Medetomidine-Induced Alterations of Intraocular Pressure and Contraction of the Nictitating Membrane

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The alpha-2 adrenoceptor agonist, medetomidine (MED), was examined for effects on: (1) intraocular pressure (IOP) in normal and sympathectomized (SX) rabbits; (2) IOP in normal rabbits pretreated with the alpha-2 antagonist idazoxan; (3) contractions of the cat nictitating membrane (CNM) elicited by nerve stimulation and intra-arterial (IA) norepinephrine. Unilateral topical administration of MED (7.5–75 \( \mu \)g) caused dose-dependent, bilateral IOP reduction in normal eyes, but MED (25 \( \mu \)g) had no appreciable hypotensive activity in SX eyes. The ocular hypotensive effect of MED (25 \( \mu \)g) was antagonized by treatment with idazoxan (100 \( \mu \)g, bilaterally), a relatively selective alpha-2 antagonist. MED and dexmedetomidine (DMED) also inhibited frequency-related contractions of CNM induced by electrical stimulation of the cervical sympathetic trunk. Rauwolscine (100 \( \mu \)g, IA) shifted the MED dose response in the CNM to the right indicative competitive antagonism, whereas SK&F 104078 (300 \( \mu \)g, IA), a relatively dose-selective postjunctional alpha-2 antagonist, had no effect on DMED suppression. These results show that MED lowers IOP in part, by interacting with alpha-2 adrenoceptors located on sympathetic nerve endings. An effect of MED on imidazoline sites may also be possible.


Stimulation of alpha-2 adrenoceptors lowered intraocular pressure (IOP) in animals and humans in 1966.1 The alpha-2 agonist prototype, clonidine, decreased the rate of production of aqueous humor in intact, arterially perfused cat eyes2 and in humans.3 Potential peripheral modes for the ocular hypotensive action of clonidine included inhibition of neurotransmitter release from sympathetic nerve endings4 and suppression of adenylate cyclase activity in the ciliary body.5

More recently, apraclonidine inhibited the transient rise in IOP in humans6 and rabbits7 after laser surgery of the anterior segment and suppressed aqueous flow in humans.8 Apraclonidine has significantly less ability than clonidine to penetrate the blood–brain barrier, suggesting a possible peripheral site of action. The clinical success of apraclonidine has renewed the interest of ophthalmologists in alpha-2 agonists as potential antiglaucoma agents.

Medetomidine (4-[1-2,3-dimethylphenyl) ethyl]-1H-imidazole) is a newly developed alpha-2 agonist that has potent and selective activity on alpha-2 adrenoceptors.9 This report describes the ocular hypotensive activity of medetomidine (MED) in normal and sympathectomized (SX) rabbits and the effects of MED and dexmedetomidine (DMED) on neuronally mediated contractions of the cat nictitating membrane.

Materials and Methods

Animals and Animal Care

Dutch belted rabbits and New Zealand white rabbits were obtained from local suppliers and maintained under controlled conditions of temperature and humidity on a 12 hr:12 hr light–dark cycle. Mongrel cats of either sex were maintained under similar conditions. Rabbits underwent surgical sympathectomy as previously reported.10 Rabbits were allowed to recover for 10 days after sympathectomy before they were used in experiments. All animal experiments were conducted in accordance with the ARVO Resolution on the Use of Animals in Research and with protocols approved by the Animal Care and Use Committee of the Baylor College of Medicine, Houston, Texas.

IOP Measurement in Rabbits

IOP of male and female rabbits was measured with a BioRad pneumotonometer, Digilab model 30D (Biorad Laboratories, Cambridge, MA). The 25 \( \pm \) 2 mm Hg factory calibration verifier was used at the beginning and end of each experiment to confirm stability of the instrument. For IOP measurements, animals were removed from their cages, and placed indi-

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Presented at the Annual ARVO meetings, May 1990, and at The Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas.

Supported in part by grant EY06338 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

Submitted for publication: October 3, 1990; accepted May 20, 1991.

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OVA statistics were applied in comparing eyes treated and control eyes in normal or SX rabbits. AN-bility of a significant difference between MED-treated and MED only.

Topical Administration of Drugs

Medetomidine (dexmedetomidine), rauwolscine, idazoxan, and SK&F 104078 were obtained from Farmos (Turku, Finland), Carl Roth KG (Karlsruhe, Germany), Reckitt and Coleman (Hull, England), and Smith Kline & French (King of Prussia, PA), respectively. T-61 (mebezonium iodide, embutramide, and tetracaine) was obtained from Hoechst/Roussel Agri. Vet (Somerville, NJ).

Topical Administration of Drugs

In rabbit experiments, drugs were dissolved in deionized water and applied topically (25 μl) at varying concentrations. In all cases of unilateral treatment with MED, the contralateral (fellow) eye received a 25-μl application of vehicle alone. In experiments involving antagonism of MED responses by idazoxan, the inhibitor was applied topically to both eyes 30 min before a challenge dose of MED.

Cat Nictitating Membrane Preparation

Preparation of the cat nictitating membrane (CNM) for use as a pharmacologic test system was described in detail previously. Animals were anesthetized with alpha-chloralose (40 mg/kg) and urethane (500 mg/kg) administered intraperitoneally. Respiration was maintained with a mechanical ventilator after tracheal cannulation. The femoral and lingual arteries were catheterized for measurement of blood pressure and intra-arterial (IA) drug administration, respectively. The preganglionic sympathetic trunk was isolated and fitted with shielded, miniature, bipolar stimulating electrodes. Subsequently, the nictitating membrane itself was attached to a force transducer with a suture that passed through the cartilaginous portion of the membrane. Frequency–response curves were produced by measuring contractions of the CNM in response to 2, 4, 6, and 8 Hz of electrical stimulation to the sympathetic trunk at 4 volts. In addition, IA injection of norepinephrine (NE, 10 μg) stimulated postjunctional alpha-1 adrenoceptors on the CNM smooth muscle. Neural stimulation, NE injection, and saline flushing (400 μl) were under automatic control with a Sinclair ZX81 microcomputer (Timex Computer Co., Waterbury, CT). The output from the force transducer was recorded on a Grass Model 7D polygraph (Grass Instrument Corp., Quincy, MA). In each experiment, the test alpha-2 agonist, MED (dissolved in 100 μl of deionized water) was injected into the lingual artery catheter and was washed through with 400 μl of saline. A series of increasing doses of MED (or DMED) was injected, and dose-related effects on the CNM frequency and NE contractions were recorded. After an increasing cumulative dose series of MED (or DMED), relatively selective alpha-2 antagonists (rauwolscine or SK&F 104078) were administered (100 μg IA). An MED (or DMED) test series was then repeated in the presence of the antagonist, with appropriate increases in doses of agonist. After completion of CNM experiments, animals were killed by injection of T-61 into the femoral vein.

Results

IOP Responses in Normal and SX Rabbits

Medetomidine (MED) was tested topically at 2.5, 7.5, 25, and 75 μg in the eyes of New Zealand white (NZW) rabbits. Rabbits were allowed to recover for 1 week before exposures to MED. As shown in Figures 1 and 2, all concentrations lowered IOP in a time- and dose-related fashion. The ocular hypotensive responses in the MED-treated (ipsilateral) eyes were slower in onset and smaller in magnitude as the dose was increased (Fig. 1). In contrast, the response in the contralateral eyes was immediate and dose related (Fig. 2). Significant hypotensive responses to 2.5 and 7.5 μg MED were seen at 1 hr in the treated eyes, whereas significant responses to higher concentrations (25 and 75 μg MED) occurred later, at 2 hr. The ocular hypotensive effect of the highest concentration (75 μg MED) lasted approximately 3 hr in the treated eye. In contrast, the IOP response in the contralateral (vehicle-treated) eyes was directly related to the dose applied (Fig. 2). The lowest concentration of MED (2.5 μg) did not lower IOP significantly in the contralateral eyes, whereas all higher concentrations (7.5, 25, 75 μg) were effective. If the ocular hypotensive response in terms of area under the curve is considered (IOP as a function of time), the contralateral IOP responses to 25 and 75 μg MED were greater than the ipsilateral IOP responses to the same doses.
SX animals were allowed 10 days to recover from surgery before they were tested with MED. MED (25 μg) was also an effective ocular hypotensive agent in the normal eyes of unilaterally SX Dutch belted (DB, pigmented) rabbits (Fig. 3). As in NZW rabbits, the onset of the IOP response in intact DB rabbits occurred earlier (0.5 hr) in the contralateral eyes than in the ipsilateral eyes at 2 hr (Fig. 4). However, the magnitude of the IOP response in DB rabbits was slightly less than that in NZW rabbits at comparable doses of MED. In contrast, the SX eyes of DB rabbits did not show an ocular hypotensive response, regardless of whether MED was applied to the normal or to the denervated eye. When MED was applied topically to the SX eyes of DB rabbits, a trend toward an increase in IOP began at 0.5 and at 1 hr (Fig. 4).

As shown in Figure 5, bilateral pretreatment with idazoxan, a relatively selective alpha-2 adrenoceptor antagonist, inhibited the ocular hypotensive effect of MED in the ipsilateral (upper panel; treated) and contralateral (lower panel) eyes of NZW rabbits. In contrast, MED alone (25 μg topically) lowered IOP approximately 8 mm Hg in the ipsilateral and contralateral eyes of normal rabbits. Therefore, Idazoxan (IDAZ 100 μg, 25 μl) antagonism was effective bilaterally in rabbits treated unilaterally with MED.
Fig. 3. Ocular hypertensive response to medetomidine when applied topically to innervated eyes of unilaterally sympathectomized Dutch Belted rabbits. The contralateral (sympathectomized) vehicle-treated eye did not respond. Asterisks denote statistical significance between intraocular pressure (IOP) values in treated compared with control eyes.

Alteration of Noradrenergic Function in the Cat Nictitating Membrane (CNM)

This preparation was used to confirm the prejunctional (neuronal) activity of MED and to assess the relative activity of MED at pre- and postjunctional sites, i.e., to determine the alpha-2:alpha-1 activity ratio. It is used because ocular adnexa and the ciliary body receive innervation from the same source—the superior cervical ganglion. Although alpha-2 activity of MED was examined in other organ systems, the CNM was used as an ocular paradigm to document neuronal activity of a variety of autonomically active drugs.

MED produced dose-related inhibition of CNM contractions elicited by frequency-related, electrical stimulation of the sympathetic trunk (Fig. 6). With increasing doses of MED, an increased response to IA NE indicative of the additive alpha-1 activity of MED and NE occurred. Because MED is a racemic mixture, the active dextrorotatory isomer, dexmedetomidine (DMED) was used as a comparison in this preparation. Although other frequencies were tested in all experiments, Figure 7 shows the effects of MED and DMED on electrically induced contractions of the CNM at 4 Hz. Rauwolscine (100 ng, IA), a pre- and postjunctionally active alpha-2 antagonist, shifted the MED dose–response curve approximately 1 log unit to the right, indicative of competitive antagonism. In contrast, SK&F 104078 (300 µg, IA), an antagonist that is purported to be relatively dose-selective for postjunctional alpha-2 receptors, did not shift the dose–response curve for DMED (Fig. 7). Similar re-
Fig. 6. Medetomidine (Med)-induced alterations in cat nictitating membrane responses to electrical stimuli (square-wave pulses of 0.8 msec duration for 5 sec at 4 volts) and norepinephrine (NE), intra-arterial (IA). Panels show: control responses (gram of force); responses to cumulative administration of medetomidine 0.3, 1.0, 3.3 μg IA, respectively; responses 2-1/2 min after 100-μg IA rauwolscine; responses to cumulative administration of medetomidine 3.3, 10.0, and 33.3 IA μg after rauwolscine.
tools for investigating the functions of alpha-2 adrenoceptors in the central nervous system (CNS) and on peripheral sympathetic nerve endings.14 Thus, pre- and postjunctional alpha-2 adrenoceptors may be important therapeutic targets for the development of novel antiglaucoma drugs.

Recently, MED, an alpha-2 agonist, was introduced in Scandinavia as a veterinary sedative-anesthetic drug with anxiolytic properties. In contrast to clonidine, MED is a specific and selective full agonist on alpha-2 adrenoceptors.13 MED is highly lipid soluble and has a rapid onset of action but shorter duration of action than clonidine at equivalent doses. In humans, MED caused dose-dependent depression of heart rate, blood pressure, salivation, and alertness, presumably by activating inhibitory alpha-2 adrenoceptors in the central nervous system (CNS) and on peripheral sympathetic nerve endings.14

In this study, MED was evaluated for activity on IOP in normal and SX rabbits and on contractions of the CNM elicited by neural stimulation and IA injection of NE. The latter model was used to assess the activity of MED on peripheral alpha-2 and alpha-1 adrenoceptors. The competitive antagonism of the action of MED on neurally mediated contractions of the CNM by rauwolscine showed a potential peripheral site of action on sympathetic nerve endings. Evaluation of MED in SX rabbits was used to confirm the level of involvement of the sympathetic nervous system in the intraocular hypotensive action.

MED produced dose-related lowering of IOP bilaterally at concentrations of 7.5 µg or greater. As the dose of MED was increased, the drop in IOP in the ipsilateral (treated) eye was delayed as described for another alpha-2 agonist, UK14, 304-18.15 Moreover, the onset of ocular hypotensive effect in the contralateral eye preceded that in the ipsilateral (MED-treated) eye by 0.5–1.5 hr, depending on the dose. At the concentrations tested, the duration of action of MED ranged from 1–3 hr. Generally, MED was slightly less efficacious in normal, pigmented rabbit eyes than in the eyes of normal albino rabbits. A similar effect was reported previously for another alpha-2 agonist (clonidine) in pigmented vs albino rabbits and was ascribed to nonspecific binding to pigment.16

Although no significant mydriatic effects to topical MED were seen in rabbits, intravenous (i.v.) doses of 3 and 100 µg/kg produced mydriasis in anesthetized rats.9 The order of potency in the rat mydriasis study was: medetomidine > detomidine > clonidine > UK14, 304-18 > xylazine. Mydriasis was attributed to alpha-2 adrenoceptor stimulation based on antagonism by idazoxan, a relatively selective alpha-2 agonist. Experiments showed that idazoxan also antagonized the ocular hypotensive effect of MED in rabbits.

MED was also tested for IOP-lowering activity in unilaterally SX Dutch belted rabbits. An intermediate concentration of MED (25 µg) produced depression of IOP in eyes with intact sympathetic innervation, regardless of whether the drug was applied unilaterally to the denervated or innervated eye. In contrast, no ocular hypotensive response was seen in the SX eyes. These results suggest that, for maximal activity, MED requires intact postganglionic sympathetic innervation, but does not preclude action within the CNS.

Bilateral topical pretreatment with idazoxan (IDAZ, 100 µg), which binds to alpha-2 and imidazoline sites,17 antagonized the lowering of IOP by topical MED (25 µg) in ipsilateral (treated) and contralateral eyes. These data show that the ocular hypotensive action of MED is mediated by alpha-2 adrenoceptors, but may also suggest action on imidazoline sites.17 Because topically applied MED and IDAZ probably reach central and peripheral sites of action, this information does not provide evidence regarding the relative importance of central vs peripheral alpha-2 adrenoceptors or imidazoline sites in the action of MED.
MED. However, unpublished data with L-659,066, a peripherally acting alpha-2 antagonist, and the current results with the CNM, suggest that MED has significant effects on peripheral alpha-2 adrenoceptors in the eye and its adnexa.

To confirm the possibility of a peripheral site of action for MED on sympathetic, dose-response curves for MED were conducted on the CNM in the absence and presence of rauwolscine, a pre- and postjunctional alpha-2 adrenoceptor antagonist, and SK&F 104078, a postjunctional alpha-2 adrenoceptor antagonist.12 Previous studies showed that unlike rauwolscine, SK&F 104078 does not inhibit prejunctional alpha-2 adrenoceptors in the autoperfused rat hindlimb, but both SK&F 104078 and rauwolscine (1 mg/kg, i.v.) antagonize postjunctional alpha-2 adrenoceptor-mediated vasoconstrictor responses to Azenpoxole (BHT 933).18 Rauwolscine (100 µg, IA)18 reversed the effects of MED and shifted the dose-response curve in the CNM more than 1 log unit to the right indicative of competitive antagonism. In contrast, a three-fold higher concentration of SK&F 104078 (300 µg, IA) had no effect on the dose-response curve to DMED, an active enantiomer of MED, in the CNM. These results show that the peripheral suppression of sympathetic activity by MED in the CNM was mediated by prejunctional (neuronal) alpha-2 adrenoceptors. In contrast, data in the rabbit iris-ciliary body preparation suggest that rauwolscine and SK&F 104078 will reverse the effects of alpha-2 agonists.12

Additional experiments are necessary to quantify the relative contributions of pre- and postjunctional alpha-2 adrenoceptors to the ocular hypotensive actions of clonidine-like agents and to substantiate the presumed presence of alpha-2 adrenoceptors subtypes12 and/or imidazoline sites17 in the eye.

Key words: alpha-2 adrenoceptors, intraocular pressure, rabbits, cat nictitating membrane, medetomidine

Acknowledgments

The authors thank Merle Husband, Zenovia Pearson, and Vereen Williams for secretarial assistance.

References


