Dorsomedial/Perifornical Hypothalamic Stimulation Increases Intraocular Pressure, Intracranial Pressure, and the Translaminar Pressure Gradient

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PURPOSE. Intraocular pressure (IOP) fluctuation has recently been identified as a risk factor for glaucoma progression. Further, decreases in intracranial pressure (ICP), with postulated increases in the translaminar pressure gradient across the lamina cribrosa, has been reported in glaucoma patients. We hypothesized that circadian fluctuations in IOP and the translaminar pressure gradient are influenced, at least in part, by central autonomic regulatory neurons within the dorsomedial and perifornical hypothalamus (DMH/PeF). This study examined whether site-directed chemical stimulation of DMH/PeF neurons evoked changes in IOP, ICP, and the translaminar pressure gradient.

METHODS. The GABA_A receptor antagonist bicuculline methiodide (BMI) was stereotactically microinjected into the DMH/PeF region of isoflurane-anesthetized male Sprague-Dawley rats ($n = 19$). The resulting peripheral cardiovascular (heart rate [HR] and mean arterial pressure [MAP]), IOP, and ICP effects were recorded and alterations in the translaminar pressure gradient calculated.

RESULTS. Chemical stimulation of DMH/PeF neurons evoked significant increases in HR ($+69.3 \pm 8.5$ beats per minute); MAP ($+22.9 \pm 1.6$ mm Hg); IOP ($+7.1 \pm 1.9$ mm Hg); and ICP ($+3.6 \pm 0.7$ mm Hg) compared with baseline values. However, the peak IOP increase was significantly delayed compared with ICP (28 vs. 4 minutes postinjection), resulting in a dramatic translaminar pressure gradient fluctuation.

CONCLUSIONS. Chemical stimulation of DMH/PeF neurons evokes substantial increases in IOP, ICP, and the translaminar pressure gradient in the rat model. Given that the DMH/PeF neurons may be a key effector pathway for circadian regulation of autonomic tone by the suprachiasmatic

nucleus, these findings will help elucidate novel mechanisms modulating circadian fluctuations in IOP and the translaminar pressure gradient. (*Invest Ophthalmol Vis Sci.* 2012; 53:7328–7335) DOI:10.1167/iovs.12-10632

Glaucoma is one of the leading causes of blindness worldwide,^{1,2} yet we are still trying to develop a fundamental understanding of the pathophysiological mechanisms underlying retinal ganglion cell loss. Although multiple factors such as age,^{3,4} ethnicity,^{5,6} and family history^{7,8} contribute to risk of glaucoma, it is well established that elevated intraocular pressure (IOP) plays a major role in the development and progression of the disease.^{6,9–15} Recently, additional potential risk factors have been identified. Evidence indicates there are significant differences in the intracranial pressure (ICP) between patients with primary open angle glaucoma (POAG), normal tension glaucoma (NTG) and nonglaucomatous controls,^{16–19} indicating that the translaminar pressure gradient (i.e., the difference between IOP anteriorly and the CSF pressure posteriorly) may be an important factor in the pathogenesis of glaucoma. Further, multiple studies have identified IOP fluctuation as an independent risk factor for the progression of glaucoma^{20–23} with the highest pressure readings often occurring in the morning hours.

Circadian fluctuation in IOP, which correlates with other known circadian increases in sympathetic outflow,²⁴ can be attenuated using drugs that target the adrenergic pathway, such as timolol maleate.^{25,26} Given the extensive autonomic innervation of the eye, there is good reason to believe that circadian IOP fluctuations are regulated, at least in part, by the sympathetic nervous system.^{27,28}

The suprachiasmatic nucleus (SCN)²⁹ is generally accepted as the key “pacemaker” center of circadian rhythms.^{30–35} The neurons within the dorsomedial hypothalamus (DMH) and surrounding perifornical area (PeF) receive strong direct and indirect projections from the SCN and in turn have a broad array of efferent projections to autonomic sympathetic relays.³⁶ We hypothesized that the DMH/PeF neurons are ideally situated to control the circadian fluctuations in IOP and ICP, which would influence or possibly regulate the translaminar pressure gradient. To test this hypothesis, we utilized site-directed chemical stimulation of DMH/PeF neurons in anesthetized rats while continuously recording IOP and ICP, allowing for calculation of the translaminar pressure gradient throughout the experiment. Furthermore, we also examined sympathoexcitatory activity by assessing HR and MAP to see if IOP or ICP increases were positively correlated.

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MATERIALS AND METHODS

Animal Preparation

All experiments were approved by the Institutional Animal Care and Use Committee and adhered to all standards set forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Sprague-Dawley rats (250–325g; Harlan, Indianapolis, IN) were housed in pairs on a 12-hour light-dark cycle (lights on at 0700), with access to food and water *ad libitum*. At least 48 hours were allowed for habituation in the animal facility before any testing. Anesthesia was accomplished using an induction box attached to the anesthesia machine (3.0% isoflurane at an O₂ flow of 1.5 L/min; Summit Medical Equipment, Bend, OR). After induction, the fur overlying the skull and inguinal area were removed using hair clippers. The animal was transferred to the surgical table, where anesthesia was maintained (2.5% isoflurane at an O₂ flow rate of 1.5 L/min) using a nosecone. Body temperature was maintained at 37°C via a homeothermic blanket (Harvard Apparatus, Holliston, MA) attached to a rectal thermometer.

Attention was then placed on cannulating the femoral artery. Briefly, an arterial cannula was fashioned by connecting a 6-cm leader of 28-gauge PTFE Teflon tubing (Zeus, Inc., Orangeburg, SC) to a 2-cm piece of 23-gauge Tygon tubing (Plastic Corp., Lima, OH). The tip of the Teflon tubing was beveled to 45 degrees and the cannula filled with heparinized saline. A skin incision was made in the inguinal area and the femoral nerve, artery, and vein were visualized. The artery was isolated and the distal end ligated with a suture. The proximal end was temporarily occluded using a looped suture. A 50% thickness incision was created exposing the lumen of the vessel between the two sutures. The Teflon tubing was inserted into the artery and advanced into the descending aorta for continuous monitoring of heart rate and blood pressure. The femoral arterial cannula was connected via PE tubing to a Microswitch high sensitivity pressure transducer (Honeywell, Morristown, NJ) that was attached to a data acquisition system (PowerLab 8/35; AD Instruments, Mountain View, CA) and a laptop running data acquisition software (LabChart 7; AD Instruments) for continuous recording of HR and MAP.

The animal was mounted into a stereotaxic apparatus (Lab Standard Stereotaxic; Stoelting Company, Wood Dale, IL) with the bite-bar elevated 5 mm above the interaural line. A midline incision overlying the skull was created to expose the bregma and carried down to the shoulder blades. The muscles were then reflected off the occipital bone exposing the atlanto-occipital membrane. An intracranial cannula was fashioned similar to those used by Kusaka and colleagues.³⁷ Consecutive sections of PE-10, PE-50, and PE-90 tubing were connected with cyanoacrylate glue. The tip of the PE-10 was beveled to 45 degrees and the entire cannula filled with normal saline. A 25-gauge needle was used to create a small opening in the atlanto-occipital membrane and the beveled tip of the PE-10 tubing was inserted into the cisterna magna space. Cyanoacrylate glue was used to create a water-tight seal around the PE tube entry site. The intracranial cannula was then connected to the data acquisition system (AD Instruments) in a manner similar to the femoral arterial line. A good sinusoidal pattern corresponding to both pulse pressure and an overlying respiratory waveform in both the femoral and intracranial lines was used as a positive indicator of precise cannulation of the desired spaces.

Micropipettes were created on a vertical micropipette puller (David Kopf Instruments, Tujunga, CA) using glass capillary tubes (1.0 mm outer diameter, 0.25 mm inner diameter; AM Systems, Sequim, WA). Pulled pipettes were beveled at 30 to 45 degrees using a BV-10 pipet grinder (Sutter Instrument Company, Novato, CA) to an outer-dimension tip of 38 to 50 μ m. A microscope reticle with 0.1 mm gradations was taped to the pipette in line with the internal lumen so that the meniscus movement could be observed through an operating microscope and volume of injectate precisely determined. The micropipette was secured to the stereotaxic arm, which was positioned 10 degrees oblique to the midsagittal plane. Once secured,

the distal end of the pipette was attached to a picoinjector (PLI-100; Harvard Apparatus, Holliston, MA) with PE tubing.

Using the bregma as a reference point, the DMH/PeF region was targeted for microinjection (0.4 mm posterior, 2.1 mm lateral, and 8.4 mm ventral to the bregma). After moving to the lateral and posterior coordinates, the skull overlying the DMH/PeF was removed using a Dremel rotary tool. The pipette was then backfilled with either the GABA_A receptor antagonist bicuculline methiodide to disinhibit the DMH/PeF region (4 mM BMI in 0.9% normal saline and 10% yellow FluoSpheres), or vehicle (0.9% normal saline and 10% yellow FluoSpheres), and lowered to the appropriate depth as reported previously.³⁸ Cyanoacrylate glue was used to create a water-tight seal around the micropipette. After completion of all surgical procedures, isoflurane concentration was reduced to 1.5% and sufficient time, 10 to 20 minutes, was allowed to ensure animals had reached a steady heart rate and core body temperature.

Microinjection Studies

Baseline HR, MAP, and ICP measurements were continuously recorded (10,000 readings/sec) for 10 minutes prior to microinjection. IOP was monitored throughout the experiment using a rodent rebound tonometer (Icare TonoLab; Icare Finland Oy, Helsinki, Finland). All IOPs were taken in triplicate every 2 minutes and averaged for each time point. Immediately after the fifth baseline IOP measurement, animals received a microinjection of either BMI (30 pmol/75 nL) or saline vehicle (75 nL) targeted to the DMH/PeF region using graded puffs of compressed nitrogen through the picoinjector to deliver the injectate. Rationale for the dose and volume of BMI used was based on our previous microinjection experience^{39,40} and studies showing the spread of radiolabeled (3H) bicuculline⁴¹ following microinjection into the hypothalamus. This dose and volume was determined to be adequate for stimulation of neurons within the DMH/PeF region but did not have excessive spread outside the region of interest. HR, MAP, ICP, and IOP were monitored for 60 minutes postinjection.

Histology

Upon completion of each experiment, the animal was transcardially perfused with 150 mL of 0.1 M PBS followed by 150 mL of 4% paraformaldehyde. The brain was post-fixed in 4% paraformaldehyde for at least 24 hours and then transferred to a 30% sucrose solution for cryoprotection until the brains were saturated with sucrose (evidenced by brains sinking to bottom of tube, in approximately 4–5 days). Brains were then blocked, mounted using mounting medium (Tissue-Tek; Sakura Finetek, Torrance, CA), and 40- μ m tissue sections cut on a cryostat (Leica Cryocut 1800; Leica, Buffalo Grove, IL) at –20°C. Sections were collected and stored in PBS at 4°C until they could be mounted on glass slides and injection sites localized and photographed in a manner to that previously reported.³⁸

Data Collection and Statistical Analysis

The mean baseline HR, MAP (defined as 1/3 systolic + 2/3 diastolic pulse pressure), and ICP readings were averaged over the entire 10-minute baseline period. The mean baseline IOP was calculated as the average value of all rebound tonometry readings taken prior to the microinjection.

To compare the parameters after the stereotaxic microinjection over time, HR, MAP, and ICP data was averaged into 2-minute bins, corresponding to the IOP readings, for the duration of the experiment. The transmural pressure gradient was calculated as IOP minus ICP for each 2-minute time bin.

Comparison of baseline values between the BMI and saline treatment groups was completed using an independent *t*-test. A repeated measures ANOVA was then used to determine whether stereotaxic microinjection of BMI or normal saline into the DMH/PeF region had an effect over time. Where a time effect was found, an

TABLE 1. Baseline Physiologic Parameters prior to Chemical Stimulation of the DMH/PeF Region

Baseline	BMI	Saline	P Value
HR, beats/min	371.1 ± 12.5	390.9 ± 7.8	<i>P</i> = 0.21
MAP, mm Hg	102.0 ± 1.9	102.3 ± 1.9	<i>P</i> = 0.99
ICP, mm Hg	7.1 ± 0.5	7.3 ± 0.6	<i>P</i> = 0.86
IOP, mm Hg	10.2 ± 0.9	11.5 ± 1.3	<i>P</i> = 0.75
TLPG, mm Hg	3.1 ± 0.9	4.2 ± 1.1	<i>P</i> = 0.08

There are no significant differences in baseline HR, MAP, ICP, or IOP between BMI and saline treatment groups (*t*-test).

unpaired 2-tailed *t*-test corrected for multiple comparisons was used to compare individual time points between the BMI and vehicle control groups. Finally, the mean time to reach peak HR, MAP, ICP, and IOP after microinjection was compared using a one-way ANOVA followed by post-hoc analysis using Tukey's method to determine differences between each pair of parameters. All data is reported as mean ± SEM. Significance was considered *P* < 0.05 for all experiments.

RESULTS

There were no significant baseline differences in HR (371.1 ± 12.5 beats per minute versus 390.9 ± 7.8 beats per minute; *P* = 0.21); MAP (102.0 ± 1.9 mm Hg versus 102.3 ± 1.9 mm Hg; *P* = 0.99); ICP (7.1 ± 0.5 mm Hg versus 7.3 ± 0.6 mm Hg; *P* = 0.86); or IOP (10.2 ± 0.9 mm Hg versus 11.5 ± 1.3 mm Hg; *P* = 0.75) between the group treated with BMI and the control group treated with saline prior to microinjection (see Table 1). Microinjection of BMI caused a significant increase in HR (+69.3 ± 8.5 beats per minute; *F* = 29.76, *P* < 0.001); MAP (+22.9 ± 1.6 mm Hg; *F* = 30.96, *P* < 0.001); ICP (+3.6 ± 0.7 mm Hg; *F* = 11.16, *P* < 0.001); and IOP (+7.1 ± 1.9 mm Hg; *F* = 9.65, *P* < 0.001; see Fig. 1). However, there were no significant changes in any of the parameters after microinjection of saline vehicle into the DMH/PeF region (see Table 2 and Fig. 1).

While BMI caused a significant increase in HR, MAP, ICP, and IOP, the time to reach the peak change was not uniform (see Fig. 1). An ANOVA with post-hoc Tukey's analysis indicated that there was no significant difference between the time to peak increase in HR (8.9 ± 0.9 minutes postinjection); MAP (8.5 ± 1.7 minutes postinjection); or ICP (4.3 ± 0.5 minutes postinjection); HR versus MAP *P* = 0.99; HR versus ICP *P* = 0.51; MAP versus ICP *P* = 0.085). However, IOP peaked significantly later than all other monitored parameters (27.8 ± 4.1 minutes postinjection; *P* < 0.001 compared with HR, MAP, and ICP). All physiologic parameters returned to baseline by the end of the 60-minute experimental period.

There was no significant difference in the baseline calculated translaminar pressure gradient in the BMI (3.2 ± 0.9 mm Hg) and saline (4.3 ± 1.1 mm Hg) treatment groups (see Table 1). Microinjection of BMI into the DMH/PeF region evoked a significant increase in the translaminar pressure gradient (+7.2 ± 1.7 mm Hg; *F* = 10.18, *P* < 0.001) while microinjection of saline had no effect (see Table 2 and Fig. 2). Comparison between the two groups showed that the translaminar pressure gradient remained significantly elevated in the BMI treatment group compared with controls between the 24- and 38-minute timepoints following microinjection (see Fig. 2) and then slowly returned to baseline by the end of the 60-minute study period.

In order to confirm accuracy of the injection sites, 40-μm coronal brain sections were examined by fluorescent and brightfield microscopy to identify the site of injection. For each experiment, the microinjection was determined to be in the

FIGURE 1. Changes in HR, MAP, ICP, and IOP after site-directed microinjection of BMI (●; 30 pmol/75 nL; *n* = 9) or saline (Δ; 75 nL; *n* = 10) into the DMH and PeF regions. All injections at *T* = 0 minutes. * Denotes significant difference between saline and BMI treatment groups, *P* < 0.05.

TABLE 2. Maximum Change in Physiologic Parameters from Baseline following Chemical Stimulation of the DMH/PeF Region

	Maximum Increase from Baseline	% Change	P Value
BMI			
HR, beats/min	+69.3 ± 8.5	18.7%	$F = 29.76, P < 0.001^*$
MAP, mm Hg	+22.9 ± 1.6	22.4%	$F = 30.96, P < 0.001^*$
ICP, mm Hg	+3.6 ± 0.7	50.7%	$F = 11.16, P < 0.001^*$
IOP, mm Hg	+7.1 ± 1.9	69.6%	$F = 9.65, P < 0.001^*$
TLPg, mm Hg	+7.2 ± 1.7	225.0%	$F = 10.18, P < 0.001^*$
Saline			
HR, beats/min	+4.7 ± 8.1	1.2%	$F = 1.94, P = 0.15$
MAP, mm Hg	+2.0 ± 1.5	2.0%	$F = 1.09, P = 0.36$
ICP, mm Hg	+0.6 ± 0.5	8.2%	$F = 1.48, P = 0.25$
IOP, mm Hg	+0.8 ± 0.8	7.0%	$F = 1.41, P = 0.26$
TLPg, mm Hg	+0.2 ± 1.4	4.8%	$F = 1.92, P = 0.14$

Microinjection of BMI, but not saline vehicle, into the DMH/PeF region evokes significant increases in HR, MAP, ICP, and IOP (repeated measures ANOVA).
* Denotes significant increase from baseline; $P < 0.05$.

DMH and PeF region according to the atlas of Paxinos and Watson (see Fig. 3).⁴²

DISCUSSION

IOP, ICP, and the Translaminar Pressure Gradient

Consistent with our hypothesis, we have demonstrated that chemical stimulation of the DMH/PeF neurons causes a robust increase in IOP and ICP (see Fig. 1). We initially predicted that the IOP and ICP increases would coincide with the increases in HR and MAP known to occur following microinjection of BMI into this region.^{38,39} However, following chemical stimulation of the DMH/PeF, we observed a rapid increase in HR, MAP, and ICP that began within 1 minute after injection and peaked between 5 to 10 minutes postinjection. Surprisingly, the increase in IOP did not begin until 20 minutes postinjection and peaked at approximately 10 minutes later. By the time IOP

FIGURE 2. Changes in the translaminar pressure gradient after site-directed microinjection of BMI (●; 30 pmol/75 nL; $n = 9$) or saline (Δ; 75 nL; $n = 10$) into the DMH and PeF regions. All injections at $T = 0$ min. * Denotes significant difference between saline and BMI treatment groups, $P < 0.05$.

FIGURE 3. A representative photo of fluorescent microspheres identifying a microinjection site (upper left) as well as a schematic of coronal sections (bottom six figures) of a rat brain adapted from the atlas of Paxinos and Watson⁴² showing the injection sites for BMI (●; 30 pmol/75 nL; $n = 9$) or saline (Δ; 75 nL; $n = 10$) in the hypothalamus. Numbers represent the anatomic level in relation to the bregma. DMN, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamus; PH, posterior hypothalamus; f, fornix; mt, mammillothalamic tract. Reprinted with permission from Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 6th ed. San Diego, CA: Academic Press; 1997. Copyright 1997 Elsevier.

had reached its peak, ICP had returned to baseline and the HR and MAP were approaching baseline levels.

Because of this temporal difference between the IOP and ICP peaks, there is a dramatic fluctuation in the calculated translaminar pressure gradient postinjection. Coinciding with the ICP peak 4 to 6 minutes postinjection, the translaminar pressure gradient drops to near zero (see Figs. 1, 2). While we do not yet know the mechanism underlying the increases in ICP following DMH/PeF stimulation, we did observe that ICP rapidly returns to baseline levels ahead of HR and MAP (see Fig. 1). This is likely due to the fact that cerebral blood flow and ICP are under tight autoregulation.^{43,44} Since IOP continues to rise while ICP is dropping, there is a corresponding increase in the calculated translaminar pressure that extends for an average of 25 minutes. The combined effect of this sequence of events is a dynamic pressure shift experienced by the lamina cribrosa equal to the sum of the ICP and the IOP fluctuations (roughly 10–11 mm Hg). To our knowledge these studies represent the first data showing that stimulating the DMH/PeF, a site well established in regulating circadian rhythms and autonomic outflow, causes an increase in IOP, ICP, and the translaminar pressure gradient.

Currently, little is known regarding daily fluctuation in ICP at the level of the optic nerves in humans. However, similar to others, we believe that ICP is likely to be highest at nighttime due to a shift from the upright to the supine position while

sleeping.⁴⁵ Data on ICP fluctuation in the rodent model has produced conflicting results, with both the presence^{46,47} and absence⁴⁸ of circadian ICP fluctuation being reported. As small-scale pressure monitoring technology advances in the future, it should enable more accurate and direct measurement of the translaminar pressure gradient in these types of models.

We are just now beginning to understand the potential importance of ICP and the translaminar pressure gradient on the development and progression of glaucoma. The effect of reduced ICP has been examined in the past. Yablonski and colleagues found that in the feline model, reduction of ICP to just below atmospheric pressure for 1 to 7 days caused compaction of prelaminar disc tissue without structural changes in the lamina cribrosa, consistent with the initial changes seen in preglaucomatous eyes (Yablonski ME, et al. *IOVS* 1978;17:ARVO Abstract 6). A follow-up study using the same model showed that lowering ICP for approximately 3 weeks resulted in prelaminar axonal swelling, optic disc enlargement, and posterior bowing of the lamina cribrosa (Yablonski ME, et al. *IOVS* 1979;18:ARVO Abstract 8) similar to models of early experimental glaucoma in primates.⁴⁹

Recent clinical studies further support the importance of ICP and the translaminar pressure gradient in the development of glaucomatous optic neuropathy. Berdahl and colleagues showed that patients with POAG had significantly lower opening pressure on lumbar puncture compared with non-glaucoma controls.¹⁶ Additionally, patients with both POAG and NTG had significantly lower ICP compared with age-matched controls, while patients with ocular hypertension had ICP that was significantly higher than their age-matched controls.¹⁷ These findings have now been corroborated in prospective studies in POAG, NTG, and ocular hypertensive patients.^{18,19} Our results show that stimulation of the hypothalamic DMH/PeF causes an increase in ICP as well as a shift in the translaminar pressure gradient. It is possible that dysregulation or loss of sympathetic tone as we age could result in a lowering of ICP and an aberrant translaminar pressure gradient, thus increasing risk for the development and progression of glaucoma.

We believe that work aimed at understanding the lamina cribrosa as a biomechanical structure^{50,51} and its physiological response to glaucomatous stress and strain^{49,52,53} (Girard MJ, et al. *IOVS* 2012;53:ARVO E-Abstract 3182) is a paradigm-shifting concept in glaucoma research. Combined with the results of recent clinical studies and our experimental results, we believe that there is strong evidence to suggest that inclusion of ICP and translaminar pressure gradient data into future biomechanical models will strengthen our understanding of the underlying pathophysiological mechanisms underlying development and progression of glaucoma.

Hypothalamic Control of Intraocular Pressure

Historically, hypothalamic control of IOP has been the subject of considerable investigation.⁵⁴⁻⁵⁷ Studies on this subject had their origins decades ago in the search for hypothalamic centers controlling the sympathetic “fight-or-flight” response.⁵⁸ A classic study in cats by Walter Hess,⁵⁹ an ophthalmologist by training, and later rodent work by DiMicco, Shekhar, Samuels and others^{39,40,60-65} mapped the posterior hypothalamic regions that evoked mobilization of the sympathetic nervous system (as evidenced by tachycardia and pressor responses) and corticosterone release when electrically or pharmacologically stimulated. In these studies, areas containing DMH/PeF neurons often produced the most robust responses.

Interestingly, at nearly the same time that Hess and colleagues were mapping the hypothalamic pathways in-

involved in sympathetic mobilization, glaucoma researchers had also begun investigating the reasons why intense emotional states and stress evoked episodes of angle closure glaucoma and increased IOP. Ophthalmic studies showed that electrical stimulation of the diencephalic centers, primarily the posterior hypothalamus, evoked immediate increases in IOP.⁵⁴⁻⁵⁷ However, much of this work was difficult to interpret due to the variability in data showing that increases in IOP were accompanied by both increases and decreases in blood pressure depending on the location stimulated. Some of this confusion may be related to the methods used in those experiments. Electrical stimulation is now known to activate both cell bodies in the region of the electrode tip as well as adjacent axons traveling through the area from other nuclei. It would not be until the advent of stereotaxic chemical microinjection techniques that scientists could more reliably perform site-directed microinjection experiments specifically targeting cell bodies.⁶⁶ By this time, much of the glaucoma research community had refocused their attention on the pathophysiology underlying increased aqueous outflow resistance in the trabecular meshwork. However, given the recent findings that IOP fluctuation, ICP levels, and changes in the translaminar pressure gradient may be important factors in the pathogenesis of glaucoma, there is renewed interest in the contribution of hypothalamic and other central nervous system centers that control these variables.

The ideal central nervous system control center would need to regulate both circadian rhythms and sympathetic outflow. The suprachiasmatic nucleus (SCN), which receives direct projections from retinal ganglion cells,⁶⁷⁻⁷⁰ is a prime candidate for regulating circadian rhythms. The SCN is known as the body’s “master time-clock,” and has a variety of functions in regulating circadian activities, including corticosteroid release,^{30,31} locomotor activity and feeding,³² and sleep.³⁴ The SCN also appears to play a role in IOP fluctuations as well. There is ample evidence demonstrating that IOP varies in a cyclical manner over a 24-hour period in both human and rodents, with the peak pressure occurring in the morning hours upon awakening.⁷¹⁻⁷⁴ Liu and Shieh have shown that SCN lesions alter circadian fluctuations in IOP.⁷⁵ Yet the SCN itself is not a critical locus for regulating sympathetic outflow. A more ideal candidate is the dorso-medial/perifornical hypothalamus (DMH/PeF).

Classically, the DMH and neurons in the surrounding PeF region have been recognized as key regulators of feeding behaviors, body weight regulation, neuroendocrine control, and metabolism (for a review, see Bellinger and Bernardis).⁷⁶ Further studies indicate that in addition to mobilizing sympathetic outflow in response to stress or threat, the DMH/PeF plays a critical role in regulating a wide range of behavioral, endocrine, and autonomic circadian rhythms.^{29,77} For instance, the DMH/PeF region receives robust projections from the SCN,²⁹ and lesions to the DMH/PeF region disrupts the sleep-wake cycles⁷⁸ and circadian behaviors.²⁹ While there are many potential efferent pathways from the SCN that could control circadian fluctuation of IOP,⁷⁹⁻⁸² the DMH/PeF is a strong candidate since this region receives strong projections from the SCN, and plays a critical role in sympathetic mobilization and regulation of circadian activity (e.g., behavior, endocrine, and autonomic activity). This information, combined with our findings that chemical stimulation of the DMH/PeF evokes increases in IOP, provides compelling rationale for the hypothesis that the DMH/PeF is the putative location for hypothalamic control of IOP. Given the strong direct and indirect connections to the SCN²⁹ and extensive efferent connections to sympathetic autonomic relays and the endocrine system through the

FIGURE 4. This illustration represents a hypothetical map showing the potential hypothalamic neural circuitry responsible for controlling IOP, including afferent and efferent connections of the dorsomedial and perifornical hypothalamic neurons. oc, optic chiasm; ac, anterior commissure; pc, posterior commissure; RVL/MPa, rostromedial medulla; MPa, raphe pallidus.

paraventricular nucleus,³⁶ we believe the DMH/PeF neurons are ideally situated to modulate the circadian fluctuations in IOP evoked by activity of SCN neurons and alterations in both ICP and the translaminar pressure gradient (see Fig. 4). Future functional studies utilizing this microinjection model will help to determine whether this pathway alone or in concert with others contribute to circadian fluctuation of IOP and ICP.

Future Directions and Conclusions

While chemical stimulation of neurons in the DMH/PeF region evoke reproducible increases in IOP, we do not know the physiologic mechanism underlying these changes nor the reason for the temporal differences between increases in IOP compared with HR, MAP, and ICP. Future studies will be aimed at identifying the physiological basis for DMH/PeF-mediated changes in IOP.

In summary, this study suggests that two potential mechanisms for glaucoma progression, fluctuation of IOP and an increased translaminar pressure gradient, may both be regulated in part by neurons located in the DMH/PeF region of the hypothalamus. This is a previously unrecognized function of these neurons. Further defining this pathway and the neurotransmitters involved may provide targets for novel glaucoma therapies aimed at reducing circadian fluctuation of IOP or stabilizing the translaminar pressure gradient.

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