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A new nasal decongestant, A-57219: a comparison with oxymetazoline

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2-(4-Amino-3,5-dichlorobenzyl)imidazoline hydrochloride (A-57219), has α_1-agonist/α_2-antagonist activity and was more effective and long-acting than oxymetazoline on canine nasal mucosa, in-vitro and in-vivo. Upon intranasal administration to dogs, the compound was devoid of systemic effects up to a concentration 1000 times that needed for local decongestant effect (1·65 μg, atomized from a 1 μg mL^{-1} solution) suggesting limited mucosal absorption. After nasal administration to rats for 15 days at a concentration 1000 times greater than that required for nasal decongestion, no mucosal tissue toxicity or systemic effects were seen.

During our investigations on new selective α-adrenergic agents, we uncovered a new compound 2-(4-amino-3,5-dichlorobenzyl)imidazoline hydrochloride (A-57219), which has the in-vitro properties of α_1-agonism/α_2-antagonism. This profile suggested a novel nasal decongestant. It has been previously shown (Anderson & Bende 1984; Berridge & Roach 1986; Hall & Jackson 1968; Ichimura & Jackson 1984) that the nasal mucosa in dogs and man contain α_1- as well as α_2-adrenoceptors. Drugs that selectively stimulate either α_1- or α_2-adrenoceptors cause nasal decongestion.

Clinically used nasal decongestants such as phenylephrine and methoxamine are selective α_1-agonists whereas oxymetazoline and naphazoline stimulate both α_1- and α_2-receptors, with preference for the latter receptor subtype. A-57219 appeared to have a very desirable nasal decongestant profile. This compound could produce decongestion by α_1-mediated contraction of capacitance vessels, but by virtue of its α_2-antagonism, would not compromise blood flow. In addition, it would block any endogenous noradrenaline-mediated α_2-constriction of the resistance vessels. Such a profile should allow for reduced rebound phenomenon associated with currently available nasal decongestants.

Materials and methods

α_1-Adrenoceptor agonist activity using rabbit isolated aorta. Female rabbits, 2–5 kg, were killed by cervical dislocation and the descending aorta removed and placed in Krebs buffer, prepared daily (mM: NaCl 119, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $CaCl_2$ 2·5, glucose 11, NaEDTA 0·03, and ascorbic acid 0·3) saturated with 95% O_2 and 5% CO_2 and adjusted to pH 7·4.

A helical strip of aorta was mounted in a 10 mL tissue bath containing continually aerated Krebs buffer at 37 ± 0·5 °C and attached to a force transducer (Grass or Statham). An initial tension of 2 g was applied, then the tissue was allowed to equilibrate for 1 h during which time it was washed four times and the tension reset to 2 g until it had stabilized. Contractions, measured by the force transducers, were recorded on a Grass Model 7 polygraph.

Phenoxybenzamine-treated dog saphenous vein. Rings (3–4 mm wide) of lateral saphenous veins excised from beagle dogs of either sex were suspended in 10 mL tissue baths containing bicarbonate buffer (mM: NaCl 19, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·5, KH_2PO_4 1·2, $NaHCO_3$ 20, dextrose 11, ascorbic acid 0·3, NaEDTA 0·03, cocaine 0·03, hydrocortisone hemisuccinate 0·04, and propranolol 0·004) gassed with 95% O_2 and 5% CO_2 at 37 °C, pH 7·40. Isometric contractions of the tissues, preloaded with a tension of 2 g, were measured with Grass FT03 strain gauges and recorded on a Grass Model 7 polygraph. Following an equilibration of 15–20 min and maximal contraction by noradrenaline (10^{-4} M), the tissues were washed for 60 min at which time they were exposed to phenoxybenzamine (PBZ) (1×10^{-7} M) for 30 min followed by a 60 min washout. Tissues were then readjusted to 2 g tension, and a control cumulative concentration-response curve obtained for the standard agonist, noradrenaline. After its washout (45–60 min), tissues were again equilibrated.

For agonists, a cumulative concentration-response curve for the test compound agonist was then obtained. For antagonists, each tissue received a single dose of the test compound for 30 min, followed by a repeat of the noradrenaline response curve. Each concentration of antagonist was tested simultaneously in four replicates, and pA_2 values for the antagonists were determined using the classical Schild analysis (Arunlakshana & Schild 1959).

Radioligand binding assays

Tissue preparation. Twenty male Sprague-Dawley rats weighing 250–350 g were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.), and the brains and livers removed and placed in assay buffer (Tris-HCl 50 mM pH 7·7 at 25 °C) at 4 °C. Cerebral cortices were separated and tissues were pooled, weighed, then separately homogenized in 20 volumes of preparation

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buffer (Tris-HCl 50 mM pH 7·7 at 25 °C containing 5 mM EDTA), using a Tekmar SDT homogenizer at full speed for two 10 s bursts. The homogenates were centrifuged at 50 000*g* (4 °C) for 10 min, and the supernatant discarded. The pellets were resuspended by homogenization as above in 20 volumes of preparation buffer, recentrifuged for 10 min, and the supernatant was again discarded. The final pellet was resuspended in 6·25 volumes of assay buffer, flash frozen in liquid nitrogen, and stored at −70 °C until the day of the experiment. Tissues were thawed at room temperature (20 °C), and thereafter maintained at 4 °C.

Assay methods. All binding assays were performed as previously described by DeBernardis et al (1985).

Nasal resistance in the dog. Following the general method of Stovall & Jackson (1967), beagle dogs of either sex, 9–12 kg, were anaesthetized with pentobarbitone (30 mg kg^{-1} i.v.) and supplemented as required. The dogs were intubated with a cuffed endotracheal tube and were ventilated with room air by means of a Harvard respiration pump. Arterial blood pressure was recorded from a femoral artery using a Statham P23Gb pressure transducer. A tachygraphic recording of heart rate was obtained from the blood pressure signal. All recordings were made on a Grass Model 7 polygraph. The dogs were maintained at 39 °C.

A constant flow of air (2 L min^{-1}; provided by an anaesthetic machine CAECO) was administered into the nasal cavity through a 6 cm long plastic tube tapered to a diameter of 6 mm to fit into the right nostril. The air perfused the nasal cavity and exited through the mouth. The resistance to the air flow exerted by the large surface area of the nasal mucosa was measured as a nasal pressure in cm of water, using a Model MP45 Validyne transducer. For drug administration, a by-pass arrangement in the air tubing permitted air to flow through a 690 Ultrasonic Humidifier containing the nebulized drugs. This instrument atomized the drug solution into a vapour which was carried by the air flow into the nasal passages. It was calibrated to nebulize 1·65 mL of fluid over 5 min perfusion. The nasal resistance, heart rate and blood pressure were monitored before and for 2 h following, drug administration.

Local toxicity in rats. Male Sprague-Dawley rats, 5 weeks old, were lightly anaesthetized with ketamine chloride (i.p.) before administration of 0·04 mL day^{-1} (0·02 mL/nostril) phosphate-buffered saline (vehicle), oxymetazoline or A-57219. Test compounds were dissolved in the vehicle at concentrations of 0·05, 0·15, 0·5 or 1·5%. All solutions were adjusted to pH 7. Dosages were administered for 15 consecutive days. At necropsy, the nasal cavity of each rat was prepared for histopathologic examination according to Young (1981). Heamatoxylin- and eosin-stained nasal cavity sections from four distinct regions were subsequently evaluated microscopically for evidence of treatment-related changes.

Results and discussion

It is known (Hall & Jackson 1968) that α_1-agonists (e.g., methoxamine) act on the nasal mucosa to produce constriction, thereby decreasing nasal airway resistance and producing a decongestive effect. Recently Anderson & Bende (1984) found that α_2- but not α_1-agonists, decrease the blood flow in the human nasal mucosa. It has been suggested (Hall & Jackson 1968) that when the nasal blood flow has been reduced to the point where tissue metabolism produces acidosis, oedema will be generated in the surrounding tissue and this could possibly account for the 'rebound' congestion which is often observed on prolonged use of nasal decongestants. Accordingly, an α_1-agonist/α_2-antagonist might be ideally suited as a nasal decongestant since this type of agent would contract the nasal mucosa (via stimulation of the α_1-receptor) thereby reducing nasal airway resistance, while at the same time ensuring an adequate blood flow to the nasal mucosa (by virtue of its ability to block the α_2-receptor). We felt this might avoid the rebound phenomena.

As shown in Table 1, A-57219 possesses α_1-agonistic activity in the rabbit aorta (α_1-selective tissue) (Docherty et al 1981) slightly more potent than noradrenaline but less potent than oxymetazoline.

Table 1. Indexes and ED50 values in the rabbit aorta (α_1) and PBZ-pretreated dog saphenous vein (α_2).

Compound	(ED50 NA/ ED50 CPD) Index[a,b]	n	E_{max}%	ED50 NA ($\times 10^7$)[a]	ED50 CPD ($\times 10^7$)[a]
A-57219[c]	1·3 ± 0·2	5	109 ± 5	0·67 ± 0·14	0·53 ± 0·08
Oxymetazoline[c]	8·5 ± 3·8	4	90 ± 1	2·03 ± 0·9	0·28 ± 0·05
A-57219[d]	—	8	—	—	ANT[e]
Oxymetazoline[d]	0·83 ± 0·09	3	47 ± 3	7·8 ± 1·2	9·3 ± 0·7

[a] Mean of n experiments ± s.e.m. NA (noradrenaline), CPD (compound). [b] Because the ED50 NA and the ED50 CPD were both determined in the same tissue (see Materials and methods) these values are 'paired', and the index number must be calculated for each experiment and then summed to produce a mean. It is not appropriate to divide the mean ED50 NA by the mean ED50 CPD since these are not independent observations. For a discussion of paired comparisons, see: Mainland (1963). [c] α_1-Activity. [d] α_2-Activity. [e] Antagonist, pA_2 6·51.

Table 2. Radioligand binding affinities.

	α_1		α_2	
Compound	K_i[a], nM	n	K_i, nM	n
A-57219	553 (424–721)	4	25 (20–32)	5
Oxymetazoline	346 (265–451)	3	27 (10–74)	5
Noradrenaline	390 (370–410)	6	37 (35–39)	6

[a] Geometric mean of n duplicate determinations with 90% confidence limit shown in parentheses.

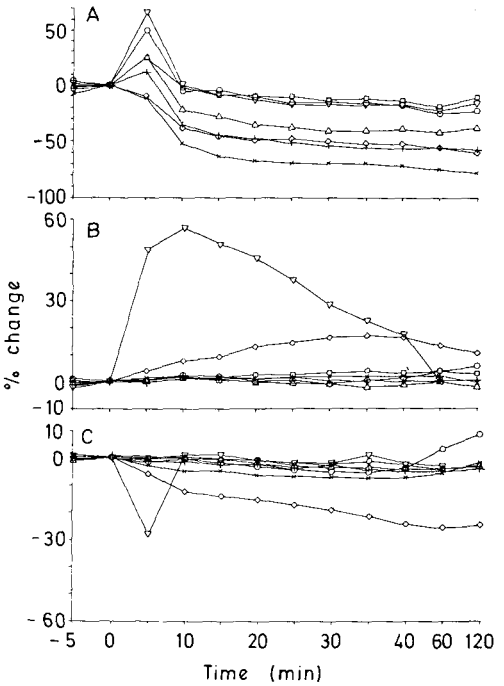

FIG. 1. Effects on airway resistance and systemic absorption in the anaesthetized dog for oxymetazoline. The initial increase in nasal airway resistance is artifactual and reflects the manipulation of the nebulizer valve. The dose in mg is the calculated amount of test compound consumed by the nebulizer during the 5 min administration period. Only a portion of that dose actually acts on the tissue in the open system. In all graphs: □ 0 mg, n = 6; ○ 0·000165 mg, n = 4; △ 0·00165 mg, n = 4; + 0·0165 mg, n = 6; × 0·165 mg, n = 4; ◇ 1·65 mg, n = 4; ▽ 16·5 mg, n = 1. For nasal resistance (A): △ $P \leq 0.05$, + $P \leq 0.005$, × $P \leq 0.001$, ◇ $P \leq 0.005$. For blood pressure (B): ◇ $P \leq 0.05$. For heart rate (C): ◇ $P \leq 0.005$.

However, unlike oxymetazoline, A-57219 possesses no α_2-agonistic activity in the phenoxybenzamine-pretreated dog saphenous vein (PBZ-DSV) (α_2-selective tissue; Constantine et al 1982; Kyncl et al 1985; Table 1). Rather, the compound showed α_2-antagonism with low to moderate potency. In radioligand binding experiments (Table 2), A-57219 exhibited affinity for both the α_1- as well as the α_2-receptor comparable with either noradrenaline or oxymetazoline.

Table 3. Effects of intranasal administration of A-57219 in the anaesthetized dog.

Dose[a]	n	Time[b]	Nasal Resistance[c]	BP[c]	HR[c]
Saline	6	15	−10	+2	−2
		60	−25	+3	−4
		120	−20	+4	−4
0·000165	1	15	−15	+2	+5
		60	−18	0	−4
		120	−20	−3	−8
0·00165	4	15	−35*	+3	−2
		60	−35*	+3	−2
		120	−28*	+2	+7
0·0165	4	15	−50**	−1	−8
		60	−50**	+3	0
		120	−38**	0	−4
0·165	4	15	−65***	+2	−8
		60	−70***	+1	−11
		120	−65***	+1	−8
1·65	4	15	−75****	+10	−6
		60	−80****	+9	−15
		120	−75****	+6	−12

[a] Dose in mg administered to each animal.
[b] Time in min after dosing was begun. Dose administered during initial 5 min.
[c] Change from predose baseline expressed as a per cent. Statistically significant differences indicated as follows: * ($P \leq 0.05$), ** ($P \leq 0.02$), *** ($P \leq 0.005$), **** ($P \leq 0.001$).

To evaluate the nasal decongestant activity associated with A-57219 we employed a modification of the rhinometric technique described by Stovall & Jackson (1967).

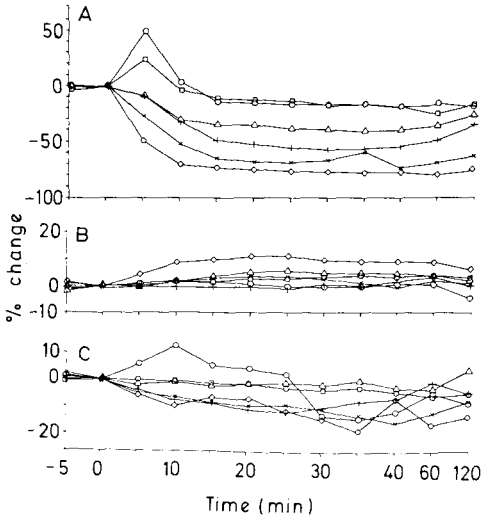

FIG. 2. Effects on airway resistance and systemic absorption in the anaesthetized dog for A-57219. In all graphs: □ saline, n = 6; ○ 0·000165 mg, n = 1; △ 0·00165 mg, n = 4; + 0·0165 mg, n = 4; × 0·165 mg, n = 4; ◇ 1·65 mg, n = 4. For nasal resistance (A): △ $P \leq 0.005$, + $P \leq 0.02$, × $P \leq 0.005$, ◇ $P \leq 0.001$. (B) Blood pressure, (C) heart rate.

As can be seen from Table 3, A-57219 shows a dose-related decrease in nasal resistance which is significant at the level of $P \leq 0{\cdot}05$ or better. In addition, the decreased nasal resistance was observed throughout the 2 h testing period. Figs 1 and 2 show a comparison of the compound with oxymetazoline. Both compounds produce a potent decrease in the nasal airway resistance. This effect was not accompanied by any changes in systemic arterial blood pressure and/or heart rate until the concentration in the aerosol was increased 1000 times above the minimally effective decongestant dose. In fact, the effect of A-57219 on blood pressure and heart rate even at the highest concentration, was minimal.

The local toxicity of A-57219 and oxymetazoline was evaluated (concentrations 0·05–1·5%) in rats following 15 days of daily intranasal administration. Although oxymetazoline at the concentration used clinically (0·05%) produced no toxicity, at higher concentrations (0·5–1·5%) toxicity (resulting in ataxia, rough coats, pale skin, possible localized haemostasis and death) was seen, and at concentrations of 0·5%, toxicity (60% deaths) suggestive of systemic absorption of the compound was observed. This was not observed with any dose of A-57219. Microscopic lesions of attenuated, ulcerated and/or ruptured mucosa with ensuing exudate were observed with oxymetazoline at the 1·5% concentration while A-57219 was devoid of any toxicity either systemically or on the mucosa at concentrations ranging from 0·5–1·5%. Since low toxicity for the cells of the mucosa upon topical administration is an essential requirement for a chronically-used nasal decongestant (Jackson et al 1976), A-57219 appears to offer a considerable advantage over oxymetazoline as a nasal decongestant.

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REFERENCES

Anderson, K. E., Bende, M. (1984) Ann. Otol. Rhinol. Laryngol. 93: 179–182

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Berridge, T. L., Roach, A. G. (1986) Ibid. 88: 345–354

Constantine, J. W., Lebel, W., Archer, R. (1982) Eur. J. Pharmacol. 85: 325–329

DeBernardis, J. F., Kerkman, D. J., Winn, M., Bush, E. N., Arendsen, D. L., McClellan, W. J., Kyncl, J. J., Basha, F. Z. (1985) J. Med. Chem. 28: 1398–1404

Docherty, J. R., Constantine, J. W., Starke, K. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 317: 5–7

Hall, L. J., Jackson, R. T. (1968) Ann. Otol. Rhinol. Laryngol. 77: 1120–1130

Ichimura, K., Jackson, R. T. (1984) Arch. Otolaryng. 110: 647–651

Jackson, R. T., Per-Lee, J. H., Rooker, D. W., Turner, J. S. (1976) in: Avery, G. S. (ed.) Drug Treatment, Principles and Practice of Clinical Pharmacology and Therapeutics. Adis Press, Sydney, pp 252–274

Kyncl, J. J., Buckner, S. A., Bush, E. N., DeBernardis, J. F., Lin, C. T., Warner, R. B. (1985) Fed. Proc. 44: 1466

Mainland, D. (1963) in: Elementary Medical Statistics. 2nd edn, Saunders, Philadelphia, pp 202–203

Stovall, R., Jackson, R. T. (1967) Ann. Otol. Rhinol. Laryngol. 76: 1051–1059

Young, J. T. (1981) Fund. Appl. Toxicol. 1: 309–312