AQUEOUS HUMOR DYNAMICS IN MONKEYS IN RESPONSE TO THE KAPPA OPIOID AGONIST BREMAZOCINE

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ABSTRACT

Purpose: To determine the effects of the kappa opioid agonist, bremazocine (BRE), on intraocular pressure (IOP) and aqueous humor dynamics in normotensive cynomolgus monkeys.

Methods: IOP, pupil diameter, refraction, aqueous humor flow, and mean arterial pressure (MAP) were measured following unilateral topical application of 1 to 100 µg BRE. IOP and MAP responses to 100 µg BRE were repeated during intravenous infusion of angiotensin II (ATII). IOP and MAP responses to BRE were also measured following pretreatment with the opioid receptor antagonists norbinaltorphimine (nor-BNI) or naloxone. Outflow facility was measured following unilateral intracameral exchange with 0.01 to 100 µg/mL BRE. IOP, aqueous humor flow, pupil, and MAP were measured after unilateral intracameral bolus injection of 1 µg of BRE.

Results: Unilateral topical BRE caused a dose-related reduction in IOP and aqueous humor flow in both eyes and in MAP. Pupil miosis occurred at the 100-µg dose. There was no effect on refraction. IOP and MAP decreases after 100 µg of BRE were eliminated by ATII infusion. Differential IOP effects after 10-µg topical BRE doses were not eliminated by nor-BNI or naloxone. Unilateral intracameral bolus injection of BRE decreased IOP in both eyes but had no effect on MAP or aqueous humor flow. Outflow facility was unchanged after intracameral exchange with BRE.

Conclusions: The IOP response to high doses of BRE in monkeys can be attributed to peripheral or central effects on MAP. The IOP-lowering response to topical BRE is due to aqueous humor flow suppression via non-opioid receptor stimulation. Some components of the IOP response are mediated by unknown mechanisms.

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INTRODUCTION

The first line of intraocular pressure (IOP)-lowering therapy for glaucoma is usually pharmacologic in the form of topical eye drops.[1] Over time, most patients will use more than one medication, comprising differing pharmacologic classes with varying mechanisms of action. There is evidence that opioid agonists may represent another class of agents that may be developed for glaucoma therapy. Opioid agonists were shown in early studies to lower IOP in humans and rabbits regardless of the method of administration.[2-4]

There are three main subtypes of opioid receptors; mu, delta, and kappa.[5] Delta and kappa agonists decrease IOP[6-9] and aqueous humor flow rates in rabbit eyes.[6,7,9] The IOP-lowering response in rabbits is dependent on intact sympathetic innervation.[9] Previous studies with the mu opioid receptor agonist heroin[2] and more recent studies with the kappa opioid receptor agonist bremazocine (BRE)[7] found an increase in outflow facility in rabbits. In rabbits, the IOP-lowering response to kappa opioid agonists is accompanied by increased levels of natriuretic peptides in the aqueous humor.[6,7,10] Increased levels of atrial natriuretic peptide correlate with activation of K^+(ATP) channels assumed to be associated with kappa opioid receptors.[10] Intravitreal injection of C-type natriuretic peptide into rabbit eyes lowers IOP and increases outflow facility and aqueous humor levels of cGMP but does not affect aqueous humor flow.[11] Intravitreal injection of atrial natriuretic peptide into rabbit eyes also lowers IOP while decreasing aqueous humor flow.[12] The IOP and outflow facility responses in rabbits are inhibited by pretreatment with the kappa opioid receptor antagonist norbinaltorphimine (nor-BNI) and the natriuretic peptide receptor antagonist isatin.[7] This suggests that kappa opioid receptors activate paracrine effects of natriuretic peptides on tissues within the ocular outflow pathways.

All of the in vivo effects of opioid agonists on aqueous humor dynamics in rabbits occur bilaterally after unilateral topical application,[6-9] suggesting they may be mediated, in part, through central or peripheral effects. BRE lowers arterial pressure by inhibition of noradrenaline release following activation of peripheral opioid receptors.[13]

In vitro studies also suggest direct effects of opioid agonists on ocular tissues. Inhibition of norepinephrine release and stimulation of cAMP accumulation in iris ciliary bodies treated with kappa opioid receptor agonists suggest that there are both prejunctional and postjunctional sites of action for kappa opioid agonists.[8,14] Stimulation of protein kinase C and phosphodiesterase is also part of the complex mechanism whereby kappa opioid receptor agonists reduce levels of cAMP in rabbit iris ciliary bodies.[15] Delta[16] and kappa[17] opioid receptor agonists produce concentration-dependent increases in the levels of inositol phosphates in iris ciliary bodies, which could contribute to alterations in aqueous humor dynamics through increases in natriuretic peptide release. Atrial natriuretic peptide and C-type natriuretic peptide increase cGMP accumulation in human trabecular meshwork cells and ciliary muscle cells in vitro.[18] Narrow dose ranges of bromo-cGMP decreased aqueous humor flow after intravitreal injection and increased outflow facility after intracameral injection into monkey eyes in vivo.[19]

These studies suggest that opioids may, via similar effects on cGMP and natriuretic peptide pathways, be effective in lowering IOP in nonhuman primates.

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Nor-BNI has been characterized as a selective kappa$_1$ antagonist in nonhuman primates.[20,21] BRE insensitivity to nor-BNI has been observed only in primate studies and not in other species.[22] Therefore BRE may exert its effects via non-kappa$_1$ opioid receptors in nonhuman primates. Naloxone has been used as a nonselective opioid antagonist in rabbit IOP studies.[9,23]

We therefore investigated the effects of topical application of the kappa opioid agonist BRE on aqueous humor dynamics in monkeys in vivo and the effects of nor-BNI and naloxone inhibition on these responses.

METHODS

All animal experiments were conducted in accordance with the University of Wisconsin IACUC and National Institutes of Health guidelines, and with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

ANIMALS, ANESTHESIA

Ocular normotensive adult cynomolgus monkeys, of either sex, weighing 3.1 to 8.4 kg were anesthetized with intramuscular ketamine HCl (10 mg/kg, supplemented with 5 mg/kg) for IOP, pupil diameter, refraction, and aqueous humor flow measurements. For outflow facility measurements, animals were also given intravenous sodium pentobarbital (15 mg/kg, supplemented with 5 to 10 mg/kg). One monkey had undergone unilateral superior cervical ganglionectomy (sympathectomy)[24] in 1988. Persistent functional ocular sympathetic denervation was verified by periodic experiments showing supersensitivity to phenylephrine,[24] in 1999, before these studies began, and again in 2002, midway through the sequence of experiments.

IOP, PUPIL DIAMETER, REFRACTION

Intraocular pressure (minified Goldmann applanation tonometer),[25] pupil diameter (digital calipers, room light [~350 lux]), and refraction (Hartinger coincidence refractometer) were measured at 0, 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours prior to any treatment and, on separate days, after unilateral topical administration of a single dose of 1, 10, or 100 μg BRE (±BRE, Sigma/RBI, Natick, Massachusetts) in 2 × 5 μL drops. Drops were administered to the central cornea, 1 minute apart, while the monkeys were in a supine position with their eyelids held open for at least 2 minutes after drop administration. Vehicle, either distilled water[9] or 0.9% saline, was administered to opposite eyes. The denervated eye of the sympathectomy monkey was the treated eye.

IOP, MEAN ARTERIAL PRESSURE

Intraocular pressure was measured at 30-minute intervals for 4 to 6 hours following unilateral topical administration of a single dose of 10 or 100 μg BRE. Systolic and diastolic blood pressure values were recorded via a cuff applied to the arm and/or leg and attached to a Dinamap PRO-100 monitor (Critikon, Tampa, Florida). Dinamap values for each time point represent the average of 2 to 4 measurements taken with the cuff. MAP is generated by the Dinamap via an oscillometric method wherein a transducer measures cuff pressure and minute pressure oscillations within the cuff. MAP during angiotensin II (ATII) studies (see below) was also recorded via an arterial line in the tail, at 15- to 30-minute intervals. MAP was calculated from the arterial line data using the formula (2/3 diastolic) + (1/3 systolic).[26]

ANGIOTENSIN II

ATII (Sigma, St Louis, Missouri) at 0.303 μg/mL in heparinized (10 U/mL) lactated Ringer's solution was infused intravenously at a rate of between 200 and 600 μL/min, equivalent to 64.5 to 150 μL/kg/min, to maintain MAP after 100 μg BRE administration. On a separate day, ATII was infused alone at the specific rate required to maintain MAP after exposure to BRE for that particular animal.

NORBINALTORPHIMINE, NALOXONE

Topical nor-BNI (nor-BNI-2HCl, Tocris Cookson Inc, Ellisville, Missouri), 200 μg (2 × 5 μL) or 600 μg (6 × 5 μL) was administered to one eye and vehicle (saline) to the opposite eye. This is twice and 6 times the dose required to completely block the IOP and outflow facility responses to BRE in rabbits.[7,9] In other experiments nor-BNI (unilateral) was given 30 minutes before treatment with 10 μg BRE (bilateral). IOP and MAP measurements were taken as described above.

Similar experiments were conducted using the broad-spectrum opioid antagonist naloxone (naloxone HCl, Tocris), 200 μg (2 × 5 μL). This is the same dose used to antagonize the BRE-induced ocular hypertensive and hypotensive effects in sympathectomized rabbit eyes.[9]

TRANSCORNEAL INJECTIONS

To further clarify whether or not there was a local effect of BRE on IOP, transcorneal intracameral injections were performed. Each ketamine-anesthetized monkey was placed supine in a head holder with the eyes directed upward. The corneas were anesthetized with 1 drop of topical 0.5% proparacaine (Alcaine, Alcon, Fort Worth, Texas). A 10-μL volume of solution containing either 0.1 μg, 1 μg, or 5 μg of BRE was injected into the anterior chamber of one eye; saline vehicle to the opposite eye. This BRE dose was roughly equivalent to a 10-, 100-, or 500-μg topical dose assuming 1% corneal penetration.[27] The solutions were injected using a micrometer syringe connected to polyethylene tubing fitted with a 30-gauge needle. Using microscopic visualization, the needle was threaded through the cornea for several millimeters, and then the tip was angled into the anterior chamber taking care not to touch the iris. The needle was left in place for 1 to 2 minutes after injection of the solutions to allow for hysteresis due to the tensile properties of the tubing to subside. No fluid leakage was observed during needle withdrawal. IOP, pupil diameter, aqueous humor flow, and MAP measurements were then taken as described above.

OUTFLOW FACILITY

Outflow facility was determined by two-level constant pressure perfusion of the anterior chamber with Bárány's perfusand.[28] Baseline outflow facility values were collected for 45 to 60 minutes followed by 2-mL anterior chamber exchanges with 2 or 3 doses of BRE, ranging from 0.01 µg/mL to 100 µg/mL in Bárány's perfusand. After each exchange, eyes were allowed to equilibrate with no external flow from reservoirs for 30 minutes to minimize resistance washout.[29] Then reservoirs were opened and outflow facility was measured for 45 to 60 minutes for each dose. The opposite eye was exchanged each time with Bárány's perfusand. MAP measurements were taken every 15 minutes as described above. IOP measurements for the 10-µg and 100-µg doses were collected after the postexchange equilibration period, while the reservoirs were still closed.

AQUEOUS HUMOR FLOW

Aqueous humor flow rate was determined by ocular scanning fluorophotometry (Fluorotron Master, OcuMetrics Inc, Mountain View, California). Scans were taken every half hour for 6 hours, beginning 30 minutes after topical administration of 10 or 100 µg BRE to one eye; vehicle (saline) was administered to the opposite eye. Baseline fluorophotometry was done three times on each animal: 5 days to 6 weeks prior to the 10-µg dose of topical BRE; 1 to 6 weeks after the 10-µg dose and before the 100-µg dose; and 4 to 6 weeks after the 100-µg BRE dose. These values were averaged to give one baseline flow value. Following intracameral injections of BRE (1 µg in 10 µL), scans were taken every half hour for 4 hours beginning 30 minutes postinjection. Comparisons following intracameral injection were to the 4-hour preinjection baseline taken on a separate day within 2 weeks prior to the injection.

ANALYSIS

Data are expressed as the mean ± standard error of the mean (SEM). Significance was determined by the 2-tailed paired *t* test for ratios compared to 1.0 or differences compared to 0.0.

RESULTS

INTRAOCULAR PRESSURE

During the 6-hour, no-drug baseline, IOP values increased slightly in both eyes (Figure 1A, B). Unilateral topical BRE caused a dose-dependent reduction in IOP in both eyes with a greater response in the treated eyes. IOP reduction in the BRE-treated eyes began within 0.5 hour. At the 100-µg dose, IOP remained significantly decreased for 5 hours. The maximum IOP reduction in BRE-treated eyes occurred at 3 hours (-36 ± 6%, $P < .005$; n = 6) and in vehicle control eyes at 2 hours (-25 ± 9%, $P < .05$, n = 6). The percent IOP difference between BRE-treated and vehicle control eyes corrected for the same-day pretreatment baseline was significant ($P < .05$) at all time points for the 10-µg dose and at 0.5 and 3 hours ($P < .05$) for the 100-µg dose. This suggested there may be local as well as systemic effects of BRE, especially at the submaximal 10-µg dose.

The BRE-treated sympathectomy and vehicle control eyes also showed IOP-lowering dose-response relationships. There was nearly a 3-fold stronger response in the sympathectomy eye (-31% change) compared to the control eye (-12% change) at the 10-µg dose (Figure 1C, D).

PUPIL DIAMETER AND REFRACTION

Unilateral topical BRE (100 µg) caused significant miosis from 0.5 to 3 hours in BRE-treated and vehicle control eyes with a maximum percent changed from baseline of 26 ± 6% (Figure 2A, B). Pupil diameter gradually returned to baseline by 5 to 6 hours after treatment. Very little or no miosis occurred at lower doses.

The BRE-treated sympathectomy and vehicle control eyes (Figure 2C, D) showed less miosis at the 100-µg dose than did the eyes in Figure 2A, B. Slight mydriasis was apparent in the 10-µg BRE-treated sympathectomy eye (Figure 2D).

Topical BRE had no effect on refraction (not shown) at any dose in either treated, control, or sympathectomy eyes.

MAP, HEART RATE, IOP, AND ATII

BRE caused a significant dose-dependent decrease in MAP from 0.5 to 2 hours after treatment with both the 10- and 100-µg doses. A maximum reduction of approximately 40% ($P < .005$, n = 5) compared to pretreatment values occurred at 1 hour following the 100-µg dose (Figure 3). There was also a dose-dependent decrease in heart rate with a similar maximum reduction at the 100-µg dose.

Following 100 µg of topical BRE, infusion of ATII (0.06 to 0.08 µg/min) to maintain MAP (Figure 4B) also eliminated the IOP-lowering response previously seen with this dose of BRE (Figure 4A). ATII infusion alone, at the monkey-specific rate required to counteract the effects of BRE on MAP, increased MAP (mean, 31 ± 2%) and IOP (mean, 9.4 ± 0.6%) from 1 to 3.5 hours (Figure 4C).

IOP AND MAP WITH NOR-BNI, NALOXONE, AND BRE

The selective kappa opioid receptor antagonist, nor-BNI (200 µg), administered unilaterally had no effect on IOP or MAP when compared to pretreatment baseline (Figure 5A, C). There was no difference in IOP between the eyes (Figure 5B). Unilateral topical nor-BNI (200 µg) given 30 minutes prior to bilateral topical BRE (10 µg) appears to partially attenuate the initial IOP-lowering response to BRE (Figure 5D, E). There was no reduction in MAP, which, conversely, was variably but not significantly increased compared to pretreatment baseline (Figure 5F). The lack of an effect of bilateral topical 10-µg BRE on MAP (Figure 5F) could

suggest antagonism by nor-BNI. The presence of an IOP-lowering response to BRE in the absence of a MAP effect suggests that other mechanisms may be involved.

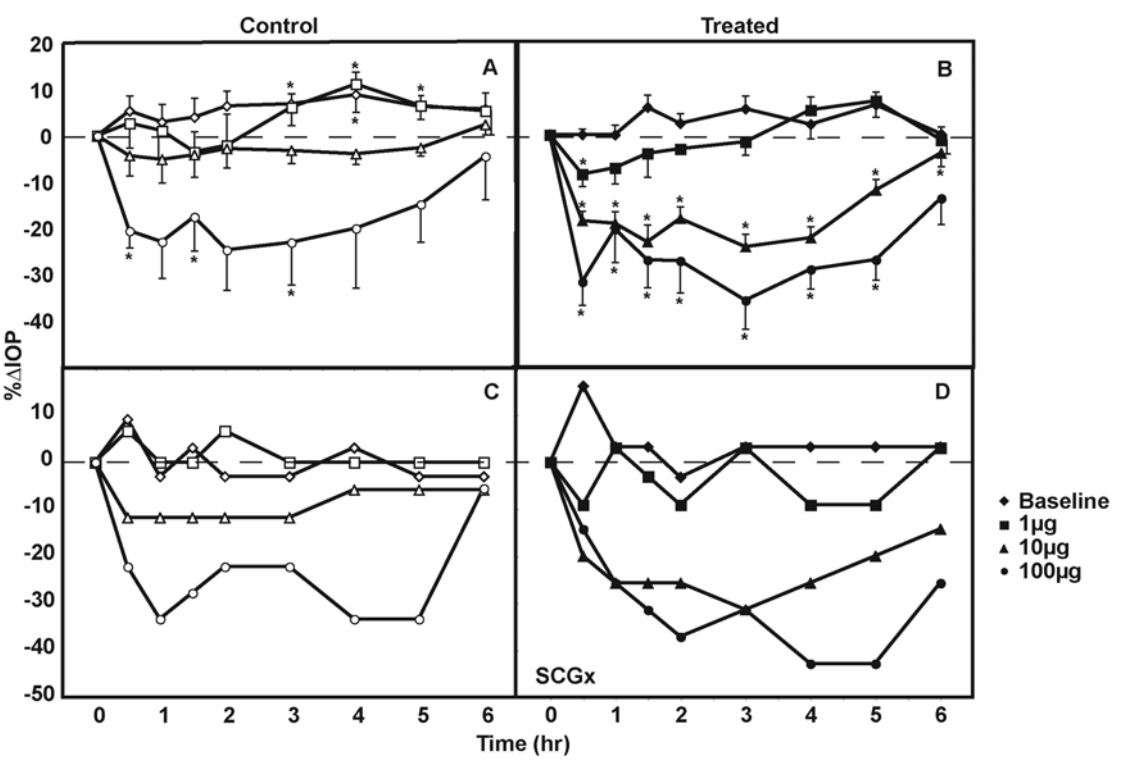

FIGURE 1

Intraocular pressure (IOP) dose-response following topical bremazocine (BRE). BRE caused a dose-related IOP reduction in both treated (B, D) and contralateral vehicle-treated control (A, C) eyes following single unilateral topical administration. Data are shown as the percent change in IOP from the same-day pretreatment time zero baseline. SEM bars may be obscured by the data point symbols in some cases. A, B, and C, Normal eyes. C is the contralateral eye for D. D, Superior cervical ganglionectomy (SCGx) eye. A and B, n = 8 (no drug baseline and 10-µg doses); n = 4 (1-µg dose); n = 6 (100-µg dose). C and D, n = 1. Significantly different from 0.0 by the 2-tailed paired *t* test: **P* < .05, minimum. Pretreatment IOPs (mm Hg) for treated (B) and control (A) eyes, respectively, are as follows: (baseline) 15.4 ± 0.3, 15.0 ± 0.5; (1 µg) 15.2 ± 1.0, 15.5 ± 0.7; (10 µg) 15.9 ± 0.6, 15.6 ± 0.6; (100 µg) 15.8 ± 0.8, 15.6 ± 0.9. Pretreatment IOPs for SCGx (D) and control (C) eyes: (baseline) 15.5, 16.5; (1 µg) 16.5, 15; (10 µg) 17.5, 17; (100 µg) 17.5, 18.

The initial magnitude of the reduction in IOP (Figure 5D) for BRE alone was similar to that seen in the Figure 1 results in the same 8 monkeys (reproduced as panels G through I in Figure 5 for ease of comparison), but the duration of the IOP effect was shorter. Also, the MAP reduction was attenuated (Figure 5F vs 5I). If MAP plays an important role in the IOP reduction, then one would expect both eyes (Figure 5G) to have a similar IOP-lowering response and duration of effect. Nor-BNI therefore must antagonize something, such as BRE-induced local vasospasm, that contributes to the prolonged IOP reduction in the BRE-treated eyes, as seen in Figure 5G. In addition, there may be another mechanism contributing to the IOP-lowering effect of BRE that remains even in the presence of nor-BNI antagonism.

The IOP and MAP response to 10-µg BRE following pretreatment with a higher dose of nor-BNI (600 µg) or after pretreatment with the nonselective opioid antagonist naloxone (200 µg) was studied in 4 animals in common to those from Figure 5. A 3-fold higher dose of nor-BNI had no effect on the BRE-induced IOP reduction (Figure 6A, B). Similarly, naloxone had no antagonistic effect (Figure 6D, E) and perhaps enhanced the IOP reduction in both eyes. Naloxone alone may have slightly reduced IOP (Figure 6G), but this was not significant. In this group of animals, there was a very strong differential IOP response to unilateral BRE with virtually no effect on the contralateral eye (Figure 6J, K). Also, in these particular animals, there was no effect of BRE on MAP when administered alone (Figure 6L) or after pretreatment with the antagonists (Figure 6C, F, I).

TRANSCORNEAL INJECTIONS

To further elucidate whether or not there was a local IOP effect of BRE, intracameral bolus injections were performed. Outflow facility studies (see below) showed that an exchange dose of BRE of 10 µg/mL (corresponding roughly to a 1-µg transcorneal bolus injection dose) did not alter MAP but that a 100-µg/mL exchange dose (roughly corresponding to a 10-µg transcorneal injection dose) did markedly lower MAP. Therefore, we chose the 1-µg dose for the trancorneal injection studies.

After transcorneal intracameral injection of 1-µg BRE, IOP decreased significantly during the first 1 to 1.5 hours in both treated

and control eyes by approximately 10% (n = 8) with no difference between the eyes when compared to preinjection baseline (Figure 7A). MAP was unchanged during this time except at 4 hours (Figure 7B). Pupil diameter increased briefly at 1 to 2 hours (Figure 7C).

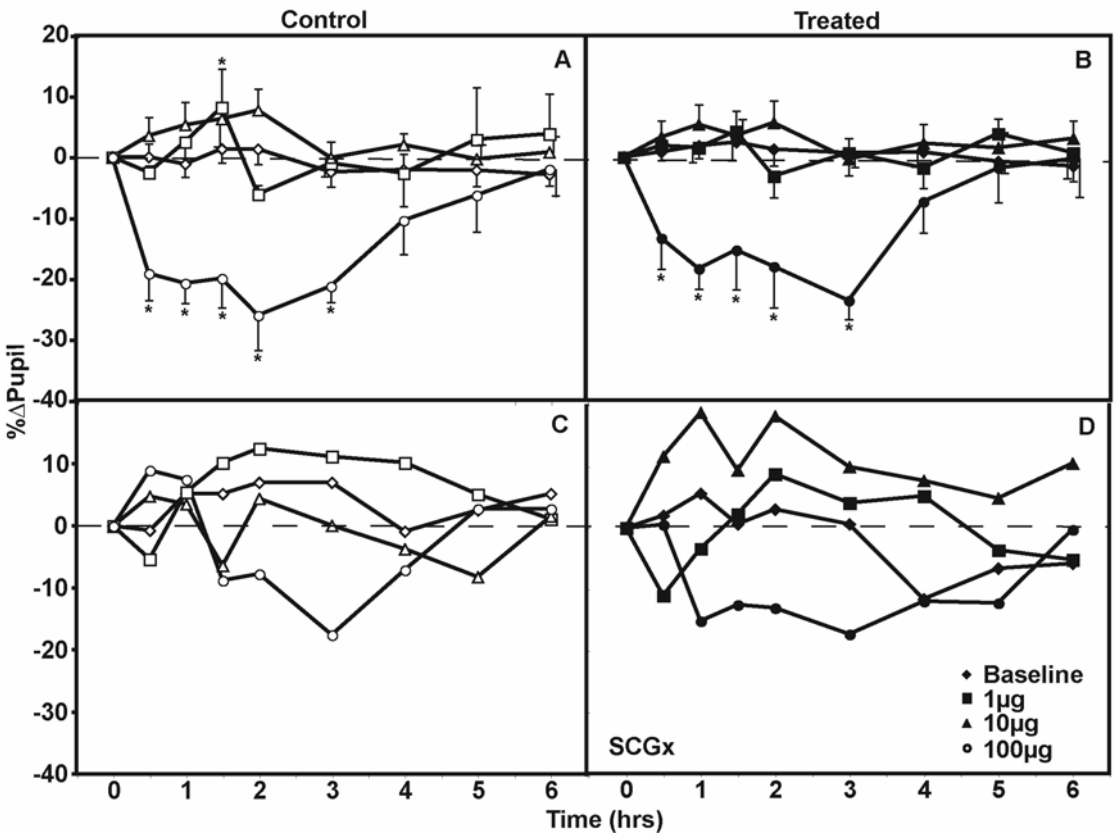

FIGURE 2

Pupil diameter dose-response following topical bremazocine (BRE). BRE produced miosis in both treated (B, D) and control (A, C) eyes only at the highest dose (100 µg) following unilateral topical administration. Data are shown as the percent change in pupil diameter from the same-day pretreatment time zero baseline. SEM bars may be obscured by the data point symbols in some cases. A, B, and C, Normal eyes. C is the contralateral eye for D. D, Superior cervical ganglionectomy (SCGx) eye. A and B, n = 8 (no drug baseline and 10-µg doses); n = 4 (1-µg dose); n = 6 (100-µg dose). C and D, n = 1. Significantly different from 0.0 by the 2-tailed paired *t* test: **P* < .05, minimum. Pretreatment pupil diameter (mm) for treated (B) and control (A) eyes, respectively, are as follows: (baseline) 5.2 ± 0.2, 5.2 ± 0.2; (1 µg) 5.5 ± 0.2, 5.4 ± 0.3; (10 µg) 5.0 ± 0.1, 4.9 ± 0.1; (100 µg) 4.8 ± 0.3, 4.8 ± 0.2. Pretreatment pupil diameter for SCGx (D) and control (C) eyes: (baseline) 4.9, 5.7; (1 µg) 4.3, 5.2; (10 µg) 3.4, 5.6; (100 µg) 3.7, 4.8.

OUTFLOW FACILITY

Outflow facility was unchanged following anterior chamber exchange with BRE (Table 1). The 10-µg/mL dose corresponds roughly to a 100-µg topical dose assuming a 1% penetration rate (reviewed in Schoenwald[30]) and 100-µL anterior chamber volume.[31] Significant increases in outflow facility compared to baseline in the control eye at the 10-µg/mL dose and in the treated eye at the 100-µg/mL dose are presumably due to washout resulting from the prolonged duration of the perfusion,[29] even though reservoirs were closed for 30 minutes after each exchange in an attempt to minimize this effect. There were also no posttreatment outflow facility differences comparing opposite eyes without correction for baseline. The magnitude of the washout in the current studies is comparable to that encountered in control eyes during other long outflow facility studies.[32] Outflow facility was not measured in the sympathectomy monkey.

IOP, measured after the postexchange waiting period and before reservoirs were opened, was decreased significantly in both BRE-treated and vehicle control eyes after 100-µg/mL BRE but not after 10-µg/mL BRE (Table 2). However, this could be a result of the duration of pentobarbital anesthesia, which would have been an hour or more by the time the 100-µg/mL dose was administered. The IOP difference between BRE-treated and vehicle control eyes corrected for time 0 was not significant at either dose. MAP after intracameral BRE during the outflow facility studies did not change significantly at the 10-µg/mL dose but decreased significantly within the first 15 minutes postexchange at the 100-µg/mL dose and continued to drop by as much as 35% to 40% (*P* < .005) at 60 to 75 minutes**.**

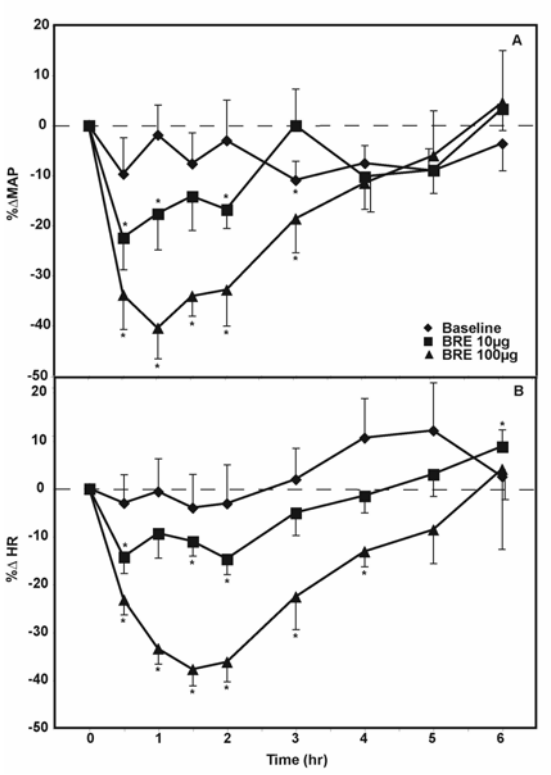

FIGURE 3

Effect of bremazocine (BRE) on mean arterial pressure (MAP) and heart rate (HR). Topical BRE caused a dose-dependent reduction in MAP (Dinamap) and HR lasting for approximately 2 hours before returning to pretreatment values. N = 9 (baseline and 10-µg doses); n = 5 (100-µg dose). Significantly different from 0.0 by the 2-tailed paired *t* test: **P* < .05, minimum. Pretreatment MAP (mm Hg): (baseline) 94 ± 4; (10 µg) 92 ± 4; (100 µg) 79 ± 8. Pretreatment HR (beats per minute): (baseline) 155 ± 10; (10 µg) 166 ± 10; (100 µg) 151 ± 17.

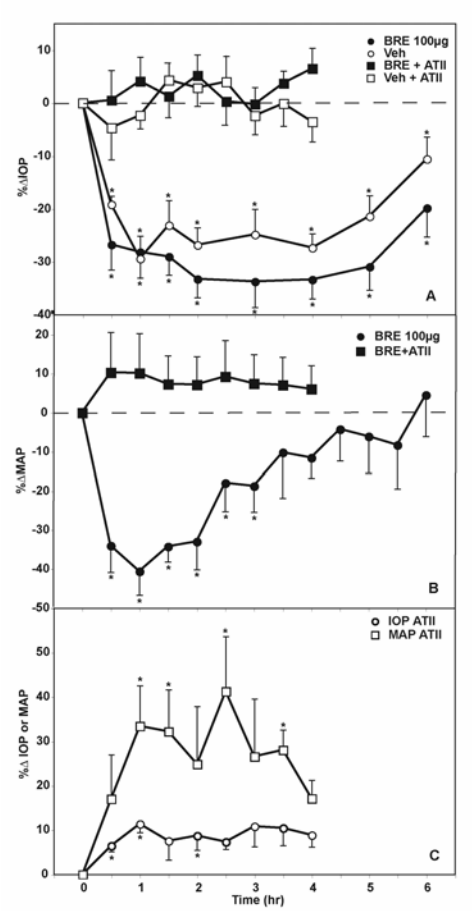

FIGURE 4

Effects of angiotensin II (ATII) on mean arterial pressure (MAP) (Dinamap) and intraocular pressure (IOP) with and without bremazocine (BRE) treatment. ATII intravenous infusion to prevent the reduction in MAP (B), which normally occurs after topical BRE, eliminated the IOP (A) lowering response to 100 µg topical BRE in both the BRE-treated and vehicle (Veh) control eyes. ATII infusion after topical BRE maintained MAP (B) within 10% of pretreatment baseline values. ATII infusion alone (C) without BRE treatment increased IOP and MAP. Data are percent change from time 0. Significantly different from 0.0 by the 2-tailed paired *t* test: **P* < .05, minimum. n = 5 (A, B), n = 3-5 (C). Baseline IOP (mm Hg) and MAP (mm Hg) prior to treatment are as follows: A (IOP), BRE 16.7 ± 0.5, Veh 16.8 ± 0.6, BRE+ATII 14.9 ± 0.9, Veh+ATII 15.2 ± 0.9; B (MAP), BRE 79 ± 1, BRE+ATII 77 ± 7; C (IOP) 16.5 ± 0.4, (MAP) 87 ± 8.

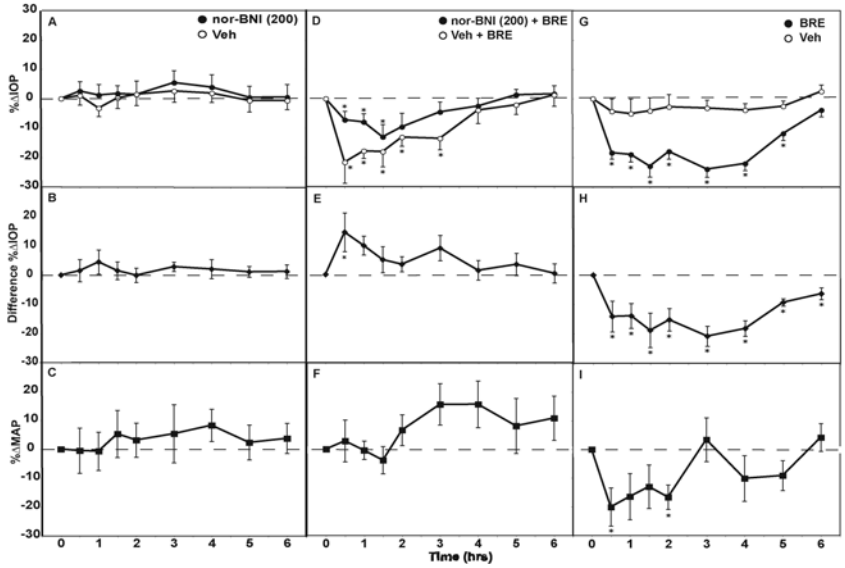

FIGURE 5

Effect of 200 µg norbinaltorphimine (nor-BNI) on intraocular pressure (IOP), mean arterial pressure (MAP), and response to bremazocine (BRE). A, B, C, Nor-BNI (200 µg) administered topically to one eye had no effect on IOP or MAP. D, E, F, Unilateral nor-BNI (200 µg) administration 30 minutes prior to bilateral BRE (10 µg) partially antagonized the initial IOP-lowering effect of bilateral BRE (D). Only the 0.5-hour IOP difference was significant (E). There was a variable increase but no significant effect of unilateral nor-BNI/bilateral BRE on MAP (F). G, H, I, Original IOP and MAP response from Figures 1 and 3 showing the magnitude of the IOP (G) and MAP (I) response to unilateral 10-µg BRE and the difference in IOP response between the eyes (H). IOP and MAP data are percent change from baseline on the same day. Significantly different from pretreatment baseline at time 0: **P* < .05, minimum. n = 8 (the same 8 animals were used throughout). Baseline IOP (mm Hg) and MAP (mm Hg) prior to treatment were: A (IOP), nor-BNI 15.4 ± 0.5, vehicle (Veh) 15.4 ± 0.5; D (IOP), nor-BNI+BRE 15.2 ± 0.8, Veh+BRE 15.0 ± 0.7; G (IOP), BRE 15.9 ± 0.6, Veh 15.6 ± 0.6; C (MAP), 85 ± 6; F (MAP), 82 ± 7; L (MAP), 92 ± 4.

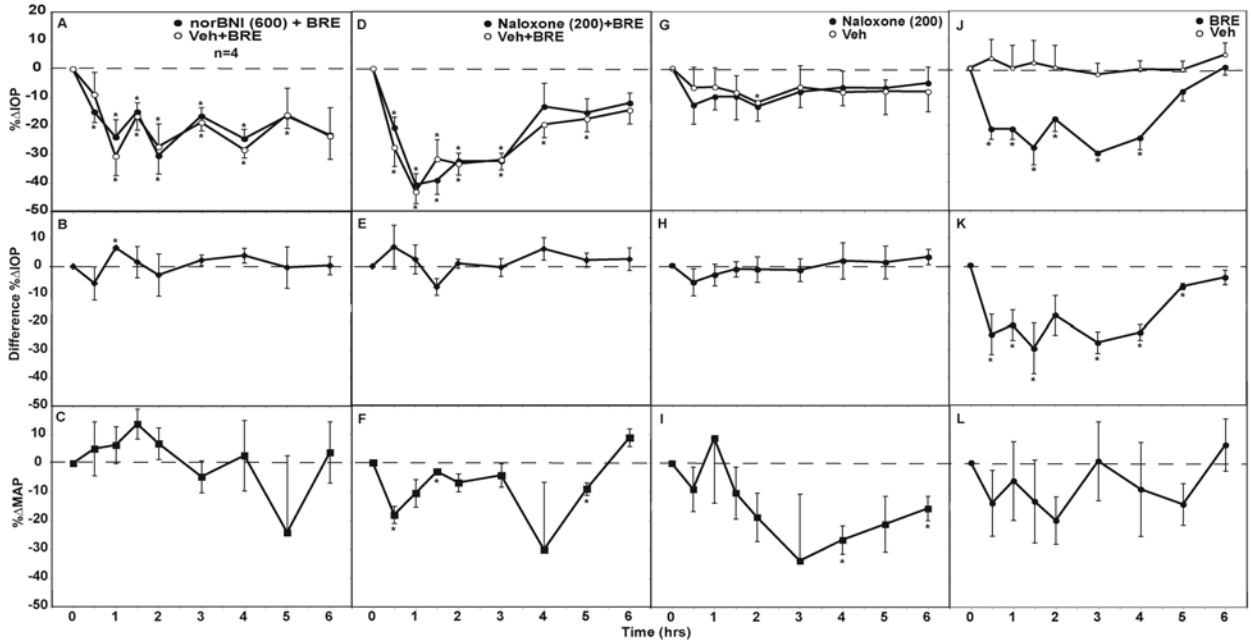

FIGURE 6

Effects of 600 µg norbinaltorphimine (nor-BNI) and 200 µg naloxone on intraocular pressure (IOP) and mean arterial pressure (MAP) after bremazocine (BRE). A,B,C, Unilateral nor-BNI (600 µg) administration 30 minutes prior to bilateral BRE (10 µg) did not antagonize the IOP-lowering effect of bilateral BRE (A, B). There was no significant effect of unilateral nor-BNI/bilateral BRE on MAP (C). D, E, F, Unilateral pretreatment with naloxone (200 µg) also did not antagonize the IOP-lowering response of bilateral BRE (D, E). There was no effect of naloxone plus BRE on MAP (F). G, H, I, Naloxone alone did not significantly alter IOP (G, H) or MAP (I). J, K, L, IOP and MAP response for these 4 monkeys showing the magnitude of the IOP (J) and MAP (L) response to unilateral 10-µg BRE and the difference in IOP response between the eyes (K). IOP and MAP data are percent change from baseline on the same day. Significantly different from pretreatment baseline at time 0: **P* < .05, minimum. n = 4 (the same 4 animals were used throughout and are a subset of those from Figures 1, 2, 3, and 5). Baseline IOP (mm Hg) and MAP (mm Hg) prior to treatment were: A (IOP), nor-BNI+BRE 16.5 ± 0.5, BRE 16.0 ± 0.7; D (IOP), naloxone+BRE 14.9 ± 0.8, vehicle (Veh)+BRE 15.9 ± 0.6; G (IOP), naloxone 15.6 ± 0.6, Veh 15.6 ± 0.6; J (IOP), BRE 15.0 ± 0.6, Veh 14.6 ± 0.6; C (MAP), 97 ± 9; F (MAP), 87 ± 6; I (MAP), 104 ± 4; L (MAP), 97 ± 6.

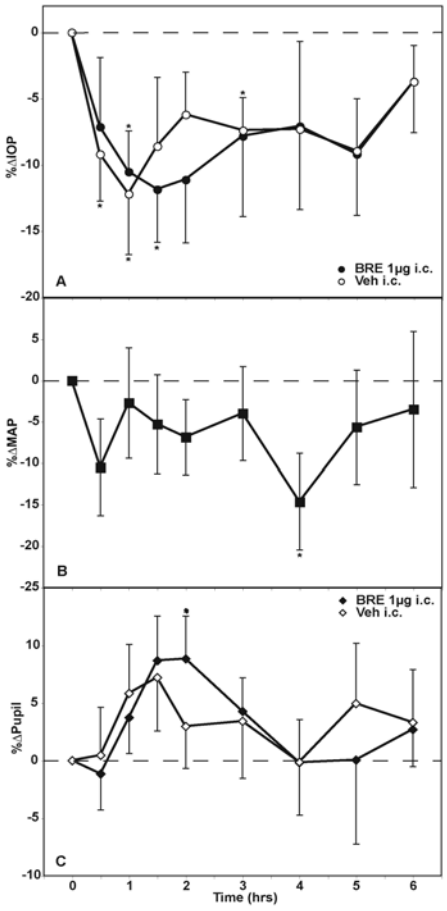

FIGURE 7

Effects of intracameral bremazocine (BRE) on intraocular pressure (IOP), mean arterial pressure (MAP), and pupil diameter. A, Following intracameral (ic) injection of 1 µg BRE, IOP decreased significantly during the 1- to 1.5-hour interval in both BRE-treated and vehicle (Veh) control eyes and remained decreased for the duration of the measurements. There was no difference in IOP between the eyes. B, MAP was unchanged except for a significant decrease at hour 4. C, Pupil diameter increased briefly at 1 to 2 hours. Data are % change from pretreatment time 0. Significantly different from 0.0 by the 2-tailed paired *t* test: **P* < .05, n = 8. Pretreatment IOP (mm Hg), MAP (mm Hg), and pupil diameter (mm) were as follows: A (IOP), BRE 18.9 ± 0.8, Veh 18.2 ± 0.7; B (MAP), 82 ± 5; C (pupil), BRE 4.8 ± 0.3, Veh 4.9 ± 0.3.

TABLE 1. OUTFLOW FACILITY AFTER INTRACAMERAL BREMAZOCINE

		OUTFLOW FACILITY*		OUTFLOW FACILITY RATIO	OUTFLOW FACILITY RATIO		
	n	**BRE**	**Veh**	**BRE/Veh**	**BRE/BL**	**Veh/BL**	**BRE/BL//Veh/BL**
Baseline	8	0.32 ± 0.08	0.37 ± 0.07	0.87 ± 0.10			
1 µg/mL	4	0.26 ± 0.08	0.37 ± 0.07	1.06 ± 0.14	0.96 ± 0.14	0.87 ± 0.04	1.11 ± 0.18
10 µg/mL	8	0.38 ± 0.08	0.51 ± 0.10	0.86 ± 0.17	1.29 ± 0.18	1.37 ± 0.10†	0.94 ± 0.11
100 µg/mL	8	0.49 ± 0.11	0.65 ± 0.14	0.92 ± 0.16	1.63 ± 0.10‡	1.91 ± 0.39	1.05 ± 0.16

BL, baseline; BRE, bremazocine; Veh, vehicle.
*Outflow facility units = µL/min/mm Hg. Only data from the three highest doses are shown.
Ratios significantly different from 1.0: †*P* < .01, ‡*P* < .001.

AQUEOUS HUMOR FLOW

Topical BRE produced a dose-dependent reduction in aqueous humor flow in both BRE-treated and vehicle control eyes of normal monkeys with a slightly greater effect in BRE-treated eyes (Table 3). The effect was greatest during the 0.5- to 3-hour interval after dosing. Compared to baseline during the same interval, 100-µg BRE decreased aqueous humor flow by 63% in treated eyes. The effect of BRE on aqueous humor flow in the sympathectomy monkey was comparable to that in normal monkeys at the 10-µg dose but less in the sympathectomy eye at the 100-µg dose.

Aqueous humor flow was also measured after intracameral injection of 1-µg BRE (Table 4), which is roughly equivalent to a 100-µg topical dose, assuming 1% corneal penetration as explained above. At this dose and mode of administration, no confounding effects on MAP were noted. Analysis of the data indicated no effect of BRE on aqueous humor flow during the interval 0.5 to 4 hours after injection when all data were considered (n = 8). Relative to baseline, there was no change in aqueous humor flow in the BRE-

treated eyes but an increase in aqueous flow in the vehicle control eyes, confounding interpretation of these data. No significant changes were detected when shorter time intervals were analyzed (not shown).

TABLE 2. SPONTANEOUS INTRAOCULAR PRESSURE (IOP) AFTER PERFUSION EXCHANGE*

		SPONTANEOUS IOP (mm Hg)		IOP DIFFERENCE		
	n	**BRE**	**Veh**	**BRE-BL**	**Veh-BL**	**(BRE-BL)-(Veh-BL)**
Baseline	4	9.2 ± 1.2	8.50 ± 1.66			
10 μg/mL	4	7.6 ± 1.3	6.75 ± 0.72	-1.63 ± 1.03	-1.75 ± 1.48	0.13 ± 0.9
%Δ				-17.3 ± 9.8	-14.7 ± 11.8	-2.6 ± 8.0
Baseline	8	9.5 ± 0.8	8.81 ± 0.72			
100 μg/mL	8	6.6 ± 0.5	6.50 ± 0.84	-2.88 ± 0.65†	-2.31 ± 0.73‡	-0.56 ± 0.70
%Δ				-27.2 ± 7.0§	-25.5 ± 8.0 ‡	-1.7 ± 8.5

BL, baseline; BRE, bremazocine; Veh, vehicle; %Δ, percentage change of difference compared to baseline.
*Data are mean ± SEM.
Significantly different from 0.0: †$P < .005$, ‡$P < .05$, §$P < .01$.

COMPARISON OF DINAMAP AND ARTERIAL LINE DATA

During some experiments where ATII infusion was used to maintain MAP after 100-μg topical BRE, blood pressure measurements were taken with both the Dinamap and via a tail arterial line on the same day (13 to 15 measurements for each of 5 monkeys). All measurements recorded and calculated from the arterial line data were significantly higher than those recorded from the Dinamap (Table 5). This was especially true of the diastolic pressure. The differences were not dependent on the pressure level; therefore the differences between the two techniques can be assumed to be constant. There was no difference in Dinamap values recorded from the arm vs the leg (not shown).

SLIT-LAMP BIOMICROSCOPY AND SEDATION

No corneal toxicity or anterior chamber cells or flare were observed at any dose by slit-lamp biomicroscopy. There seemed to be an effect on sedation, especially at the 100-μg dose, where monkeys were less responsive to stimulation and required less ketamine anesthesia.

DISCUSSION

Kappa opioid receptor stimulation following topical BRE decreases IOP, aqueous humor flow, and MAP in the monkey. IOP and aqueous humor flow–lowering effects can be significant in the contralateral control eyes, although not to the extent seen in the treated eyes. The correlation in time and dose-dependent nature of ocular and systemic hypotensive effects suggest that these events may be related. Blocking the reduction in MAP with ATII eliminates the IOP decrease. However, at lower doses, differences in IOP between the treated and control eyes, as well as the longer duration of the hypotensive IOP response compared to the MAP response, suggest a local effect in addition to the systemic effect.

There is evidence for a complete renin-angiotensin system in the ciliary body.[33,34] However, since the angiotensin levels in ocular tissue are unlikely to be derived from the circulation,[35] the systemic infusion of ATII during the current studies is unlikely to have masked any substantial local effects of BRE. However, small IOP effects due to other mechanisms may have been overshadowed by the slight ocular hypertension resulting from ATII alone (Figure 4C).

The MAP/IOP relationship in general is complex. In rabbit studies, for every 10-mm Hg decrease in the mean arterial pressure, there was approximately a 1-mmHg decrease in IOP.[12] The effect of ocular application of BRE on MAP in rabbit studies was not reported. In our studies there was a 1-mm Hg reduction in IOP for every 6- to 9-mm Hg reduction in MAP during the first 2 hours after treatment with the 100-μg dose of BRE.

We acknowledge that not all agents that alter blood pressure are equally effective in altering IOP. However, MAP decreases of the magnitude found in our studies, especially after the 100-μg dose of BRE, could affect IOP, as has been shown in monkey and rabbit studies.[36-38] Deprivation of oxygen and nutrient supply to the ciliary body as a result of poor perfusion could delay the restoration of

TABLE 3. AQUEOUS HUMOR FLOW (AHF) AFTER TOPICAL BREMAZOCINE*

	0.5 to 3 hours			3.5 to 6 hours			1 to 6hr		
	AHF		AHF ratio	AHF		AHF ratio	AHF		AHF ratio
	BRE	Veh	BRE/Veh	BRE	Veh	BRE/Veh	BRE	Veh	BRE/Veh
BL (n = 8)	1.55 ± 0.15	1.36 ± 0.07	1.14 ± 0.10	1.80 ± 0.15	1.52 ± 0.11	1.18†± 0.04	1.90 ± 0.28	1.66 ± 0.17	1.13 ± 0.06
10 µg BRE (n = 8)	1.41 ± 0.10	1.05 ± 0.08	1.01 ± 0.09	1.32 ± 0.13	1.22 ± 0.14	1.13 ± 0.12	1.22 ± 0.09	1.21 ± 0.10	1.03 ± 0.06
10 µg/BL	0.72† ± 0.07	0.82‡ ± 0.07	0.88 ± 0.05	0.76‡ ± 0.08	0.84 ± 0.13	0.97 ± 0.09	0.77‡ ± 0.08	0.85 ± 0.09	0.92 ± 0.05
100 µg BRE (n = 4)	0.53 ± 0.13	0.76 ± 0.12	0.74 ± 0.19	1.49 ± 0.30	1.38 ± 0.24	1.12 ± 0.24	1.21 ± 0.18	1.17 ± 0.12	1.04 ± 0.09
100 µg/BL	0.37‡ ±	0.50‡ ± 0.11	0.68 ± 0.16	0.66‡ ± 0.09	0.73 ± 0.10	0.98 ± 0.21	0.64‡± 0.11	0.69 ± 0.12	0.93 ± 0.06
N = 1	**BRE**	**Veh**	**BRE/Veh**	**BRE**	**Veh**	**BRE/Veh**	**BRE**	**Veh**	**BRE/Veh**
Baseline	1.25 ± 0.18	1.62 ± 0.32	0.90 ± 0.25	1.83 ± 0.22	1.94 ± 0.27	0.96 ± 0.11	1.61 ± 0.14	1.77 ± 0.22	0.93 ± 0.07
10 µg BRE	0.76	1.13	0.67	0.97	1.11	0.88	0.90	0.97	0.93
10 µg/BL	0.65	0.79	0.82	0.49	0.57	0.86	0.55	0.58	0.95
100 µg BRE	1.12	0.95	1.18	1.74	1.50	1.17	1.31	1.12	1.16
100 µg/BL	0.84	0.52	1.59	0.92	0.69	1.34	0.76	0.57	1.34

BL, baseline; BRE, bremazocine, Cont, control; SCGx, superior cervical ganglionectomy; Veh, vehicle.
*Units for aqueous humor flow are µL/min; ratios are unitless. Data are mean ± SEM. Baseline values are the mean of 2 to 3 separate predrug and postdrug baselines. For n = 4-8, each animal contributed one mean value based on their 2 to 3 baselines. For comparison to BL for each dose, the average BL values before and after that dose are used (ie, average of 2 BLs).
Significantly different from 1.0 by the 2-tailed paired *t* test: †$P < .01$; ‡$P < .05$.

TABLE 4. AQUEOUS HUMOR FLOW (AHF) AFTER INTRACAMERAL BREMAZOCINE*

	0.5 TO 4 HOURS		
	AHF		AHF RATIO
	BRE	VEH	BRE/VEH
BL	1.75 ± 0.22	1.66 ± 0.17	1.04 ± 0.05
BRE 1 µg	1.80 ± 0.23	2.01 ± 0.36	0.91 ± 0.06
BRE/BL	1.07 ± 0.11	1.25 ± 0.15	0.90 ± 0.09

BL, baseline; BRE, bremazocine; Veh, vehicle.
*Units for aqueous humor flow are µL/min; ratios are unitless. n = 8. Data are mean ± SEM. BL measured on a separate day within 1 week prior to the injection.

aqueous humor production, even though MAP returned to normal. The pressure-dependent ultrafiltration component of aqueous humor flow is thought to account for no more than 10% to 20% of aqueous humor flow.(reviewed in Gabelt and Kaurman[39]). However, if MAP reduction were responsible for all of the IOP response to BRE, then the IOP reduction should be similar in both eyes. This was clearly not the case at the 10-µg dose, where a differential effect on IOP was found.

TABLE 5. COMPARISON OF DINAMAP AND ARTERIAL LINE DATA

PRESSURE MEASUREMENT	AVERAGE DIFFERENCE (ARTERIAL – CUFF [MM HG[)	DIFFERENCE 95% CI	PAIRED TEST *P* VALUE
Systolic	2.9	(0.5-5.2)	.0172
Diastolic	15.0	(12.7-17.4)	<.0001
MAP	4.4	(2.2-6.7)	.0002

An increase in central venous pressure might cause underperfusion of the ciliary body as well. Although we did not measure central venous pressure, calculation of intrascleral venous pressure (ISVP) according to Bill[40] using our data indicated a dose-dependent reduction in ISVP after BRE. Therefore, it is unlikely that blood flow through the ciliary body was blocked by elevated recipient venous pressure.

Vasoconstriction is another possible mechanism for the IOP reduction due to BRE. However, in studies with topical brimonidine, which has been found to cause ocular vasoconstriction in rabbit,[41] maintenance of MAP with ATII in monkeys after topical brimonidine did not alter the aqueous humor flow and IOP-lowering effects.[42] Therefore, one may expect that if BRE were producing ciliary body vasoconstriction, the IOP response would still be evident even during ATII infusion.

The bilateral IOP response following unilateral intracameral injections of BRE could also indicate that other mechanisms are involved. Afferent-to-efferent and other central pathways have been suggested to be involved in the regulation of IOP.[43] The effects of barbiturates and hyperosmotic agents are thought to be mediated by central efferent fibers running through the optic nerve.[44] Aqueous humor flow results (Table 4) after unilateral intracameral BRE also suggested no local effect when all animals were considered.

The results of pretreatment with the kappa opioid antagonist nor-BNI on the IOP response to topical BRE are difficult to interpret. The 200-µg dose of nor-BNI we used in our initial studies was based on that used to inhibit IOP and aqueous humor flow responses to BRE in rabbits.[7,9] The relative dose of nor-BNI to BRE is comparable to that used in other studies where local responses to BRE were antagonized by local administration of nor-BNI.[45,46] However, 100 to 1000-fold higher doses of nor-BNI relative to BRE are reported to be required when nor-BNI and BRE are not administered to the same location.[47,48] Therefore, we included additional studies in 4 monkeys with a 600-µg dose of nor-BNI.

Following unilateral nor-BNI and bilateral BRE (10 µg), there appeared to be a slight antagonism of the initial IOP-lowering effect compared to the opposite eye at the 200-µg dose of nor-BNI but no antagonism at the higher 600-µg dose. In these studies there was also no MAP-lowering response.

Nor-BNI has been characterized as a selective $kappa_1$ antagonist in nonhuman primates.[20,21] BRE may exert some of its effects on IOP via non $kappa_1$-opioid receptors, and therefore nor-BNI may be ineffective at doses selective for antagonism of $kappa_1$ opioid receptors. BRE insensitivity to nor-BNI has been observed only in primate studies and not in other species.[22] Therefore, the IOP response to BRE in rabbits, which is completely blocked by nor-BNI, may be due to $kappa_1$ opioid receptors, whereas at least part of the IOP-lowering response in monkeys may be due to non-$kappa_1$ opioid receptors.

Antagonism studies with the nonselective opioid receptor antagonist naloxone were undertaken to attempt to clarify this species discrepancy. However, pretreatment with naloxone, at a dose shown to antagonize IOP responses to BRE and other opioid agonists in rabbits,[9,23] was also ineffective in blocking the IOP response to BRE in monkeys. Some antagonists labeled as nonselective have been found to be somewhat selective in other cases[49] or can act via nonopioid mechanisms.[50]

The IOP reduction and aqueous humor flow suppression found in normal rabbits after topical, unilateral BRE is dependent on intact sympathetic nerves.[9] A transient hypertensive response to BRE was seen in sympathectomized rabbits.[9] In macaque monkeys, the hypotensive effect does not seem to be dependent on sympathetic innervation, although only one monkey was studied. The sympathectomized eye used in this study showed a dose-dependent reduction in IOP comparable to, or perhaps even greater than, that in nonsympathectomized eyes. Therefore, the IOP and aqueous humor flow suppression in monkeys may be due only to postjunctional effects, whereas in rabbits it is due to both prejunctional and postjunctional effects.[8] Another postjunctional effect could result from humoral modulation as a result of systemic effects. This species difference is similar to what was found with the α_2-adrenergic agonist brimonidine, in which sympathetic innervation was necessary for the IOP-lowering response in cats and rabbits[51] but not in monkeys.[42]

BRE does not appear to significantly alter outflow facility in monkeys. This is in contrast to the results obtained in rabbits. In rabbits, the outflow-facility-increasing and IOP-lowering effects of BRE were mediated, in part, through increased levels of cGMP and natriuretic factors.[7] The outflow-facility-increasing and aqueous-humor-flow-decreasing effects of cGMP[19] in monkeys and the increase in cGMP in response to natriuretic factor treatment of human trabecular meshwork cells[18] suggest the possibility that opioid

agonists might affect aqueous humor dynamics in ways similar to what was found in rabbits. However, the lack of an outflow facility response to BRE in our studies suggests that the signaling pathways for opioids in nonhuman primate eyes may be different than in rabbits. Also, the rabbit lacks a true trabeculum and the vascular anatomy of the outflow pathways differs from that of the primate.[52]

It is unlikely that factors that were released in response to BRE, which may have altered outflow facility, were washed out by our perfusion technique unless the factors were only released immediately upon BRE administration. During outflow facility studies, after each anterior exchange with BRE, the reservoirs were closed for 30 minutes so that only endogenous aqueous humor formation would be driving the egress of factors from the aqueous humor. The effective dose of BRE would have been largely maintained throughout the 30-minute waiting period, which would have been sufficient time for any factors to be generated that were involved in the very rapid IOP-lowering response to topically administered BRE. At the lowest doses of BRE, it is possible that low levels of factors generated while the reservoirs were closed, and which might have been effective if maintained at those levels, might be washed out and thus found to be ineffective. However, the highest dose of BRE, 100 μg/mL, which corresponds roughly to 10 times the highest dose given topically, would surely have generated sufficient levels of factors to have an effect during the 45 minutes of the outflow facility measurement.

Pentobarbital anesthesia used during outflow facility studies in monkeys can lower the aqueous humor formation rate and IOP as compared to ketamine anesthesia.[31,53] However, MAP is unaffected by pentobarbital anesthesia alone.[31]

If sympathetic innervation were required for atrial natriuretic factor generation in the monkey eye, the finding that there was a large IOP response to BRE in our sympathectomized monkey suggests that atrial natriuretic factor is not necessary for the IOP response. In earlier studies, intracameral administration of atrial natriuretic factor (81 to 162 pmol/mL) to monkeys led to an increase in aqueous humor formation. A slight reduction in IOP was attributed to a slight increase in uveoscleral outflow.[54] Thus, it is unlikely that the large IOP decrease in monkeys is due to an increase in atrial natriuretic factor levels. Nonetheless, measurement of atrial natriuretic factor levels in monkey aqueous is an additional study that could be done for the purpose of distinguishing species differences. Miosis occurred only at the highest dose of BRE (100 μg) in both normal eyes and the SCGx eye, suggesting mediation by a central effect, possibly through humoral or some other parasympathetic or other nonsympathetic pathway affecting the sphincter agonistically rather than the dilator antagonistically.

In conclusion, topical BRE lowers IOP in monkeys as a result of aqueous humor formation suppression. At high doses of BRE (100 μg), some of this IOP-lowering effect may be due to MAP reduction, which can be eliminated by ATII administration. At lower doses of BRE (10 μg), a differential IOP-lowering response in ipsilateral compared to contralateral eyes is found, which is not blocked, or is perhaps only slightly blocked, by pretreatment with the selective $kappa_1$ (nor-BNI) or nonselective (naloxone) opioid antagonists. A third component of the IOP reduction occurs bilaterally after unilateral intracameral injection. The mechanism for this IOP reduction is unknown and does not appear to be due to aqueous humor formation suppression. Other opioid subtype agonists and antagonists need to be evaluated for their effects on aqueous humor dynamics in nonhuman primates. Studies in additional sympathectomized monkeys are also warranted. Our results emphasize the importance of the selection of species for ocular physiology studies that are relevant to the human situation.

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PEER DISCUSSION

DR LOUIS B. CANTOR: In this study by Dr. Kaufman and colleagues, in the monkey eye the kappa opioid receptor agonist bremazocine, given topically, results in a dose-dependent decrease in intraocular pressure (IOP), a decrease in aqueous flow, a decrease in mean arterial pressure, and a decrease in IOP and flow in the contralateral control eye following unilateral topical and intracameral injection. Selective $kappa_1$ opioid antagonist pretreatment has a variable effect based on the dose of bremazocine, suggesting that at least some of the IOP-lowering effect is due to non-$kappa_1$ opioid receptors.

Nonselective opioid antagonist pretreatment has no effect in blocking the IOP response in monkeys, also suggesting that nonopioid receptors may be involved in the activity of this agent. Angiotensin II can be used to block the decrease in blood pressure, which may also lower the IOP. Despite using angiotensin II, there is little change in the effect of bremazocine on IOP. Sympathectomy also did not alter the IOP response, suggesting that the IOP and aqueous flow decreases associated with bremazocine may be due to postjunctional effects.

The most intriguing result from this study is the observation that unilateral injection or topical administration of bremazocine decreases IOP in the contralateral eye. Is this a systemic effect? Might this be the result of central mechanisms of IOP control? If so, this would raise interesting questions about our understanding of the central control of IOP. What afferent-to-efferent central pathways regulate intraocular pressure? Research exploring the autonomic and central control mechanisms that might in part regulate IOP exist, but are limited.[1,2]

Evidence exists that suggests that bremazocine increases C-type natriuretic peptide, which increases outflow facility via protein kinase C.[3] Natriuretic peptides are involved in neuroendocrine fluid homeostasis throughout the body. Could there be a neural pathway for release of C-type natriuretic peptide in the fellow eye following unilateral administration? Sympathetic innervation is known to lower aqueous production, increase flow, decrease outflow resistance, and increase the permeability of the blood-aqueous barrier.[4]

. Parasympathetic activation via cranial nerve III is known to increase outflow.[5] Might pathways exist for afferent-to-efferent

alteration of these autonomic influences in the contralateral eye following unilateral administration? The mechanism of bilateral IOP reduction following unilateral administration of bremazocine remains unknown and challenges the authors and others to shed light on this finding and the larger question of central control of IOP.[6]

The authors are to be congratulated for exploring a novel class of medications for IOP control. As is often the case, their work has raised interesting questions and highlights our incomplete understanding to the control of aqueous humor dynamics.

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RESOURCE LIST

1. Bergmanson JP. Neural control of IOP. *Am J Optom Physiol Opt* 1982;59:94-98.
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DR. ALLAN J. FLACH: I have no conflicts whatsoever. As usual, your papers are so exciting I can barely contain my enthusiasm. For many years I attempted to do complete sympathectomies in many different animal species, but I have never succeeded. If you have a way you can do it, then I sure would like to know about it. I know cannabinoid receptors have been identified in humans and other animal species, but has anyone really identified opioid receptors, and if so, what are they like? Regarding the angiotensin observations and remembering that simply a millimeter increase in episcleral venous pressure results in a change of IOP by a millimeter, would it have helped to determine the episcleral venous pressure in considering your observations about the angiotensin. The thought is that vascular changes might affect the intraocular pressure. I am glad that you reminded us of the importance of the experimental animal species. If we had used rabbits for the initial timolol maleate studies, then it would have been classified as an ineffective drug. You are so wise in your conclusion to say that part of the IOP lowering response is aqueous humor suppression. This brings to mind all the years we watched Keith Green working with the cannabinoid derivatives and how during each successive year another aspect of the pharmacodynamics became apparent. Just before his unfortunate death, I had a list of ten different mechanistic actions, all of them involving agonist and antagonist activity of one or another part of the autonomic nervous system. I really want to thank you for a very exciting paper and for having the courage to work with the controlled substance, which is very difficult to do.

DR. PAUL L. KAUFMAN: I thank Lou for his discussion and Allan for his comments. I have presented at many meetings and I find the discussion here to be the most insightful and interesting of all those that I attend. I do not think people appreciate that. Why is there a bilateral effect of a unilateral intracameral injection? What does this say about mechanism of action? It is a difficult problem that we see with many, albeit not all, of the compounds we use. Systemic effects following topical ocular administration in a small animal are easier to understand in terms of getting a systemic level. We had the same issue with brimonidine. If you reduce the dosage sufficiently, then you can dissect the effect in the treated eye from the control eye. Whether this is due to systemic levels, which as you noted we did not measure, or whether this says something about central effects and reflexes, we really do not understand and have not explored. Performing sympathectomies in monkeys is a challenge, but is actually easy to do. Eye surgeons can master it, but it may be better to have a real neck surgeon perform the procedure. With a little practice and knowledge of monkey neck anatomy, you can remove the ganglion and trunks on both sides and demonstrate by pharmacological means that the eye has no sympathetic tone for years. It was not as if this was the only one we were able to do, but it was the only available sympathectomized monkey at the time we were doing these experiments. We have performed many of these with the help of a good neck surgeon and it is not that difficult to do. We are happy to show the technique if you are really bold enough to want to learn how to do it. Regarding the identification of the opioid receptors in the eyes of nonhuman primates, I believe that this has not been explored as much as one would like. There is more information in the rabbit, but not much information in the monkey. Episcleral venous pressure is the forgotten parameter. You would find this difficult to measure accurately even in man, although we do have techniques to do that. It is very difficult to measure in monkeys because of the highly pigmented conjunctiva and the smaller amount of sclera that is exposed in the palpaebral fissure compared to the human eye. You are correct in stating that we did not measure episcleral venous pressure. Usually the intraocular pressure effects are larger than what you would expect to see on the basis of a change in episcleral venous pressure, but nonetheless this is a possibility. Regarding cannabinoid receptors, there has been a whole new generation of studies of cannabinoid receptors and actions. Keith Green did great work at a time when much less was known. I wish he were with us today. He would have better techniques to work with in terms of compounds and understanding of the receptors. Working with controlled substances is challenging, but it is not so difficult. I have a separate DEA and Schedule I DEA for those compounds. We have tough security clearance for everybody in the laboratory. Thank you all very much for allowing me to present this work and for the very intelligent discussion. I really appreciate it.