 10-112, 33 Avenue Louis Pasteur, Boston, MA, USA 02115

Tel: 617-992-3049; Fax: 617.992.2486; E-mail: robbie.mcleod@merck.com

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Abbreviations:
bpm: beats per minute
CV: Cardiovascular
KO mice: knockout mice
PD: pharmacodynamics
PK: pharmacokinetic
QD: once daily administration
Abstract

Nasal congestion is one of the most troublesome symptoms of many upper airways diseases. We characterized the effect of selective α2c-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 (PK)/pharmacodynamics (PD) relationship, duration of action, potential development of tolerance and topical efficacy of α2c-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%. Compound A (0.1 – 3.0 mg/kg, p.o), Compound B (0.3 – 5.0 mg/kg, p.o) and d-pseudoephedrine (0.3 and 1.0 mg/kg, p.o.) produced a 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 duration of action of around 4.0 hrs. 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 α2c-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 (Cobanoglu 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 et al., 2008; Meltzer et al., 2009). Being more than 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 et al., 1986; Lung and Wang, 1989). Congestion occurs in part, as a consequence of vasodilation of venous sinusoids that then becomes 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 post-junctional α1- and α2-adrenergic receptors subtypes are found prevalently distributed on capacitance and resistance blood vessels where they mediated 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 decongestant 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 which decreases blood flow through the mucosa, shrinks nasal erectile tissue and improves cavity patency (Anderssen and Bende, 1984). While topical and oral $\alpha$-adrenergic sympathomimetics are effective nasal decongestants they 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 non-selective $\alpha$-adrenergic sympathomimetics there are unmet needs for the development of novel decongestants, especially agents without the systemic or central liabilities of currently available $\alpha$-adrenergic sympathomimetics.

In situ mRNA studies by Stafford-Smith et al., (2007) in human nasal turbinates suggested that the $\alpha_{2c}$-receptor subtype is the only $\alpha_{2}$-receptor localized to nasal vasculature, specifically showing a high degree of expression in the sinusoids and arteriovenous anastomoses. Consequently, selectively targeting and stimulating $\alpha_{2c}$-receptors may provide a novel approach for the treatment of nasal decongestion (Stafford-Smith et al., 2007; Corboz et al, 2011). As previously described, non-selective $\alpha_{2}$ agonists can elicit constriction of nasal vessels (Corboz et al, 2007). More importantly, non-selective $\alpha_{2}$ 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; Berkowitz et al., 2005). Unfortunately, non-selective α2 agonists, particularly if they cross the blood brain and enter the CNS will likely precipitate unwanted side effects. Clonidine, quanafacine and guanabenz are prototypic examples of central acting non-selective α2 agonists that produced 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 has for the first time allowed the examination of the proposal that these drugs can elicit nasal decongestion independent of hypertensive or hypotensive actions in preclinical models.

The chemical structure 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) is 12nM and 18nM, respectively. Both drugs display greater than 100X selectivity over α2a and α2b receptors with Ki activities on α1- adrenoceptors around 10mM. 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 to 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

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). Briefly, pig snouts from male and female domestic pigs (110 - 230 kg) were provided by local abattoir, Animal Parts (Scotch Plains, NJ, USA). Nasal mucosa was removed from turbinates and cut into strips (0.5x1.5 cm). Mucosa strips were fixed in 6% low melt agarose in Krebs buffer at 37°C in a 3 ml syringe and cool down on ice until agarose become solid. The fixed tissues are cut into slices of 200-300 µm thick in Krebs buffer at 4°C, using Krumdieck Tissue Slicer (Alabama Research and Development, Munford, AL, USA). Tissue slices, freed of agarose, were then incubated in tissue culture dishes with Clonetics SmGM-2 culture medium (Biowittaker, Walkersville, MD, USA) in the presence of 1% penicillin/streptomycin (Biowittaker, Walkersville, MD, USA) at 37°C in humidified air containing 5% CO₂. The following day, nasal mucosa slices were equilibrated for 15 min 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, USA) before and 20 minutes after the addition of each concentration of compounds (0.01 - 100 µM) at 37°C. Cross-section area of vein or artery lumen was measured using computer software Image J. Vessel constriction was expressed as % 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 evaluated nasal cavity patency in the cat have been described previously (McLeod et al, 1999a, 1999b). Briefly, for feline nasal decongestant studies male Harlan short haired cats (1.5-3.0 kg, Harlan Sprague Dawley, Madison, WI, USA) 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 trigger 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, recorded and the data obtained were converted to area-distance curves. The acoustic rhinometer was calibrated to measure a distance of 0 to 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, USA). 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. Compound 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 α2c adrenergic antagonist in this species.

**Oral Dose Response Characteristics and PK Relationship in the Cat:** Oral 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, USA) one 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 post oral treatment. PK samples were examined for drug plasma concentrations at 90 and 120 minutes post 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 oral administration and nasal efficacy measurements (note that the time between Compound 48/80 challenge and efficacy assessment remained the same as 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 post 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 LC-MS/MS as previously described (Corboz et al., 2011). PK/PD modeling was conducted using Phoenix WinNonLin 5.3 program from Pharsight, Cary, NC.

**Decongestant Tolerance:** We conducted studies to determine if the nasal decongestant effects of Compound A (1.0 mg/kg, p.o.) was diminished after a 5 day QD dosing paradigm (i.e. sub-acute dosing paradigm). In these experiments Compound A was given each day at 8:00 AM. On the fifth day the drug was administered one hour before Compound 48/80 challenge. Acoustic measurements were taken 60 minutes after Compound 48/80. Results from the sub-acute dosing paradigm was compared to the decongestant efficacy of Compound A (1.0 mg/kg, p.o.) given only once. We also investigated if 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%, 50µl), Compound B (0.1 – 1.0%, 50µl), or phenylephrine (0.03 – 0.3%, 50µl), was administered thirty 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 $\alpha_{2c}$ Antagonist:**

JP 1302 is a competitive selective $\alpha_{2c}$ antagonist (Tricklebank 2007; Sallinen et al., 2007). We used this tool to inform on the $\alpha$-adrenergic subtype responsible for the decongestant actions of Compound A. Topical Compound A (3%, 50µl), JP 1302 alone (0.1%, 50 µl), control (physiological saline, 50 µl) or JP 1302 (0.1%, 50 µl) plus Compound A (3%, 50µl) was given thirty 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 impact 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 activity of Compound A and Compound B was also evaluated in conscious, adult male, purpose-bred beagle dogs (C and C Kennels, Wewoka, OK) weighing 9–11 kg). Similar to the feline studies acoustic rhinometry was used to estimate nasal cavity volumes changes after intranasal Compound 48/80 (3%, 500ul) challenge. 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 hr before Compound 48/80 and the generated data was expressed as the percent change from baseline nasal cavity volume values.

Drugs

Compound 48/80, and d-pseudoephedrine were purchased from Sigma Chemical Co. (St. Louis MO, USA). Phenylephrine hydrochloride was purchased from Research Biochemicals International (Natick MA, USA). Drug doses refer to their respective free bases. All drugs were dissolved in physiological saline (0.9%) or delivered in a gelatin capsule (size # 1, Torpac Inc., Fairfield, NJ, USA). The selective α2-adrenergic agonists, Compounds A and B were synthetized by Merck Chemistry.

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; McLeod et al., 1999b). Values displayed in the table and the figures represent the Mean ± SEM of 5-8 animals per group. Data were evaluated using an Kruskal Wallis in conjunction with a Mann Whitney-U. 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 (Figure 1). Compound A (10 nM - 0.1 mM) induced vessel constrictions, in dose dependent manners, in nasal mucosa with superior effect on veins than arteries. Similarly, compound B induced a concentration dependent constriction in veins with minimum effect on arteries (Figure 1). These results indicate that Compound A and Compound B preferentially contracts 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 while baseline minimum cross-sectional areas lied between 0.037 ± 0.002 to 0.042 ± 0.003 cm². These values were not different from controls baseline values. Figure 2 shows that 60 min after topical application Compound 48/80 significantly decreased nasal volume ratios to 0.23 ± 0.03, representing a 77% decrease in cavity volume. Oral 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 (Figure 2. and Table 1). The minimum dose of Compound A required to produce a significant nasal decongestant
effect was 0.3 mg/kg. The drug dosed up to 3.0 mg/kg had no effect on systolic blood pressure. A similar dose-related decongestant effect was observed with Compound B (Figure 2 and 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 when compared to it perspective controls (Figure 2). However, in 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 or d-pseudoephedrine, displayed a statistical relevant effect on heart rate at 60 minutes post Compound 48/80 exposure (Table 2, 3). To estimate the plasma exposure of Compound A required for efficacy, two blood samples were taken at 90 and 120 min post oral drug administration. The concentration required to produce a robust decongestant efficacy (EC80) in the cat was approximately 0.5 µM (Figure 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 hrs (Figure 4), which was consistent with a plasma exposure above 0.5 µM (Figure 3). Figure 5 shows that Compound A did not produced tolerance when given using a sub-acute dosing paradigm. Particularly, the nasal decongestant effect of Compound A (1.0 mg/kg, p.o.; QD) given for 5 days was equivalent to efficacy of Compound A (1.0 mg/kg, p.o.; QD) given only once. The decongestant action of Compound A was also not attenuated when dosed six hours apart (Figure 5).

Effect of Topical and Therapeutically Administered Compound A and Compound B
Often nasal decongestant are 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 (Figure 6). Specifically, the decongestant activity of Compound A and Compound B was determined after maximal congestion was elicited by topical Compound 48/80 provocation. The mean nasal cavity for volume ratio for control animals 30 minutes after Compound 48/80 (1%, 75 µl) was 0.23 ± 0.04. Figure 6 shows that Compound A (0.03 – 0.3%, 50µl), Compound B (0.1 – 1.0%, 50ul) and phenylephrine (0.03 – 0.3%, 50µl) 60 minutes (90 minutes post compound 48/80 challenge) after delivery, reversed the nasal effects of Compound 48/80. The minimum effective concentration of Compound A and Compound B to produce a statistically significant effect was 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).

*Effect of Compound A in the Absence and Presence of a Selective α2c Antagonist*

Figure 7 shows that a high dose 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 α2c agonist JP 1302 (alone) did not alter the congestive effect of Compound 48/80 (Figure 7). However, pretreated with JP 1302 ten minutes before Compound A fully blocked the nasal decongestion produced by the α2c agonist.
Acoustic Rhinometry in the Dog

The decongestant activity of Compounds A and B was also evaluated in conscious dogs (Figure 8). Similar to the feline studies (see Section 3.3), acoustic 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 hr before Compound 48/80 and the generated data was expressed as the percent change from baseline 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 min after topical intranasal delivery (Figure 8). There was a tendency for Compound A to produce nasal decongestion across all dose level 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 due to limited PK sampling, high variable PD responses an informative relationship could not be established.
Discussion

The present studies are the first to characterize the effect of selective α2c-adrenergic stimulation in experimental models of nasal congestion. The in vitro profile of Compound A and Compound B has been described previously (Corboz et al., 2011, 2013). Notwithstanding, these compounds are adrenoceptor agonists that display significant preference for α2c over α2 and α1 adrenergic receptor subtypes (Corboz et al., 2011, 2013). In humans, α2-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 α2c-receptors by both compound A and Compound B produced vascular constrictions of arteries and veins, however, a superior pharmacological effect was noted on capacitance vessels.

The major focus of this manuscript relates to the profiling of selective α2c-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 non-selective α2-adrenergic agonists such as BHT-920 produced nasal decongestion in our feline model of congestion (McLeod et al., 2001). We also previously demonstrated, using a selective α2c-adrenergic antagonist, that α2c subtypes appear to play a role in the regulation of basal patency in the cat.
Presently, we used acoustic rhinometry which is a highly sensitive, non-invasive 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). We were the first laboratory to apply this technology to the assessment of preclinical drugs targets in large animals, such as cats and dogs (McLeod et al. 1999a, 1999b; Erickson et al., 2001; Koss et al., 2002; Rudolph et al., 2003). The method allows evaluation of changes in the geometry (cross section 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). In our experiments we provide evidence that selective \( \alpha_{2c} \)-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 \( \alpha_{2c} \)-agonists was not achieved. Specifically, the nasal cavity volume ratio after treatment was not restored to a baseline value of one. With that being said, the maximum efficacy of Compound A and Compound 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 \( \alpha_{2c} \)-agonists is realized across the cavity and presumable as a consequence of nasal blood vessel constriction lessening 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 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. Additionally, these agents increase 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., 2010). 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 consistently improved minimum cross sectional areas across a number of experimental paradigms.

We examined the PK/PD relationship of Compound A. An Emax model with a fixed E0 of 0.22 (baseline Compound 48/80 response) was used to relate drug plasma exposure to nasal decongestion effect (changes in nasal cavity dimensions). The model parameters for (EC50) and (Emax) was found to be 0.13 ± 0.04 µM and 0.57 ± 0.03 µM, respectively. An estimated EC80 value was 0.5 µM produced a near maximum decongestive response and appears to associate well with the duration study showing that exposures of Compound A above 0.5µM (approximately 3.5 – 4.0 hours) related to the drug’s impact on nasal cavity volumes and minimum cross-sectional areas. Our topical Compound A experiments indicate that maximum nasal decongestion can be achieved by this route. In addition, the topical estimated doses (on a mg/kg bases = 0.025-0.05) is 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 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 level 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 volumes 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 future acoustic studies with large conscious animals, such as dogs, care be taken to ensure greater population sizes that will minimized 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, nasal burning, stinging, dryness, mucosal ulceration, tolerance and rebound congestion. Neither Compound A nor Compound B, at doses that produced nasal decongestion, altered blood pressure in the cat. Likewise, α2c-
adrenergic agonists did not produce CV 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 KO mice suggesting that α2c- receptors appear not to play a role in modulating CV hemodynamic response to adrenergic stimulation. In general peripheral postsynaptic α2-receptors likely play a subordinate role (compared to α1-receptors) in regulating resistance blood vessels. Aleixandre 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 were 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 increasing systemic blood pressure. Currently, there is a medical need for the development of nasal decongestants without hypertensive liabilities. Adrenergic α2c 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
References:


ylmethyl)-2H-1, 4-benzoazin-6-yl]-N-ethyl-N'-methylurea (compound A). *J Pharmacol Exp Ther.* 337:256-266.


Legends for figures

Figure 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. α1/α2-adrenoceptor agonist norepinephrine (NE) was added at the end of experiments for the maximal constriction in both arteries and veins. Panel A: The representative images of compound A-induced differential constriction in artery and in vein. Panel B: Summary results of compound A on nasal mucosa vessel constriction. Panel C: Summary results of compound B on nasal mucosa vessel constriction. Results are Mean ± SEM; n=11-16 in vein recording. 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.

Figure 2. Nasal decongestant effect of orally administered Compound A, Compound B and d-pseudoephedrine in the anesthetized cat. 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 ± SEM of 5-6 animals (*p<0.05 compared to time 0; **p<0.05 compare to compound 48/80 alone). Mean heart rate values (beats per min) are in parenthesis.

Figure 3. Relationship between plasma PK and nasal decongestion the cat. Figure displays an Emax 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 min post treatment.
Figure 4. Nasal duration of Compound A in the cat. Panels A and B displays 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 µl). Also shown are the plasma exposures of Compound A (1.0 mg/kg, p.o.) between 1.5 and 6.0 hours (Panel C). Each point represents the Mean ± SEM of 4-6 animals (*p<0.05 compared to a control Compound 48/80 response denoted by dashed line. Dash/dotted line denotes exposure based on Emax model estimated to produce an 80% nasal decongestive effect.

Figure 5. Potential nasal decongestant tolerance effect of Compound A. Sown is the nasal effect ofCompound A administered using a sub-acute paradigm; 1 mg/kg QD orally for 5 days compared to responses generated after a single dose (Panel A). In separate experiments Panel B demonstrates that the efficacy of compound A is not diminished when Compound A (1 mg/kg, p.o.) is given BID six hours apart. In both Panels A and B, nasal cavity volume ratios 60 minutes after nasal exposure to compound 48/80 (1%, 75 µl) are presented. Each bar represents the Mean ± SEM of 5-6 animals (*p<0.05 compared to time 0 [not shown] **p<0.05 compare to compound 48/80 alone).

Figure 6. Nasal decongestant effect of topically and therapeutically administered Compound A, Compound B and phenylephrine in the anesthetized cat. 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 µl). Note that test drugs were given by the intranasal route 30 minutes after Compound 48/80. Each bar represents the Mean ± SEM of 5-6 animals (*p<0.05 compared to time 0 – not shown); **p<0.05 compare to compound 48/80 alone). Mean heart rate values (beats per min) are in parenthesis.
Figure 7. Effect of Compound A in the absence and presence of JP 1302. Depicted is the impact of the selective α2c antagonist JP 1302 (0.1%, 50 µl) on nasal volume (Panel A) and minimum cross-sectional area (Panel B) produce by topical Compound A (0.3%). Each bar represents the Mean ± SEM of 5-6 animals (*p<0.05 compared to time 0 – not shown); **p<0.05 compare to compound 48/80 alone).

Figure 8. Nasal decongestant effect of orally administered Compound A and Compound B in the conscious dog. Panel A displays the time related actions of Compound 48/80 (3%, 500 µl) on nasal cavity volume size. The maximum congestive action of Compound 48/80 was observed between 120 and 150 minutes post treatment. Panel B illustrates the actions of oral 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 post Compound 48/80 treatment. Each bar represents the Mean ± SEM of 6 animals (*p<0.05 compared to time 0; **p<0.05 compare to compound 48/80 alone).
Table 1 Effect of Oral Selective α2c-Adrenergic Agonists on Minimum Nasal Cross-Sectional Areas in the Cat

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Minimum Cross-Sectional Area (cm²) (60 min post Compound 48/80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.016 ± 0.003</td>
</tr>
<tr>
<td>Compound A</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.023 ± 0.003*</td>
</tr>
<tr>
<td>0.3</td>
<td>0.021 ± 0.005</td>
</tr>
<tr>
<td>1.0</td>
<td>0.035 ± 0.003**</td>
</tr>
<tr>
<td>3.0</td>
<td>0.033 ± 0.005**</td>
</tr>
<tr>
<td>Compound B</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>0.025 ± 0.002*</td>
</tr>
<tr>
<td>1.0</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>3.0</td>
<td>0.036 ± 0.003**</td>
</tr>
<tr>
<td>5.0</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></td>
</tr>
<tr>
<td>0.3</td>
<td>0.030 ± 0.002**</td>
</tr>
<tr>
<td>1.0</td>
<td>0.029 ± 0.004**</td>
</tr>
</tbody>
</table>

Table 1. Minimum-Nasal Cross Sectional Areas in animals treated with Compound A, Compound B or d-pseudoephedrine. Values represent the Mean ± S.E.M. *p<0.05 compared to baseline (data not shown); **p<0.05 vs. control responses.
Table 2. Effect of Oral Selective α2c-Adrenergic Agonists on Heart Rate in the Cat

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

Table 2. Heart rates in animals treated orally with Compound A, Compound B or d-pseudoephedrine. Values represent the Mean ± S.E.M.
Table 3 Effect of Topical Selective α<sub>2c</sub>-Adrenergic Agonists on Heart Rate in the Cat

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Heart rate (bpm) (60 min post Compound 48/80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>204 ± 11</td>
</tr>
<tr>
<td>Compound A</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>210 ± 7</td>
</tr>
<tr>
<td>0.1</td>
<td>202 ± 13</td>
</tr>
<tr>
<td>0.3</td>
<td>207 ± 10</td>
</tr>
<tr>
<td>Compound B</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>210 ± 7</td>
</tr>
<tr>
<td>0.3</td>
<td>212 ± 10</td>
</tr>
<tr>
<td>1.0</td>
<td>208 ± 15</td>
</tr>
<tr>
<td>phenylephrine</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>212 ± 10</td>
</tr>
<tr>
<td>0.1</td>
<td>205 ± 13</td>
</tr>
<tr>
<td>0.3</td>
<td>200 ± 18</td>
</tr>
</tbody>
</table>

Table 3. Heart rates in animals treated topically with Compound A, Compound B or phenylephrine. Values represent the Mean ± S.E.M.
Figure 1

A

Baseline

Compound A
0.1μM

Compound A
1μM

NE 1 mM

Artery

Vein

B

Compound A (logM)

Vessel Constriction (% area decrease)

Vein

Artery

C

Compound B (log M)

Vessel Constriction (% area decrease)
Compound A

Nasal Cavity Volume

Blood Pressure and Heart Rate

Compound B

d-pseudoephedrine

Figure 2
Compound A

Figure 3
Figure 4

A. Nasal Volume

B. Minimum Cross-Sectional Area

C. PK Exposure

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Figure 5

5 day QD dosing paradigm

A

- Cpd 48/80 congestive response
- Day 1: Compound A (1.0 mg/kg)
- Day 5: Compound A (1.0 mg/kg)

1 day BID dosing paradigm

B

- Compound 48/80 congestive response
- Compound A (1.0) + vehicle
- Compound A (1.0) + Compound A (1.0)

Nasal Volume Ratio (Treated/Untreated)

60 Minutes Post Compound 48/80
**Compound A**

**Nasal Volume**

![Graph showing nasal volume changes](graph1.png)

**Minimum Cross-Sectional Area**

![Graph showing minimum cross-sectional area changes](graph2.png)

**Blood Pressure and Heart Rate**

![Graph showing blood pressure changes](graph3.png)

**Compound B**

**Nasal Volume**

![Graph showing nasal volume changes](graph4.png)

**Minimum Cross-Sectional Area**

![Graph showing minimum cross-sectional area changes](graph5.png)

**Blood Pressure and Heart Rate**

![Graph showing blood pressure changes](graph6.png)

**phenylephrine**

**Nasal Volume**

![Graph showing nasal volume changes](graph7.png)

**Minimum Cross-Sectional Area**

![Graph showing minimum cross-sectional area changes](graph8.png)

**Blood Pressure and Heart Rate**

![Graph showing blood pressure changes](graph9.png)

Figure 6
Figure 7

A. Nasal Volume

B. Minimum Cross-Sectional Area

Treatment (mg/kg)

controls
JP 1302 (0.1%)
Compound A (3%)

Minimum Cross-Section Area (cm²)

controls
JP 1302 (0.1%)
Compound A (3%)

*p<0.05

**
Figure 8

A

- ○ Untreated nares
- ● controls - compound 48/80 treated nares

Minutes (Post compound 48/80)

Nasal Volume (cm³)

Maximum congestion

B

Compound A

- controls
- Compound A (1 mg/kg)
- Compound A (3 mg/kg)
- Compound A (5 mg/kg)

150 min post Compound 48/80

Nasal Cavity Volume (% of Baseline)

C

Compound B

- controls
- Compound B (1 mg/kg)
- Compound B (2 mg/kg)
- Compound B (3 mg/kg)
- Compound B (5 mg/kg)

150 min post Compound 48/80

Nasal Cavity Volume (% of Baseline)