

Thyrotropin Releasing Hormone Increases Intraocular Pressure

Mechanism of Action

John H. K. Liu, Angela C. Dacus, and Stephen P. Bartels

Intravenous injections of 1–100 μ g thyrotropin releasing hormone (TRH) in rabbits elevated intraocular pressure (IOP). The 2–5 mm Hg increase of IOP lasted for less than 2 hr. No change of pupil size was observed. This IOP elevation was not due to a direct effect of TRH on ocular tissues since intravitreal injections of 0.1 and 1 μ g TRH did not change IOP. Concentrations of thyroid stimulating hormone (TSH), triiodothyronine (T-3), epinephrine (Epi) and norepinephrine (NE) in the plasma were elevated at 30 min after an i.v. injection of 10 μ g TRH. Plasma levels of prolactin and thyroxine were not changed. Bolus i.v. injections of 0.1–1 μ g TSH and 0.1–1 μ g T-3, which would produce an equivalent increase of relevant hormones in the circulation, did not increase IOP. However, similar i.v. injections of 10–100 ng Epi and 100 ng NE caused a 1.5–3 mm Hg IOP elevation for 15–30 min. Thus, the IOP elevation following TRH administration probably is caused by the increase of circulating endogenous catecholamines and not by the stimulation of the TSH–thyroid hormone axis. Heart rate, but not blood pressure, was increased with 10 μ g TRH. After unilateral transection of the cervical sympathetic trunk, the IOP elevation in the decentralized eye was larger than that in the intact eye. Topical treatment of 0.1% or 1% timolol in the decentralized eye inhibited the IOP elevations in both eyes, but 0.1% prazosin was not effective. Topical 1% atropine and atropine given subcutaneously at 0.6 mg/kg decreased the bilateral IOP elevations. These observations indicate that beta-adrenergic and muscarinic mechanisms, not an alpha-1-adrenergic mechanism, are involved. Invest Ophthalmol Vis Sci 30:2200–2208, 1989

The pituitary hormone releasing factors are small peptides which control the release of pituitary hormones. Recent studies in our laboratory demonstrate that two pituitary hormone releasing factors can affect intraocular pressure (IOP) through mechanisms in the central nervous system (CNS). An increase of corticotropin releasing factor or luteinizing hormone releasing hormone in the cerebrospinal fluid of rabbits causes a decrease of IOP for days.^{1,2} Another pituitary hormone releasing factor, thyrotropin releasing hormone (TRH), has been reported to have the opposite effect on IOP. Third ventricular microinjection of TRH in rabbits was found to elevate IOP for hours.³ Its mechanism of action was unclear. The present study explores the possible mechanisms by which TRH elevates IOP.

TRH is widely distributed in the CNS.⁴ This tripeptide can affect a variety of physiological functions through CNS mechanisms. One of them is to work as a pituitary hormone releasing factor. Endogenous TRH secreted from the hypothalamus is transported via the portal vessel to the anterior pituitary gland for the release of thyroid stimulating hormone (TSH). TSH then stimulates the secretion of triiodothyronine (T-3) and thyroxine (T-4) from the thyroid gland. It is well documented that an i.v. injection of TRH in humans increases the circulating TSH and T-3 but the increase of T-4 is less readily detected.⁵ Similarly, circulating TSH in rabbits can be elevated after an i.v. TRH injection.⁶ Exogenous TRH can also release prolactin (PRL) from the anterior pituitary gland in mammals,⁴ including the rabbit.⁷ Human serum PRL levels can be elevated by TRH in both males and females and this effect is more readily detectable in females.⁸ In addition, recent studies demonstrate that TRH functions as a CNS neurotransmitter that consequently causes a release of catecholamines from the adrenal medulla into the circulation.^{9–11} Whether the elevation of IOP by TRH is related to any of these mechanisms was investigated in the present study.

From the Eye Research Institute of Retina Foundation and the Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts.

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Reprint requests: John Liu, PhD, Eye Research Institute of Retina Foundation, 20 Staniford Street, Boston, MA 02114.

Materials and Methods

TRH was purchased from Sigma Chemical Company (St. Louis, MO). Male New Zealand albino rabbits (3–5 kg) were used in accordance with the ARVO Resolution on the Use of Animals in Research.

IOP after i.v. TRH

Rabbits were given i.v. injections of 0.1–100 μg TRH. IOP and pupil size were monitored. IOP was measured with a modified Digilab (Cambridge, MA) pneumatonometer calibrated previously for rabbit eyes. Topical 0.5% proparacaine (Ophthaine, Squibb, Princeton, NJ) was used as the local anesthetic. For each IOP measurement, the value was averaged from three successive readings. Pupil size was measured with a ruler under constant illumination. Rabbits were acclimated to tonometry prior to the beginning of each experiment. Bolus injection of 0.1, 1, 10 or 100 μg TRH in 100 μl sterile saline was made via the marginal ear vein. Sterile saline (100 μl) was given in the control group. Injections were made at 9–10 AM and six to nine rabbits were used for each dose. One week of rest was allowed between repeated injections in individual rabbits. It has been shown that tolerance in the response of serum TSH to repeated i.v. injections of TRH does not develop in humans.¹² We measured bilateral IOPs at 30 min intervals for 2 hr, at 3, 4 and 5 hr on day 1, and then at 9 AM and 4 PM for 3 more days. IOPs from both eyes were averaged. At each time point, the mean IOP difference from the IOP value at 0 time was compared with that in the control group using the paired t-test. The level of significance for a change was set to be $P < 0.05$.

IOP after Intravitreal TRH

Topically anesthetized eyes in restrained conscious rabbits were given intravitreal injections of 0.1 and 1 μg TRH in 10 μl sterile saline. Sterile saline (10 μl) was injected into the contralateral eye as the control. Injections were made at 9–10 AM and six rabbits were used for each dose. We measured bilateral IOPs at 15 min intervals for 90 min, and at 2, 3, 4 and 5 hr. At each time point, the mean IOP change in the TRH-treated eye was compared with that in the control eye using the paired t-test.

Hormone Assays

Plasma concentrations of TSH, PRL, T-3 and T-4 after an i.v. injection of 10 μg TRH were determined in groups of six to nine rabbits. Blood was collected from the opposite ear vein prior to the i.v. injection, at 30 min, 2 hr and 5 hr after the injection. Heparin-

ized blood was centrifuged and plasma was prepared and stored at -20°C until assayed.

TSH, PRL and T-4 were determined by radioimmunoassay (RIA) and T-3 was determined by enzyme immunoassay (EIA). Homologous rabbit TSH and PRL double-antibody RIAs were used. Highly purified rabbit TSH, PRL, guinea pig anti-rabbit TSH and rat anti-rabbit PRL were generously provided by Dr. Parlow (Harbor–UCLA Medical Center). TSH was radioiodinated with I-125 by the chloramine T method.¹³ PRL was radioiodinated using the same method except the iodination was halted by adding 62.5 μg sodium metabisulfite.¹⁴ The labeled TSH was isolated by Con A-Sepharose affinity chromatography¹³ and the labeled PRL by G-100 gel filtration.¹⁵ TSH RIA was performed using a modified procedure described by Kakita et al.¹⁶ The only modification was that a smaller amount of labeled TSH (approximately 4000 cpm) was used in each RIA tube. PRL RIA followed the procedure by Muccioli et al.¹⁵ The sera and antisera needed for the RIA were purchased from Sigma Chemical Co. (St. Louis, MO). T-4 was determined using the Total T-4 RIA kit from BioClinical Group (Cambridge, MA). T-3 was measured using the EIA kit from Immunotech (Boston, MA). Each sample was assayed in duplicate. Hormone concentrations after the TRH injection were compared with the initial values using the student t-test.

Catecholamines Assay

Plasma epinephrine (Epi) and norepinephrine (NE) were determined in eight rabbits given an i.v. injection of 10 μg TRH. The rabbit was put in a restraint. A catheter (Angiocath; Deseret, Sandy, UT) was placed in the central ear artery¹⁷ under local anesthesia using cetacaine (Cetylite Industries, Inc., Pennsauken, NJ) at least 30 min before the TRH administration. TRH was given intravenously via the vein of the other ear. Five milliliters blood was collected from the ear artery at 0 min, 30 min and 2 hr after the TRH injection. Plasma was prepared and Epi and NE were determined using a Waters (Milford, MA) HPLC system including a model 460 electrochemical detector, following the procedure described by the manufacturer.¹⁸

IOP after i.v. TSH, T-3, Epi, and NE

Seven rabbits were given bolus i.v. injections of 0.1 and 1 μg rabbit TSH in sterile saline. Two groups of seven rabbits were given bolus i.v. injections of 0.1 and 1 μg T-3 (Sigma) in 95% ethanol respectively. Groups of five to six rabbits were given i.v. injections

of 10 and 100 ng Epi (International Medication Systems, So. El Monte, CA) and 10 and 100 ng NE (Sigma) in sterile saline. At least 1 week of rest was allowed between injections. We measured bilateral IOPs at 15 min intervals for 90 min, and at 2, 3, 4 and 5 hr. IOPs from both eyes were averaged. At each time point, the mean IOP and pupil size were compared with the initial values using the student t-test.

Cardiovascular Effects

Cardiovascular functions after an i.v. injection of TRH were studied in seven conscious rabbits. Each rabbit was restrained and the central ear artery was cannulated as described previously. The arterial catheter was connected to a pressure transducer and blood pressure was recorded on a Grass (Quincy, MA) 7D polygraph. Heart rate was determined by the arterial pulse. The rabbit was acclimated for 30 min before the i.v. injection of 10 µg TRH in the opposite ear. IOP, blood pressure and heart rate were then monitored for 2 hr.

Transection of Cervical Sympathetic Trunk

Unilateral transection of the cervical sympathetic trunk was performed in nine rabbits under general anesthesia. The sympathetic trunk was identified by the pupillary dilation responding to an electric stimulation (5–10 V, 20 Hz) from a Grass stimulator.¹⁹ Several millimeters of preganglionic fibers distant from the superior cervical ganglion were removed. Two weeks later, the success of this procedure was confirmed by unilateral miosis. The postganglionic sympathetic innervation was intact, confirmed by the pupillary response to one drop of 1% hydroxyamphetamine (Paredrine; Smith Kline & French, Philadelphia, PA). One week of rest was allowed following the hydroxyamphetamine test. These rabbits (N = 7–9) were given i.v. injections of 0.1–100 µg TRH using the protocol described previously and IOP was measured for 5 hr.

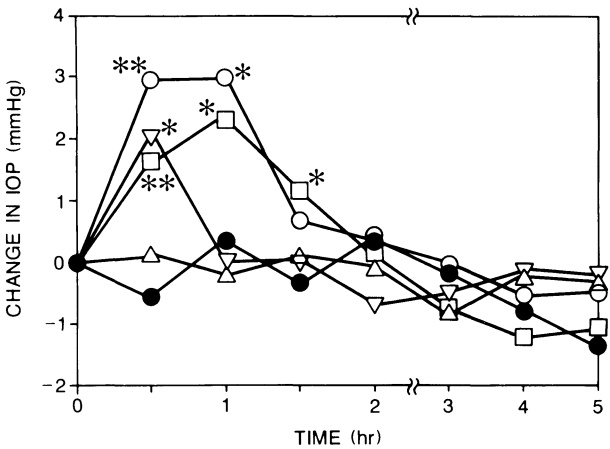


Fig. 1. Change in IOP after i.v. injection of TRH. ●, control experiment with injection of saline; △, 0.1 µg TRH; ▽, 1 µg TRH; □, 10 µg TRH; ○, 100 µg TRH. **, *P* < 0.01, *, *P* < 0.05, paired t-test (*N* = 6–9). IOP values prior to the injection range from 16.6 to 19.3 mm Hg.

Pharmacological Studies

Regional adrenergic and cholinergic neurotransmitters may participate in the IOP response to the i.v. injection of 10 µg TRH. This was investigated in experiments using three selective blocking agents; timolol (beta-adrenergic antagonist from Merck Sharp & Dohme), prazosin (alpha-1-adrenergic antagonist from Pfizer), and atropine (muscarinic antagonist from Sigma). Rabbits with unilateral transection of cervical sympathetic trunk were used. All rabbits were atropinesterase-positive, as identified by their short (<24 hr) mydriatic response to 1% atropine.²⁰ The doses used were topical 0.1 and 1% timolol, topical 0.1% prazosin, topical 1% atropine and 0.6 mg/kg atropine given subcutaneously. The topical agent was given in 20 µl saline (timolol and atropine) or in suspension with 20 µl distilled water (prazosin) unilaterally on the decentralized eye. Prazosin and atropine were given 1 hr before the TRH injection. Timolol was administered twice because of its short activity in

Table 1. Plasma hormone concentrations after i.v. 10 µg TRH

Hormone	Conc.	N	Time			
			0 min	30 min	2 hr	5 hr
TSH	ng/ml	8	0.9 ± 0.4	3.2 ± 1.3†	0.9 ± 0.4	ND
PRL	ng/ml	8	25 ± 9	23 ± 4	12 ± 7	ND
T-3	ng/ml	9	0.97 ± 0.07	1.62 ± 0.16*	1.26 ± 0.10†	1.00 ± 0.05
T-4	µg/ml	6	2.36 ± 0.42	2.33 ± 0.42	2.45 ± 0.32	2.19 ± 0.46
Epi	pg/ml	8	109 ± 20	155 ± 17†	134 ± 23	ND
NE	pg/ml	8	565 ± 81	741 ± 124†	650 ± 63	ND

* *P* < 0.01.

† *P* < 0.05, student t-test.

ND, not determined.
Values are mean ± SEM.

Table 2. IOP after i.v. injections of TSH, T-3, Epi, and NE

	Dose	N	Time (min)							
			0	15	30	45	60	75	90	120
TSH	0.1 µg	7	18.0 ± 0.3	17.8 ± 0.3	18.1 ± 0.7	18.4 ± 0.4	17.7 ± 0.3	17.4 ± 0.5	17.1 ± 0.6	17.6 ± 0.7
	1 µg	7	17.4 ± 0.3	16.9 ± 0.5	16.2 ± 0.3*	16.3 ± 0.2*	17.1 ± 0.2	16.4 ± 0.3†	16.6 ± 0.5	17.3 ± 0.8
T-3	0.1 µg	7	17.0 ± 0.6	17.7 ± 0.9	17.3 ± 0.7	17.0 ± 0.6	16.4 ± 0.6	16.9 ± 0.5	16.4 ± 0.5	16.6 ± 0.5
	1 µg	7	17.5 ± 1.1	17.4 ± 0.8	16.9 ± 0.6	17.0 ± 0.8	17.0 ± 0.5	17.2 ± 0.7	17.2 ± 0.6	17.6 ± 0.8
Epi	10 ng	5	18.3 ± 0.5	19.8 ± 0.5†	20.4 ± 0.4*	19.2 ± 0.7	19.0 ± 0.4	19.0 ± 0.7	19.0 ± 0.4	19.1 ± 0.6
	100 ng	5	17.2 ± 0.3	19.5 ± 0.5*	19.5 ± 0.6*	18.0 ± 0.6	18.5 ± 0.7	17.4 ± 0.5	18.3 ± 0.6	17.9 ± 0.3
NE	10 ng	5	20.1 ± 1.0	21.5 ± 0.6	21.0 ± 0.8	21.1 ± 1.8	21.3 ± 1.8	20.5 ± 1.6	20.0 ± 0.9	20.4 ± 0.9
	100 ng	6	18.8 ± 0.8	21.8 ± 0.9†	19.8 ± 0.4	20.5 ± 0.9	20.0 ± 0.7	20.4 ± 0.5	20.0 ± 0.6	19.1 ± 0.4

* $P < 0.01$.† $P < 0.05$, student t-test.

Values are mean ± SEM.

albino rabbits²¹; once immediately prior to the TRH injection and at 30 min later. Bilateral IOPs were measured at various time points before the administration of the blocking agents and until 4 hr after the i.v. TRH injection.

Results

Intravenous injections of 1–100 µg TRH caused a significant IOP elevation shortly after the injection and this effect lasted for less than 2 hr (Fig. 1). The threshold dose was 1 µg and the IOP elevation was dose-dependent. The peak of IOP elevation appeared at 30–60 min after the injection. There was no delayed IOP change afterward. In general, these TRH doses caused tachypnea and behavioral excitement for hours. No significant change of pupil size was observed. Intravitreal injections of 0.1 and 1 µg TRH did not change IOP (data not shown).

Changes in plasma hormone concentrations after an i.v. injection of 10 µg TRH are shown in Table 1. Thirty minutes after the injection, the plasma TSH concentration increased from 0.9 ± 0.4 (mean ± SEM) to 3.2 ± 1.3 ng/ml. It returned to normal at 2 hr. The plasma concentration of T-3 increased from 0.97 ± 0.07 to 1.62 ± 0.16 ng/ml at 30 min. At 2 hr, the T-3 concentration (1.26 ± 0.10 ng/ml) was still higher than the baseline. It returned to normal at 5

hr. No significant change in plasma PRL or T-4 concentration was observed. Thirty minutes after the TRH injection, the plasma concentrations of Epi and NE increased. The Epi concentration increased by 42% (from 109 ± 20 to 155 ± 17 pg/ml) and the NE increased by 31% (from 565 ± 81 to 741 ± 124 pg/ml). At 2 hr, Epi and NE concentrations were not different from the baseline values.

The IOP responses to i.v. injections of TSH, T-3, Epi and NE are presented in Table 2. Injection of 0.1 µg TSH did not change IOP. An IOP decrease occurred after the injection of 1 µg TSH. Injections of 0.1 and 1 µg T-3 did not affect IOP. An increase of IOP by 1.5–2.3 mm Hg for 30 min was observed after i.v. injections of 10 and 100 ng Epi. Similarly, IOP increased by 3 mm Hg at 15 min after the injection of 100 ng NE. Pupil size was not changed by these agents.

Heart rate increased by 16–20 beats/min during 30–90 min after the i.v. injection of 10 µg TRH (Table 3). The time course of this tachycardia corresponded well with the IOP elevation in these rabbits. No change of blood pressure was noted during this period.

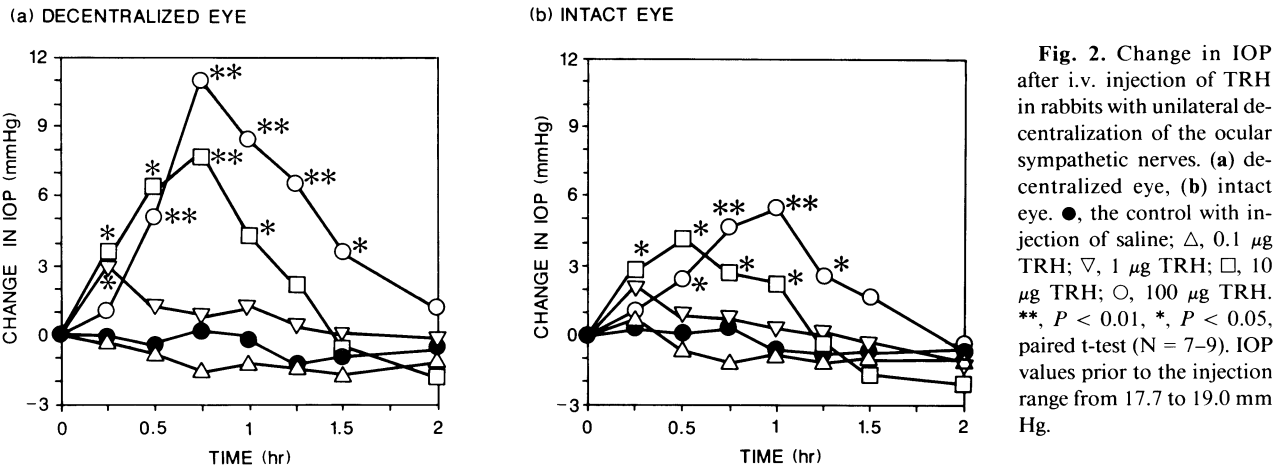
The IOP elevation after the i.v. 1–100 µg TRH was affected by the transection of the cervical sympathetic trunk. The IOP elevation in the decentralized eye was larger than that in the intact eye (Fig. 2). In these

Table 3. Cardiovascular functions after i.v. 10 µg TRH

	Time (min)							
	0	15	30	45	60	75	90	120
IOP (mm Hg)	17.4 ± 0.3	18.1 ± 0.4	20.0 ± 0.9†	22.0 ± 1.0*	20.8 ± 1.0†	19.5 ± 0.6†	18 ± 0.4	17.5 ± 0.5
BP: systolic (mm Hg)	100 ± 3	101 ± 4	100 ± 4	101 ± 4	100 ± 4	96 ± 3	97 ± 3	95 ± 3
diastolic (mm Hg)	69 ± 3	69 ± 2	69 ± 2	70 ± 2	70 ± 1	69 ± 1	67 ± 1	67 ± 2
Heart rate (beats/min)	215 ± 8	218 ± 5	231 ± 6†	234 ± 9†	235 ± 9†	233 ± 7†	233 ± 5†	226 ± 5

* $P < 0.01$.† $P < 0.05$, student t-test (N = 7).

Values are mean ± SEM.



rabbits with unilateral decentralization of the ocular sympathetic nerves, topical administrations of 0.1% and 1% timolol in the decentralized eye partially blocked the bilateral IOP elevations which occurred 15–60 min after the i.v. injection of 10 μ g TRH (Table 4). Topical treatment of 0.1% prazosin alone in the decentralized eye caused a small IOP decrease in both the treated eye and the contralateral, untreated eye. However, this treatment did not prevent the occurrence of the bilateral IOP elevations in response to the i.v. 10 μ g TRH. Figure 3 shows the IOP response in the decentralized eye under various experimental conditions. Unilateral administration of 1% atropine inhibited the bilateral IOP elevations following the i.v. 10 μ g TRH (Table 5). In this experiment, a significant mydriasis occurred in the atropine-treated eye. The average pupil size increased from 6.0 mm to 7.6 mm. Systemic atropine (0.6 mg/kg s.c.) also decreased the IOP elevations in both eyes (Table 5). No change of pupil size was observed. The inhibitory effect by the systemic atropine is stronger than that by the topical 1% atropine.

Discussion

Intravenous injections of 1–100 μ g TRH cause a significant IOP elevation in rabbits. The magnitude and the pattern of this IOP elevation are quite similar to those seen after third ventricular microinjection of TRH.³ Following i.v. injection, TRH is distributed via the blood stream and it reaches the CNS.²² Thus, the IOP elevation could be either a peripheral or a CNS effect. Similarly, a CNS or a peripheral mechanism could be responsible for the IOP elevation after third ventricular microinjection of TRH. The site of action causing the IOP elevation by these two types of TRH administrations needs to be clarified.

An analysis of the data from the i.v., intraventricular and intravitreal injections of TRH indicates that TRH has no direct, peripheral mechanism to cause the IOP elevation. The threshold dose of i.v. TRH causing an IOP elevation is 1000 ng seen in this study and that of the third ventricular microinjection of TRH is 10 ng.³ Therefore, 100 ng TRH given into the third ventricle causes an IOP increase while the same

Table 4. IOP after i.v. injection of 10 μ g TRH in rabbits treated with timolol

Treatment	Time (min)							
	0	15	30	45	60	75	90	120
Decentralized eye:								
TRH	18.0 \pm 0.7	21.1 \pm 1.3*	24.1 \pm 2.0*	25.3 \pm 2.2*	21.9 \pm 1.9*	19.8 \pm 1.7	17.0 \pm 0.8	15.3 \pm 0.5
0.1% timolol + TRH	17.6 \pm 0.3	17.5 \pm 0.8‡	20.0 \pm 0.9‡	22.1 \pm 1.9	20.9 \pm 1.7	18.0 \pm 1.2	17.2 \pm 0.8	15.7 \pm 0.6
1% timolol + TRH	17.7 \pm 0.7	16.9 \pm 0.5†	17.9 \pm 0.5†	21.4 \pm 1.5	20.7 \pm 1.3	18.5 \pm 0.9	17.0 \pm 0.7	15.8 \pm 0.8
Intact eye:								
TRH	18.7 \pm 0.9	22.3 \pm 1.5*	23.0 \pm 2.0*	21.3 \pm 0.8*	20.9 \pm 1.4*	18.3 \pm 0.8	17.0 \pm 0.6	15.6 \pm 0.5
0.1% timolol + TRH	17.8 \pm 0.6	17.6 \pm 0.6†	17.8 \pm 0.6‡	19.8 \pm 1.3	20.2 \pm 1.8	17.3 \pm 1.2	16.4 \pm 0.7	15.0 \pm 0.8
1% timolol + TRH	17.5 \pm 0.5	16.5 \pm 0.4†	16.9 \pm 0.4†	19.0 \pm 1.0‡	19.1 \pm 1.1	17.2 \pm 0.9	16.1 \pm 0.7	15.5 \pm 0.6

* Statistically significant IOP elevation from the initial value.
† $P < 0.01$.
‡ $P < 0.05$ compared with the value at the same time point in the TRH

experiment.
Values are mean \pm SEM (N = 9).
Timolol was given only to the decentralized eye at 0 min and 30 min.

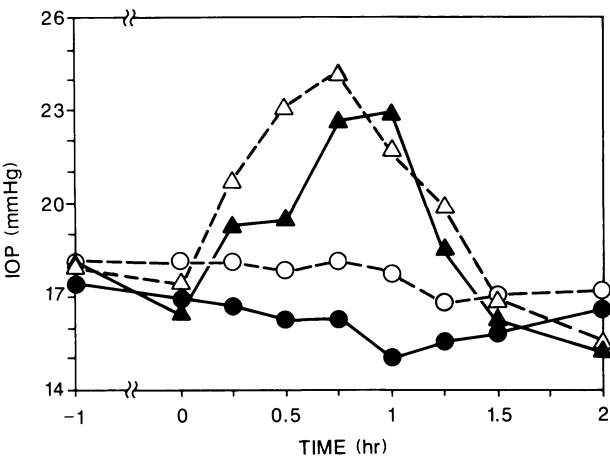


Fig. 3. IOP after i.v. TRH injection in rabbits treated with prazosin. ○, i.v. injection of saline; △, i.v. injection of 10 µg TRH; ●, topical 0.1% prazosin with i.v. injection of saline; ▲, topical 0.1% prazosin with i.v. injection of 10 µg TRH. Prazosin was given 1 hr before the i.v. injection. Only eight of nine rabbits completed all four experiments.

dose given by the i.v. route does not. Even if all of the 100 ng TRH given to the third ventricle entered the systemic circulation, the plasma TRH concentration would not be enough to cause an IOP elevation. This indicates that the site of action for the third ventricular microinjection of TRH is not peripheral. Our data show that 1–100 µg TRH given into the systemic circulation (approximately 150 ml plasma) causes a significant elevation of IOP. If the IOP elevation is due to a direct ocular action of TRH, an intravitreal injection of TRH at a sufficient dose should produce a similar IOP elevation. We injected 0.1–1 µg TRH into the vitreous body, which has a volume of 1.5 ml,²³ expecting that TRH would diffuse easily to various ocular tissues. Assuming the enzymatic degradation of TRH is minimal, these intravitreal injections should produce an intraocular TRH concentration

(0.1–1 µg/1.5 ml) within the range of plasma TRH concentrations (1–100 µg/150 ml) after those i.v. injections. However, IOP was not affected by the TRH injected intravitreally. This result does not support the theory that a direct action of TRH in the eye is responsible for the IOP elevation following its i.v. injection.

Therefore, we conclude that a CNS mechanism is responsible for the IOP elevation. TRH has several CNS mechanisms and not all are well understood.⁴ In this study, we investigated two of them. As the first mechanism, TRH acts as a pituitary hormone releasing factor. TSH and/or PRL released from the anterior pituitary gland by the TRH then affects IOP either directly, or indirectly through the thyroid hormones released subsequently. The second mechanism is that TRH acts as neurotransmitter in the CNS. A large number of TRH-positive cells have been found in various cerebral areas and the majority are extrapituitary cells.²⁴ Although the physiological role of these extrapituitary TRH-positive cells is not fully understood, it has been reported that the release of catecholamines from the adrenal medulla can be stimulated by a neurotransmitter mechanism of TRH in the CNS.^{9–11}

Our data indicates that this IOP elevation by TRH is not via the release of PRL or the stimulation of TSH–thyroid hormone axis. Plasma PRL concentration in these male rabbits did not change at 30 min after the i.v. injection of 10 µg TRH. Therefore, this IOP increase is not related to circulating PRL. TSH and T-3 concentrations at 30 min were significantly higher than the baseline values, whereas T-4 concentration did not change within the first 5 hr after the TRH injection. This response of TSH–thyroid hormones to an i.v. injection of TRH in rabbits is similar to that observed in humans⁵: an increase of TSH and T-3 in 2 hr. T-4 may increase later and may be less

Table 5. IOP after i.v. injection of 10 µg TRH in rabbits treated with atropine

Treatment	Time (min)							
	0	15	30	45	60	75	90	120
Decentralized eye:								
TRH	18.2 ± 0.8	22.0 ± 1.5*	25.2 ± 2.4*	26.1 ± 2.7*	21.6 ± 2.4*	19.3 ± 2.1	16.8 ± 0.9	15.5 ± 0.7
s.c. atropine + TRH	18.0 ± 0.3	17.2 ± 0.4†	18.6 ± 0.6†	20.9 ± 2.8	20.4 ± 1.8	18.5 ± 1.5	16.1 ± 1.3	16.1 ± 0.6
1% atropine + TRH	17.9 ± 1.0	18.2 ± 0.9‡	21.1 ± 2.7	24.6 ± 3.4	20.5 ± 2.3	18.2 ± 1.3	16.7 ± 0.7	15.6 ± 0.7
Intact eye:								
TRH	19.1 ± 1.1	23.3 ± 1.7*	24.2 ± 2.4*	21.2 ± 1.0*	20.9 ± 1.7*	18.3 ± 1.0	17.1 ± 0.8	16.0 ± 0.6
s.c. atropine + TRH	19.6 ± 0.8	19.3 ± 0.8‡	19.7 ± 0.8‡	19.7 ± 1.0	19.9 ± 1.2	18.6 ± 1.0	18.0 ± 0.6	17.4 ± 0.6
1% atropine + TRH	19.3 ± 1.0	18.6 ± 0.9‡	21.4 ± 2.4	22.1 ± 1.7	21.4 ± 2.2	17.7 ± 0.8	17.0 ± 0.8	16.3 ± 1.0

* Statistically significant IOP elevation from the initial value.
† $P < 0.01$.
‡ $P < 0.05$ compared with the value at the same time point in the TRH experiment.

Values are mean ± SEM (N = 7).
Atropine was given 1 hr before the TRH injection. Topical atropine was given only to the decentralized eye.

readily detected.⁵ We calculated that there was a total increase of 345 ng TSH and 97.5 ng T-3 based on 150 ml plasma. We then gave 0.1 and 1 μ g TSH and 0.1 and 1 μ g T-3 intravenously and observed no IOP elevation. These results indicate that the IOP elevation by TRH is unlikely to be due to the stimulation of the TSH-thyroid hormone axis. Another report demonstrates that subconjunctival injection of 5 μ g T-3 actually decreases the IOP.²⁵

The CNS TRH mechanism causing the increase of circulating catecholamines is related to the IOP elevation. Circulating Epi and NE increased 30 min after the i.v. injection of 10 μ g TRH. The only source of circulating Epi is the adrenal medulla. Stimulation of the preganglionic fibers to the adrenal medulla causes the release of large quantities of Epi and NE into the blood. We calculated that there was a total increase of 6.9 ng Epi and 26.4 ng NE in the plasma. Since the circulating catecholamines are cleared rapidly by uptake into nerve endings and by metabolism, a relatively larger dose is needed for a bolus i.v. injection of catecholamines to reach an equivalent concentration in the circulation. I.v. injections of 10–100 ng Epi and 100 ng NE caused a short increase of IOP. This result indicates that the increase of circulating catecholamines by TRH is probably responsible for the IOP elevation. It is well documented that topical administration of Epi or NE causes a biphasic IOP response in rabbits, an initial short IOP increase followed by an IOP decrease.²⁶ To our knowledge, an IOP elevation in response to the increase of endogenous circulating catecholamines has not been reported previously. However, a series of studies on human aqueous flow strongly indicates that endogenous catecholamines, probably through its beta-adrenergic activity, regulates the rate of aqueous flow.^{27–29} The present study supports this hypothesis that endogenous catecholamines are important mediators in the regulation of aqueous humor dynamics.

It is unclear whether the rate of aqueous flow is changed by TRH during this IOP elevation. Pharmacological studies in aqueous humor dynamics using invasive techniques are generally conducted in urethane-anesthetized rabbits. However, we observed that urethane anesthesia interfered with this IOP elevation. Thus, it may not be suitable to study the aqueous humor dynamics during this IOP elevation using urethane-anesthetized rabbits. Noninvasive aqueous fluorophotometry is used routinely to study aqueous flow in conscious rabbits. Our preliminary data using fluorophotometry³⁰ show that any change in aqueous flow during this IOP elevation is too small and brief to be readily detected. This is probably due to the short duration of the IOP elevation. The half-life of TRH in the blood is only 5 min,³¹ which can-

not sustain TRH's effect in the CNS and the subsequent increase of circulating catecholamines for a long period.

Our hypothesis that endogenous catecholamines released by a CNS mechanism of TRH cause the elevation of IOP is compatible with the results from the cardiovascular experiments and from the IOP experiments in rabbits with unilateral transection of the cervical sympathetic trunk. The cardiovascular response to i.v. TRH is dose-dependent. Changes in cardiovascular functions after an i.v. injection of 3.4–5.8 mg TRH³² are quite different from those effects seen in the present study. After the i.v. injection of 10 μ g TRH, there was a small increase of heart rate within 2 hr and no change in arterial blood pressure. During the same period, circulating Epi and NE were increased. Heart rate can be regulated by the beta-1-adrenergic activity of the circulating catecholamines and blood pressure by alpha- and beta-2-adrenergic activity. The beta-1-adrenergic activity of the circulating catecholamines appears to be responsible for the tachycardia. On the other hand, the alpha-adrenergic activity (vasoconstriction) of the circulating catecholamines is not evident at this TRH dose. Probably, it is offset by beta-2-adrenergic activity (vasodilation).

Supersensitivity of the ocular adrenergic receptors to Epi and NE develops following superior cervical ganglionectomy.³³ However, after transection of the cervical sympathetic trunk (decentralization), supersensitivity in terms of the effective dose of these neurotransmitters does not develop.³⁴ Since TRH increases the circulating catecholamines, the threshold TRH dose causing the IOP elevation in the decentralized eye is not expected to change dramatically. As we observed in Figure 2, the threshold dose causing IOP elevation is still 1 μ g. However, the magnitude of this IOP elevation is enlarged. We speculate that this potentiation of IOP elevation in the decentralized eye is related to the loss of neuronal vasoconstriction of the dilated vessels caused by the beta-2-adrenergic activity of catecholamines. It has been shown that an electrical stimulation of the ocular sympathetic nerves causes vasoconstriction and a decrease in IOP.¹⁹

Following an i.v. or intraventricular injection, TRH functions as a neurotransmitter in the CNS, possibly causing a series of neuronal reactions. At the end of these reactions, the preganglionic fibers to the adrenal medulla are stimulated and large amounts of Epi and NE are released into the circulation. The increase of circulating catecholamines stimulates the ocular beta-2-adrenergic receptors, causing a vasodilation and possibly an increase of aqueous flow. The ocular fluid dynamics balance changes and an IOP elevation occurs. Along this pathway from the CNS

to the eye, regional mechanisms may be complicated. However, we have used three pharmacological blocking agents to sort out some of the regional mechanisms.

Topical timolol at 0.5–4% concentration has minimal effect on normal IOP in rabbits.^{21,35} In this study, bilateral IOP elevations by TRH were partially blocked by the unilateral administration of 0.1% and 1% timolol in the decentralized eye. This indicates that beta-adrenergic receptors participate in the mechanism of IOP elevation by TRH. One possible action is that timolol blocks the vasodilation mediated by the ocular beta-2-adrenergic receptors. A previous study has shown that unilateral application of 1% timolol eyedrops in rabbits causes beta-adrenergic blockade in both eyes.³⁵ However, topical 0.1% timolol causes beta-adrenergic antagonism on the treated eye and has less effect on the contralateral, untreated eye.³⁵ Because we did not observe differential inhibition of the IOP elevations in the treated eye over the untreated eye using the lower dose of timolol, the beta-adrenergic antagonism responsible may not be located exclusively inside the eye.

Unilateral application of 0.1% prazosin significantly blocks the postganglionic alpha-1-adrenergic receptors in the eye for several hours and produces a small, bilateral IOP decrease.³⁶ However, IOP increased in the prazosin-treated eye following the i.v. injection of 10 µg TRH (Fig. 3). We conclude that ocular alpha-1-adrenergic receptors do not participate in the mechanism of IOP elevation by TRH. Another report shows that third ventricular microinjection of TRH does not increase the episcleral venous pressure,³ which is compatible with our results.

It is known, and confirmed by us, that atropine treatment alone does not change IOP. Both topical and systemic atropine administrations significantly inhibit IOP elevation by TRH. Topical 1% atropine caused mydriasis on the treated eye and the systemic atropine did not. Judging by the mydriasis, the ocular concentration of atropine after the topical treatment is higher than that after the subcutaneous injection. Since the subcutaneous injection of atropine has a stronger inhibitory effect on the IOP elevation than the topical atropine application (Table 5), the site of action of atropine should not be inside the eye. Where is the location? The preganglionic fibers for the release of catecholamines at the adrenal medulla are nicotinic. Thus, a muscarinic blocking agent like atropine would not work here. It is very likely that the site of action for atropine is in the CNS. Studies have indicated the existence of muscarinic neurons in the CNS that can be activated by TRH. For example, it was reported that TRH potentiates the excitatory ac-

tions of acetylcholine on cerebral cortical neurons.³⁷ In addition, TRH shortens the barbiturate-induced sleeping time that can be antagonized by atropine.³⁸ We speculate that the muscarinic mechanism of TRH responsible for the IOP elevation is related to these known CNS actions; the specific one remains to be identified.

In summary, we conclude that the IOP elevation after an i.v. or intraventricular injection of TRH is a CNS-mediated effect. Although TRH works at the pituitary gland to stimulate the TSH–thyroid hormone axis, this pathway is not responsible for the IOP elevation. TRH also functions as a neurotransmitter in the CNS to stimulate the release of Epi and NE from the adrenal medulla into the circulation. Intravenous injections of equivalent catecholamines increase IOP. We hypothesize that the increase of circulating catecholamines by TRH causes the elevation of IOP. Beta-adrenergic and muscarinic mechanisms are involved in the pathway. The site of action of the muscarinic mechanism is probably in the CNS, and the ocular alpha-1-adrenergic mechanism is not involved.

Key words: thyrotropin releasing hormone, intraocular pressure, catecholamine, thyroid stimulating hormone, mechanism of action

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