Hemodynamic and Neural Mechanisms of Action of Thyrotropin-Releasing Hormone in the Rat

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The mechanisms mediating the effects of thyrotropin-releasing hormone (TRH) on the cardiovascular system were studied in the conscious rat. Intracerebroventricular (i.c.v.) injection of TRH (8 pmol–80 nmol/kg) induced dose-dependent increases in mean arterial pressure, heart rate, and cardiac index. Hindquarter blood flow increased due to vasodilation, while an increase in renal and mesenteric vascular resistance caused a decrease in blood flow in the respective organs. The plasma levels of norepinephrine and epinephrine were increased by TRH, while there was no change in plasma renin activity or vasopressin. The cardiovascular actions of i.c.v. TRH were not influenced by blockade of the renin-angiotensin system or vasopressin receptors. The ganglion blocker chlorisondamine and the α_1- and α_2-adrenoreceptor antagonist phentolamine (2 mg/kg i.v.) abolished the increase in blood pressure and mesenteric vasoconstriction after i.c.v. TRH. Propranolol (2 mg/kg i.v.) blocked the TRH-induced increase in cardiac index, heart rate, and hindquarter blood flow. The hindquarter vasodilation induced by TRH was also blocked by the selective β_2-adrenoceptor antagonist ICI 188,551 (1 or 2 mg/kg i.v.), while the β_1-adrenoceptor blocker practolol (10 mg/kg i.v.) had no effect on the hindquarter vasodilation produced by TRH but totally blocked the increase in cardiac index. In adrenal demedullated rats, the systemic hemodynamic effects of i.c.v. TRH were diminished along with the decrease in renal blood flow and increase in renal vascular resistance; however, the increase in hindquarter blood flow was attenuated only in adrenal demedullated rats pretreated with the sympathetic blocker bretylium. The renal vasoconstriction induced by i.c.v. TRH was not abolished by renal denervation. In sinoaortic debuffered rats, the pressor, tachycardic, and mesenteric vasoconstrictor responses to centrally administered TRH were significantly potentiated. Taken together, these data suggest that the putative neurotransmitter TRH may play a role in central regulation of cardiac functions and organ blood flow distribution through both the sympathetic nerves and the adrenal medulla. A pivotal role for β_2-adrenoceptors in mediation of hindquarter vasodilation is also demonstrated. (*Circulation Research* 1988;62:139–154)

Thyrotropin-releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide) was the first hypothalamic releasing factor to be isolated, chemically characterized, and synthesized (for review, see Prasad[1]). In addition to its endocrine actions (thyrotropin, prolactin, and growth hormone release), TRH elicits multiple autonomic effects (e.g., arousal, analeptic effect, increased locomotor activity, and changes in body temperature, respiration, blood pressure, and heart rate) that are unrelated to its action on the hypothalamic pituitary axis (for review, see Horita et al,[2] Griffiths,[3] and Prasad[1]). Thus, cardiorespiratory responses to intracerebroventricularly (i.c.v.) administered TRH have been reported in hypophysectomized[4,5] and thyroidectomized rats.[6] Furthermore, TRH and TRH receptors are widely distributed throughout the central nervous system (CNS)[7–11] with more than 70% of the total CNS TRH found in extrahypothalamic areas.[7] The localization of TRH in synaptic nerve endings, its release by synaptic terminals, its binding to high-affinity receptors, and the presence of brain peptidases capable of inactivating the tripeptide[1,7] strongly suggest that TRH may serve as a neurotransmitter or neuromodulator in the CNS.

Increases in mean arterial pressure (MAP) and heart rate by TRH have been demonstrated in several studies and by various routes of TRH administration.[12–17] The cardiovascular effects of TRH are likely to be of central origin, since the doses needed to elicit rises of MAP and heart rate after systemic injections[12,16] are 1,000 times higher than the doses used i.c.v.[14,18,19] or for injections into discrete brain nuclei.[15,17] Also, transections in the cervical spinal cord of anesthetized rabbits abolished the pressor response to i.c.v. administered TRH[18]; furthermore, the pressor effects of TRH are also totally abolished in the pithed rat in which the entire CNS and cardiac reflexes are eliminated.[15,16] In addition, microinjections of picomolar doses of TRH into preoptic or hypothalamic nuclei of anesthetized rats produced increases in blood pressure and heart rate without changes in body temperature or respiration.[17] A role for TRH in central regulation of the cardiovascular system is also suggested from its presence in cells and fibers

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along with high-affinity receptors in brain nuclei important for cardiovascular homeostasis (e.g., periventricular and arcuate nuclei of hypothalamus, amygdala, nucleus of the solitary tract, and dorsal motor nucleus of the vagus).[7,8,10,11]

At the present time, the neural and humoral mechanisms involved in the cardiovascular actions of TRH are not clear. Stimulation of the sympathoadrenomedullary system by TRH seems evident because, concomitantly with its pressor effect, TRH produces rises of plasma catecholamines in both animals[15,20] and man.[21] Recently, increased efferent sympathetic activity in the renal, cervical, and splanchnic nerves of the anesthetized rat was reported after TRH i.c.v.[5] The cardiovascular effects of TRH have been shown to be attenuated by adrenal demedullation and by sympathetic blockers in some studies,[5,12,15,17] while no involvement for sympathetic or parasympathetic nervous system was found in others.[4,18] Also, although the pressor effect of TRH has been repeatedly reported in many experimental animals (see above) and in man,[21-23] little is known about the discrete hemodynamic mechanisms involved in the cardiovascular actions of TRH.

In view of the present controversy over the role of the sympathetic nervous system in mediation of TRH effects and the apparent lack of knowledge on the discrete hemodynamic actions of TRH, we decided to examine the effect of TRH on cardiac output and regional blood flow of conscious rats after selective sympathetic denervation or adrenal demedullation and by pharmacologic tools such as adrenolytic and adrenergic blocking agents.

Materials and Methods

Male Sprague-Dawley rats (260–340 g) from Taconic Farms (Germantown, N.Y.) were used in all experiments. In a separate series of experiments, bilaterally adrenal demedullated (Adm-x) rats (from the same source) were also used. After the surgical procedures, rats were housed individually in plastic cages (21 × 27 × 16 cm, W × L × H) with food and water ad libitum.

Measurement of Cardiac Output

The effect of TRH on cardiac output and total peripheral resistance (TPR) was studied as follows: Rats were anesthetized with an intramuscular injection of 0.13 ml/100 g of 100 mg/ml ketamine and 1 mg/ml acepromazine, and PE-50 tubing was inserted into the femoral arteries. The catheters were tunneled beneath the back skin and exteriorized at the back of the neck. Then, an incision was made at the midline of the neck from the cricoid to the clavicle, and PE-50 tubing was inserted into the right atrium through the external jugular vein. Then, the left common carotid artery was exposed and ligated, and a thermistor (model MX2-780-33 THMP f#1.5, Teflon reusable, Columbus Instruments, Columbus, Ohio) was advanced through the carotid into the ascending aorta (placement above the aortic valve was confirmed in each animal at the end of the experiment and by the shape of the dilution curve before the probe was finally sutured to the neck muscles). The jugular vein catheter and the thermistor were tunneled under the skin to the back of the neck. All lines were secured by a soft spring wire (attached by an adhesive collar) from the back of the neck and outside of the cage. The animals were allowed to recover from surgery for 24–48 hours. On the day of the experiment, the arterial line was connected to a blood pressure transducer (Narco RP 1500i), and continuous recordings of blood pressure (systolic, diastolic, and mean) and heart rate were recorded by the Narcotrace 80 computerized dynograph. The cardiac output was measured by thermodilution technique by the thermistor attached to the computerized Cardiomax II (model CM × 2-780-k with the microprobe option R, Columbus Instruments). The dead space of the venous line was first flushed with 0.05 ml of 0.9% (wt/vol) NaCl (saline) at room temperature, 22° C; after a brief stabilization period (10 seconds to ensure normal core temperature), an additional injection of 0.2 ml normal saline (22° C) was rapidly injected using a 1-ml syringe. Cardiac output was recorded in the following manner: A control period of 15 minutes included 2 or 3 cardiac output measurements to test for consistency and placement of the probe and to get control values for MAP and heart rate. The timer on the automatic data collection system was started, and data points were taken at t_0, t_2, t_5, t_{10}, t_{15}, t_{30}, and t_{45} minutes. Increasing doses of TRH or saline were injected i.c.v. at 45-minute intervals. TPR was calculated by dividing MAP by the cardiac output; values of cardiac output and TPR were further indexed per unit of weight (kg).

In a separate set of rats ($n = 6$), central venous pressure was monitored via the jugular catheter with the tip advanced into the upper thoracic vena cava. The thermodilution probe was also inserted into the aorta to allow concomitant measurements of cardiac output. A single bolus dose of TRH (8 nmol/kg) was injected i.c.v. 24–48 hours after surgery, and the changes in central venous pressure and MAP were followed for 45 minutes. Cardiac output measurements were taken 5, 15, and 30 minutes after TRH administration. Control levels for central venous pressure were taken while the pressure gauge was placed 3 cm above the animal's cage floor.

In the past, we have used the thermodilution method for cardiac output measurements in both anesthetized and conscious rats.[24] Although the cardiac output values obtained by the thermodilution technique are somewhat higher than those determined by electromagnetic flowmetry[25,26] or by radioactive microsphere method,[27,28] the values reported by us are within the range of cardiac output in the conscious rat obtained by other investigators using the thermodilution technique.[25,29-31]

Regional Blood Flow Measurements With Directional Pulsed Doppler Technique

Another set of rats was used to study the effect of TRH on regional blood flow and vascular resistance. The animals were anesthetized with ketamine-

acepromazine (see above). Miniaturized Doppler flow probes (Valpey-Fisher, Hopkinton, Mass.) were sutured around the abdominal aorta, the superior mesenteric artery, and the left renal artery according to the method described earlier by Haywood et al.[32] Briefly, a midline laparotomy was made, and 4-mm lengths of the lower abdominal aorta below the left renal artery, the superior mesenteric artery, and the left renal artery were carefully isolated with the aid of a dissecting microscope. Miniaturized Doppler flow probes were then sutured around each vessel. The wire leads were tunneled beneath the skin and exteriorized at the nape of the neck where they were soldered to a connector plug that was fixed to the animal's skull with small screws and dental acrylic cement. Polyethylene catheters (PE-50) were implanted in the left femoral artery and vein for measurements of blood pressure and heart rate and for intravenous drug injections. The catheters were led beneath the back skin to exit at the nape of the neck and were then secured by a spring wire as described above. The animals were allowed to recover for 3–5 days after surgery.

On the day of the experiment, the rat was connected to the flow probe wire connector, and the connector line was suspended from the top of the animal's home cage to allow freedom of movement during the experiment. Regional blood flow was measured with a pulsed Doppler flowmeter (model #545c-3, University of Iowa Bioengineering Facility, Iowa City, Iowa). The arterial line was attached to a pressure transducer (as above), and blood pressure, heart rate, and regional blood flows were continuously recorded on the Narco dynograph. Vascular resistance was calculated by dividing MAP by blood velocity (Doppler shift in kilohertz). Changes in blood flow and vascular resistance are expressed as a percent of control values.

Intracerebroventricular Injections

Rats were anesthetized with ketamine-acepromazine (see above) and placed on a stereotaxic device (David Kopf Instruments, Calif.). A stainless steel guide cannula was inserted through the skull and fixed with glue (Eastman 910 adhesive). Coordinates for the injections into the right lateral brain ventricle (i.c.v.) were measured from the bregma: AP = −0.8 mm and L = 1.2 mm. On the day of the experiment, injections of saline or TRH were made using a premeasured 30-g (7.5-mm) cannula inserted into the ventricular space through the guide cannula. The injection cannula was then connected via polyethylene tubing to a Hamilton microliter syringe, and a volume of 10 μl of the control or drug solution was injected over a period of 30 seconds. The proper position of the i.c.v. cannula was determined at the end of each experiment by an injection of dye (methylene blue) into the cerebral ventricles.

Experimental Protocols

Dose-response studies. The effect of TRH on systemic and regional hemodynamics was studied by injections of increasing doses of TRH (8 pmol–80 nmol/kg) or saline i.c.v. at 30–45-minute intervals.

Influence of adrenergic blockers. The effect of blockade of autonomic ganglia or α- and β-adrenergic receptors on the TRH-elicited hemodynamic changes were studied in a separate series of rats. First, the rat received i.c.v. injections of saline and TRH (8 nmol/kg) at 30-minute intervals. Chlorisondamine (5 mg/kg), phentolamine (2 mg/kg), propranolol (2 mg/kg), practolol (10 mg/kg), or ICI 118,551 (1–2 mg/kg) were injected intravenously 15–20 minutes before a single i.c.v. dose of TRH (8 nmol/kg); a separate group of rats was used for each of these experiments. This dose of TRH was chosen because in the preliminary experiments it produced a significant and reproducible change in cardiovascular variables. The effectiveness of the sympathetic blockade was tested each time against bolus i.v. doses of norepinephrine or epinephrine (0.3 and 1 μg/kg, respectively).

Effect of captopril and Sar^{1}Ile^8-angiotensin II. The potential role of the renin-angiotensin system in the cardiovascular actions of TRH was studied in 6 additional rats. Captopril (1.5 mg/kg) and Sar^1,Ile^8-angiotensin II (Sar^1,Ile^8-AngII) (0.1 mg/kg) were injected intravenously 10 and 15 minutes, respectively, before a single i.c.v. dose of TRH (8 nmol/kg).

Effect of a vasopressin antagonist. The influence of a vasopressin antagonist, PMP^1-O-methyl-Tyr^2-[Arg^8]vasopressin, on the TRH actions was studied in 5 additional rats. The vasopressin antagonist (150 μg/kg) was injected intravenously 5 minutes before a single i.c.v. dose of TRH (8 nmol/kg). This dose of the antagonist effectively blocked the pressor and vasoconstrictor effects of [Arg^8]vasopressin (0.03–0.1 μg/kg i.v.).

Effect of TRH in adrenal demedullated rats. The effect of TRH on hemodynamic and biochemical variables was also studied in bilaterally Adm-x rats. The rats were used 10 days after the adrenal demedullation. Some of these animals were treated with bretylium. Bretylium (30 mg/kg) was injected slowly (over 45 minutes) in 3 separate doses (10 mg/kg each) 120 minutes before i.c.v. administration of saline (10 μl) or TRH (8 nmol/kg). Complete demedullation was confirmed in all the demedullated rats by direct assay of plasma epinephrine, and only rats with undetectable levels of epinephrine were used.

Sinoaortic denervation. In a separate group of rats, sinoaortic baroreceptors deafferentation (SAD) was performed according to the method described by Krieger.[33] One week before the experiments, the rats were anesthetized with ketamine-acepromazine (see above) and given an injection of atropine (0.4 mg/rat i.p.). A midventral neck incision was made, and both of the carotid sinuses were exposed. The superior laryngeal nerve and cervical sympathetic chains were cut. Nerve segments of 0.5 cm were removed to prevent the possibility of reconnection; all connective tissue and nerves were stripped from the internal, external, and common carotid arteries and the occipital and thyroid arteries along 0.5 cm of their length extending from the carotid bifurcation region. In addition, the

vessels were painted with 10% phenol, and special attention was devoted to avoid damage to the vagus nerve or other nearby structures. In addition to the SAD surgery, head cannulas for i.c.v. injections and Doppler flow probes were implanted as described above. Twenty-four hours before the experiments, the animals were anesthetized with halothane (2% in oxygen), and arterial and venous lines were inserted into the femoral vessels as described above. The adequacy of the baroreceptor deafferentation procedure was assessed prior to any interventions by evaluating the maximal heart rate changes to phenylephrine (1 and 3 μg/kg i.v.). For all studies, only SAD animals exhibiting a decline in heart rate of less than 15 beats/min were selected. Saline (10 μl) and TRH (8 nmol/kg) were injected i.c.v. at 60-minute intervals.

Renal denervation. Another group of rats underwent unilateral renal denervation (ketamine-acepromazine anesthesia) 1 week before the experiment. Through a midline ventral laparotomy, left renal denervation was performed by stripping the renal artery and vein of adventitia, cutting the renal nerve bundles, and coating the vessels with a solution of 10% phenol in absolute ethanol. Implantation of head cannulas for i.c.v. injections and of Doppler flow probes on both left and right renal arteries was also performed. One day before the experiment, the rats were anesthetized with halothane (2% in oxygen), and arterial and venous lines were implanted into the femoral vessels as described previously. To confirm the success of renal denervation, the tissue catecholamine content was measured from both kidneys. The norepinephrine content of the denervated side was significantly reduced to less than 5% of that of the intact kidney. TRH (8 nmol/kg) and saline were injected i.c.v. at 60-minute intervals. Before the start of the i.c.v. injections, an intravenous dose of norepinephrine (1 μg/kg) was injected to test the reactivity of the Doppler lines.

Hemodynamic effects of systemically administered TRH. The effect of a single systemic dose of TRH (5.5 μmol/kg) on the cardiovascular system was studied in rats equipped with thermodilution probe for cardiac index measurement or Doppler flow probes for discrete organ blood flow measurement.

Effect of TRH on plasma catecholamines, vasopressin, and renin activity. To evaluate the effect of TRH on plasma catecholamines, vasopressin, and renin, PE-50 tubings were implanted in the femoral vessels as described. Blood samples (0.5 ml) were collected prior to and at the peak of TRH effect (3 minutes after TRH) and 30 minutes later. Each blood sample withdrawn was replenished with fresh blood from donor rats.

ASSAY OF PLASMA CATECHOLAMINES. In experiments in which the influence of TRH on plasma catecholamines and renin levels was studied, a single systemic dose of TRH (5.5 μmol/kg) was injected into the arterial line. Blood samples for measurement of plasma norepinephrine and epinephrine concentrations were obtained before and 5 and 30 minutes after TRH injection, as previously described.[34] Blood specimens were collected in chilled heparinized test tubes, placed on ice, and centrifuged within 10 minutes of collection, and the plasma was separated and stored at −70° C until assayed. The same experimental protocol was used to study the effects of TRH on plasma catecholamines in Adm-x rats (see below).

Plasma norepinephrine and epinephrine concentrations were measured by a radioenzymatic technique in which a partially purified enzyme, catechol-O-methyltransferase (COMT), catalyzed the transfer of a [^{3}H]S-adenosylmethionine to the metahydroxyl group of endogenous norepinephrine and epinephrine, forming [^{3}H]normetanephrine and [^{3}H]metanephrine, respectively. The resultant measurements were accurate above the value of 20–30 pg norepinephrine or epinephrine/ml plasma (2–3 pg/tube).

In a separate set of rats ($n=6$), increasing doses of epinephrine (0.1–1 μg/kg) were injected intravenously, and blood pressure and regional blood flows as well as plasma epinephrine levels were monitored. Blood samples (0.5 ml) were withdrawn before epinephrine injection and during the peak of the hemodynamic changes. Each blood sample withdrawn was replenished with fresh blood from a donor rat.

ASSAY OF PLASMA RENIN ACTIVITY. A single dose of TRH (5.5 μmol/kg) was injected intra-arterially, and blood samples were collected before and 5 and 30 minutes after TRH injection. Plasma was collected by dripping 300 μl of blood from arterial line directly into ice-cold vials containing 50 μl of 10% EDTA. The samples were then immediately centrifuged, and the plasma frozen on dry ice. The plasma samples were sent frozen on dry ice to Dr. K.B. Brosnihan (Cleveland Clinic, Cleveland, Ohio) for analysis. The plasma renin activity (PRA) was assayed as previously described by Sen et al.[35] The generated angiotensin I was measured by radioimmunoassay (RIA for Angiotensin I, New England Nuclear, Boston, Mass.). The baseline levels of PRA in our study are in accord with the PRA in conscious rats reported earlier by Sen and coworkers.[35]

ASSAY OF ARGININE VASOPRESSIN. Plasma remaining after removal of a fraction for catecholamine determination was stored at −20° C and sent frozen on dry ice to Dr. R.L. Zerbe (Eli Lilly & Co., Lilly Research Labs, Indianapolis, Ind.) for subsequent measurement of plasma vasopressin by RIA.[36] The assay consistently detects 0.2 pg of arginine vasopressin/tube and cross-reacts less than 1% with oxytocin. The intraassay coefficient of variation is 24% at 1 pg, 4% at 5 pg, and 6% at 20 pg. All plasma samples in this study were extracted at the same time and measured in the same assay.

Drugs Used

The following drugs were used: Thyrotropin-releasing hormone (TRH) (Sigma Chemical Co., St. Louis, Mo.), bretylium tosylate (kindly provided by American Hospital Supply Co.), *d,l*-propranolol hydrochloride, phenylephrine, *l*-epinephrine, *l*-norepinephrine, dopamine hydrochloride, [Arg^8]vasopressin, PMP^1-O-methyl-Tyr^2-[Arg^8]vaso-

pressin (Peninsula), captopril (Squibb), Sar[1], Ile[8]-angiotensin II (Peninsula), phentolamine (Ciba), and practolol and ICI 118,551 hydrochloride (ICI Pharmaceuticals, England). All drugs were dissolved in 0.9% saline.

Statistical Analysis of Data

Data in text and figures are presented as mean ± SEM for the indicated number of rats. Analysis of variance (ANOVA) with repeated measures andANOVAfollowed by Dunnett's or Student-Newman- Keuls test as well as Student's *t* test were used for statistical evaluation of data.

Results

Cardiovascular Effects of i.c.v. TRH in Intact Rats

Blood pressure and heart rate (Figure 1). Administration of increasing doses of i.c.v. TRH (8 pmol–80 nmol/kg) increased MAP and pulse pressure dose-dependently; the increase in heart rate was small and did not follow a dose-response relation. The maximum increases in all these variables were achieved 2–5 minutes after the injections, and the effect was completely abolished in 15–20 minutes. Intravenous injections of TRH (0.8–80 nmol/kg) had no significant effect on blood pressure or heart rate.

Cardiac output and total peripheral resistance (Figure 1). Injection of TRH (8 pmol–80 nmol/kg) dose-dependently increased the cardiac index. The maximum increase in cardiac index was achieved by the 8-nmol/kg dose. The increases in cardiac performance became apparent concomitantly with the increments in blood pressure and heart rate reaching their peak effect 5 minutes after TRH administration. The 8-nmol/kg dose of TRH slightly decreased TPR index (TPRI) ($p<0.05$ versus saline, Student-Newman-Keuls test). The other doses of TRH also tended to reduce TPRI, but the effect did not reach statistical significance. Repeated injections of saline i.c.v. (10 μl/rat) had no significant effect on cardiac index or TPRI.

In a separate set of rats, the central venous pressure was also monitored (Table 1). TRH (i.c.v.) has no significant effect on venous pressure in these rats despite the marked increment in cardiac index. Vali-

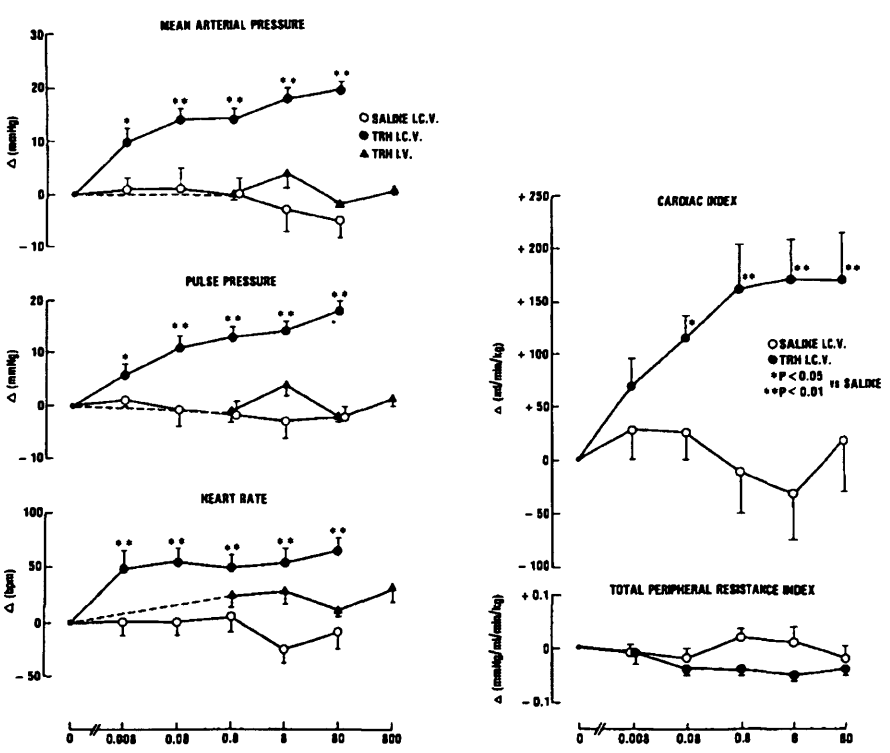

FIGURE 1. *Maximum changes in cardiovascular variables after i.c.v. or i.v. administrations of TRH. Saline or increasing doses of TRH were administered i.c.v. or i.v. at 20–45-minute intervals. Values represent mean ± SEM, n = 8–23. *p<0.05, **p<0.01, statistical significance (Student-Newman-Keuls test). Compared with TRH i.v., increases in mean arterial and pulse pressure induced by TRH i.c.v. are significant at the doses of 800 pmol–80 nmol/kg (p<0.05–0.01), and the increase in heart rate is significant at the highest dose (p<0.05). Baseline levels for mean arterial and pulse pressure were 112 ± 2 and 50 ± 3 mm Hg in the control group, 115 ± 2 and 52 ± 3 mm Hg in the i.c.v. TRH group, and 114 ± 1 and 50 ± 3 mm Hg in the i.c.v. TRH group, respectively. Levels of heart rate in the corresponding groups were 432 ± 1, 400 ± 8, and 452 ± 1 beats/min, respectively. Baseline levels of cardiac index and total peripheral resistance index in the control group were 442 ± 15 ml/min/kg and 0.25 ± 0.01 mm Hg/ml/min/kg, respectively. Corresponding values for the TRH i.c.v. group were 462 ± 13 ml/min/kg and 0.26 ± 0.01 mm Hg/ml/min/kg, respectively.*

Table 1. Effect of TRH on Arterial and Venous Pressure in the Conscious Rat

	Control	Maximal change after TRH	Volume loading	Hemorrhage
MAP (mm Hg)	118±7	+21±3†	+9±3†	−68±6†
HR (beats/min)	414±9	+75±9*	−13±12	+66±27
PP (mm Hg)	47±5	+22±9*	+6±2	+23±7*
CVP (mm Hg)	1.45±0.61	0.9±0.6	+2.51±0.83*	−2.38±0.65*
CI (ml/min/kg)	350±22	+121±23*	+149±16*	−190±37*
TPRI (mm Hg/ml/min/kg)	0.34±0.03	−0.09±0.02*	−0.08±0.01*	−0.05±0.01*

A single dose of TRH (8 nmol/kg) was injected i.c.v. For comparison, the effects of volume loading (0.2 ml saline/100 g/min over 15 minutes) and hemorrhage (6 ml/300 g/5 min) are shown. Number of rats is 6 in TRH group and 4 in the 2 other groups. MAP, mean arterial pressure; HR, heart rate; PP, pulse pressure; CVP, central venous pressure; CI, cardiac index; TPRI, total peripheral resistance index.

*$p<0.05$, †$p<0.01$ statistically significant from control (Dunnett's test).

dation of central venous pressure measurements by volume load and hemorrhage revealed an increase in central venous pressure after the former stimulus and a decrease of central venous pressure after the latter stimulus (Table 1).

Regional blood flow and vascular resistance (Figure 2). TRH (8 pmol–80 nmol/kg) induced dose-dependent decreases in mesenteric and renal blood flow accompanied by simultaneous increases in the renal vascular resistance. The blood flow to hindquarter increased, but there was only a slight decrease in the hindquarter vascular resistance. Intravenous injection of 0.8–800 nmol/kg TRH had no effect on blood flow or vascular resistance in any of the blood vessels studied (results not shown).

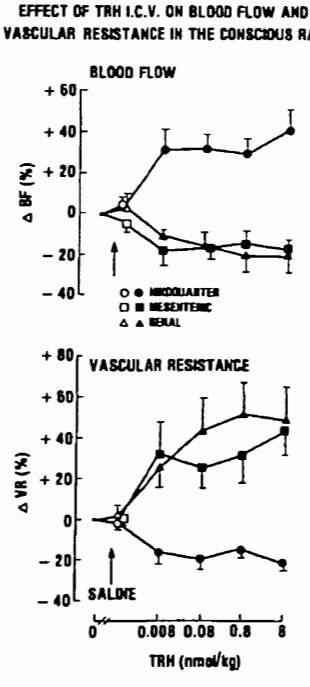

FIGURE 2. *Maximum changes in regional blood flow and vascular resistance induced by i.c.v. TRH. Saline and increasing doses of TRH were administered i.c.v. at 20–40-minute intervals. Values represent mean±SEM, n=8–14. Each group comprised 10–15 rats. Statistical significance is given as p values by Student-Newman-Keuls test. TRH-induced changes in hindquarter and mesenteric blood flow at doses of 8 pmol–8 nmol/kg and in renal blood flow at doses of 80 pmol–8 nmol/kg were significant compared with saline i.c.v. (p<0.05–0.01). Increments in mesenteric and renal vascular resistance were significant (p<0.05–0.01) at all doses, and the decrease in hindquarter resistance was significant at doses of 80 pmol–8 nmol/kg (p<0.05).*

Influence of Adrenergic Blockers on Hemodynamic Responses to TRH

Effect of phentolamine and propranolol. The effects of phentolamine (2 mg/kg i.v.) and propranolol (2 mg/kg i.v.) on systemic and regional hemodynamic variables are shown in Table 2. Baseline levels of blood pressure, heart rate, cardiac index, and TPRI before phentolamine were 121±5 mm Hg, 418±35 beats/min, 458±35 ml/min/kg, and 0.25±0.02 mm Hg/ml/kg/min. The corresponding values before propranolol treatment were 118±5 mm Hg, 411±8 beats/min,

Table 2. Effect of Phentolamine and Propranolol on Hemodynamic Variables in the Conscious Rat

	Phentolamine (n=5–13)	Propranolol (n=6–18)
ΔMAP (mm Hg)	−30±5†	+12±3*
ΔHR (beats/min)	+120±20†	−51±11†
ΔCI (ml/min/kg)	+30±29	−35±29
ΔTPRI (mm Hg/ml/min/kg)	−0.09±0.01	+0.02±0.01
ΔBlood flow (%)		
HQ	+40±14*	−24±7*
R	−17±11	0±8
M	−21±7*	—
ΔResistance (%)		
HQ	−39±8†	+76±21*
R	+11±15	+10±8
M	+9±11	+18±19

Phentolamine (2 mg/kg) or propranolol (2 mg/kg) was injected i.v. Values (mean±SEM) indicate changes 15 or 20 minutes after phentolamine or propranolol, respectively.

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; TPRI; total peripheral resistance index; HQ, hindquarter; R, renal; M, mesenteric.

*$p<0.01$, †$p<0.001$ vs. baseline (Student's paired t test).

Table 3. Effect of Phentolamine and Propranolol on Hemodynamic Actions of TRH i.c.v. in the Conscious Rat

	n	ΔMAP (mm Hg)	ΔHR (beats/min)	ΔCI (ml/min/kg)	ΔTPRI (mm Hg/ml/min/kg)
Saline	11	+5±1	+23±9	−8±12	+0.02±0.01
TRH	11	+18±2‡	+118±14§	+163±19§	−0.05±0.01‡
Phentolamine + TRH	5	+3±4†	+16±7†	+113±26§	−0.02±0.01‡
Propranolol + TRH	6	+16±4‡	+29±12†	+48±9†‡	0.00±0.01*

Phentolamine or propranolol (2 mg/kg) was injected i.v. 15–20 minutes before TRH (8 nmol/kg i.c.v.). Values (mean ± SEM) represent maximum changes within 15 minutes after saline or TRH injection. MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; TPRI, total peripheral resistance index.
*$p<0.05$ and †$p<0.01$ vs. TRH i.c.v.; ‡$p<0.05$ and §$p<0.01$ vs. saline (Student-Newman-Keuls test).

464±28 ml/min/kg, and 0.24±0.02 mm Hg/ml/min/kg. Phentolamine significantly lowered blood pressure and TPR but increased heart rate and hindquarter blood flow. Propranolol increased blood pressure and hindquarter vascular resistance and caused a slight bradycardia.

The influence of phentolamine or propranolol on the hemodynamic effects of TRH is demonstrated in Table 3 and Figure 3. Phentolamine totally abolished the pressor and vasoconstrictor effects of i.c.v. TRH. The TRH-induced tachycardia was also attenuated in phentolamine-treated rats. The baseline level of heart rate was, however, markedly increased after phentolamine treatment (586±5 beats/min versus 397±10 beats/min, $p<0.01$ by Student-Newman-Keuls test). Propranolol partially blocked the increase in cardiac index, heart rate, and hindquarter blood flow and the decrease in TPR by i.c.v. TRH. The renal blood flow increased after TRH in phentolamine-treated rats.

Effect of practolol and ICI 188,551. The influence of practolol (10 mg/kg i.v.) and ICI 188,551 (1 or 2 mg/kg i.v.) on the systemic and regional hemodynamic responses to epinephrine i.v. and TRH i.c.v. are demonstrated in Table 4. The baseline levels of MAP and heart rate in saline-, practolol-, and ICI 188,551-treated groups were 119±3 mm Hg and 387±10 beats/min ($n=25$), 119±4 mm Hg and 371±12

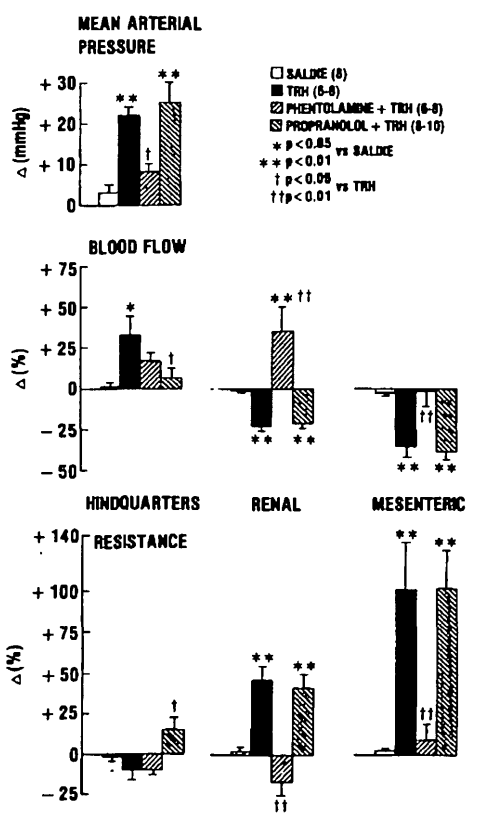

FIGURE 3. *Influence of phentolamine and propranolol on hemodynamic actions of TRH. Phentolamine (2 mg/kg) or propranolol (2 mg/kg) was injected i.v. 15–20 minutes before TRH (8 nmol/kg i.c.v.). Values (mean ± SEM) represent maximum changes within 5 minutes after TRH administration (mean ± SEM). Number of rats is given in parentheses. Asterisks and daggers denote statistical significance by Student-Newman-Keuls test.*

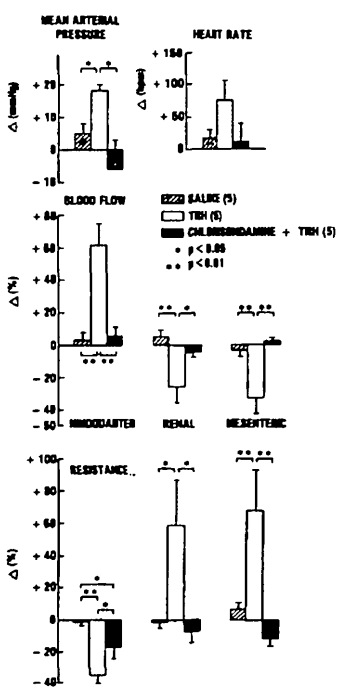

FIGURE 4. *Influence of chlorisondamine on hemodynamic actions of TRH. Chlorisondamine (5 mg/kg) injected i.v. 20 minutes before TRH (8 nmol/kg i.c.v.). Values (mean ± SEM) represent maximum changes within 5 minutes after TRH administration. Number of rats is given in parentheses. Asterisks denote statistical significance by Student-Newman-Keuls test.*

Table 4A. Influence of Practolol (β_1-Adrenoceptor Blocker) and ICI 188,551 (β_2-Adrenoceptor Blocker) on Systemic Hemodynamic Responses to EPI and TRH in the Conscious Rat

	n	ΔMAP (mm Hg)	ΔHR (beats/min)	ΔCI (ml/min/kg)	ΔTPRI (mm Hg/ml/min/kg)
EPI i.v.	12–23	+34±4	−24±11	+111±27	−0.01±0.02
EPI after PRACT	6–13	+39±5	−23±10	−4±19†	+0.07±0.02
EPI after ICI	6–12	+47±9	−3±16	+128±15	+0.03±0.03
TRH i.c.v.	12–23	+18±2	+21±11	+67±15	+0.03±0.04
TRH after PRACT	6–13	+14±4	+8±8	−87±57†	+0.06±0.02
TRH after ICI	6–12	+27±4**	−2±7	+43±14	+0.07±0.02

Practolol (10 mg/kg) or ICI 188,551 (1 or 2 mg/kg) were injected i.v. 10 minutes before epinephrine (EPI) (1 μg/kg i.v.) and 20 minutes before TRH (8 nmol/kg i.c.v.). Values (mean ± SEM) indicate peak changes for EPI and changes 2 minutes after TRH or saline. MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; TPRI, total peripheral pressure resistance index; PRACT, practolol; ICI, ICI 188,551.
*$p<0.05$, †$p<0.01$ vs. changes before PRACT or ICI 188,551 (Student-Newman-Keuls test).

beats/min ($n=13$), and 124±5 mm Hg and 362±18 beats/min ($n=12$), respectively. The levels of cardiac index and TPRI before norepinephrine and TRH administration in these groups were 439±16 ml/min/kg and 0.26±0.01 mm Hg/ml/min/kg (control, $n=12$), 482±22 ml/min/kg and 0.23±0.02 mm Hg/ml/min/kg (practolol, $n=6$), and 453±19 ml/min/kg and 0.25±0.01 mm Hg/ml/min/kg (ICI 188,551, $n=6$). There were no significant differences in the baseline levels of blood flow and vascular resistance between these groups except for the mesenteric vascular resistance, which was significantly increased in the ICI 188,551-treated rats (18±2 mm Hg/kHz in control versus 31±8 mm Hg/kHz, $p<0.05$). Practolol (10 mg/kg i.v.) blocked the increase in cardiac index induced by epinephrine (1 μg/kg i.v.) but had no effect on the blood pressure or regional hemodynamic effects of epinephrine (Table 4). At the peak of the hindquarter vasodilation 2 minutes after TRH (8 nmol/kg i.c.v.), practolol totally blocked the increase in cardiac index but had no effect on the regional hemodynamic responses to i.c.v. TRH (Table 4). The selective β_2-adrenoceptor blocker ICI 188,551 (1 or 2 mg/kg i.v.) totally abolished the increase in hindquarter blood flow and reversed the decrease in hindquarter vascular resistance induced by epinephrine (1 μg/kg i.v.). ICI 188,551 selectively blocked the hindquarter vasodilation produced by TRH and significantly potentiated its pressor response but had no effect on the rise in cardiac index induced by TRH. The TRH effects on cardiovascular variables were significant as compared with i.c.v. saline except for the heart rate, which was not significantly increased 2 minutes after TRH administration.

Influence of Ganglion Blocker Chlorisondamine

The effects of chlorisondamine on TRH-induced hemodynamic changes are demonstrated in Figure 4. Chlorisondamine (5 mg/kg i.v.) decreased MAP but had no significant effect on the baseline levels of heart rate or regional blood flow before TRH administration. Chlorisondamine totally blocked the pressor and vasoconstrictor effects of i.c.v. TRH and effectively inhibited the hindquarter vasodilation by TRH (Figure 4).

Influence of Captopril and Sar^1,Ile^8-Angiotensin II (Table 5A)

Pretreatment with captopril (1.5 mg/kg i.v.) and Sar^1,Ile^8-AngII (0.1 mg/kg i.v.) had no significant effect on the baseline levels of blood pressure or regional blood flow before TRH injection. TRH-induced changes in blood pressure and regional blood flow were not altered by captopril and Sar^1,Ile^8-AngII.

Influence of Vasopressin Antagonist (Table 5B)

Treatment of rats with vasopressin pressor antagonist PMP^1-O-methyl-Tyr^2-[Arg^8]vasopressin had no effect on the resting values of blood pressure or regional blood flow. The vasopressin antagonist also failed to alter the pressor and regional blood flow responses to i.c.v. TRH.

Table 4B. Influence of Practolol (10 mg/kg i.v.) and ICI 188,551 (1 or 2 mg/kg i.v.) on Regional Hemodynamic Changes to Epinephrine (1 μg/kg i.v.) and TRH (8 nmol/kg i.c.v.) in the Conscious Rat

		ΔBlood flow (%)			ΔVascular resistance (%)		
	n	HQ	R	M	HQ	R	M
EPI i.v.	13	+56±8	−62±10	−98±2	−10±6	+799±110	+5,070±1,168
EPI after PRACT	7	+49±9	−66±18	−96±9	−7±7	+891±506	+3,312±1,531
EPI after ICI	6	0±18*	−83±10	−99±1	+51±23*	+1,140±900	+4,416±2,451
TRH i.c.v.	13	+53±7	−22±11	−31±3	−25±4	+60±34	+66±5
TRH after PRACT	7	+33±7	−19±5	−30±5	−19±7	+39±12	+50±14
TRH after ICI	6	0±5†	−28±13	−30±8	−19±6†	+95±69	+88±27

HQ, hindquarter, R, renal; M, mesenteric; EPI, epinephrine; PRACT, practolol; ICI, ICI 188,551.
*$p<0.05$, †$p<0.01$ vs. changes before PRACT or ICI 188,551 (Student-Newman-Keuls test).

Table 5A. Effect of Captopril and Sar[1],Ile[8]-Angiotensin II (Sar[1],Ile[8]-AngII) on Hemodynamic Responses to TRH i.c.v. in the Conscious Rat

	ΔMAP (mm Hg)	ΔResistance (%)		
		HQ	R	M
Saline	+3±3	−3±3	+2±3	+4±3
TRH before treatment	+17±2†	−26±5*	+37±16	+73±24†
TRH after treatment	+16±4†	−20±5*	+20±12	+55±24*

Captopril (1.5 mg/kg) and Sar[1],Ile[8]-AngII (0.1 mg/kg) were administered intravenously 15 and 5 minutes, respectively, before TRH (8 nmol/kg i.c.v.). Values represent maximum changes after the administration of TRH (mean ± SEM), $n=6$.

*$p<0.05$, †$p<0.01$, statistical significance from saline i.c.v. (Student-Newman-Keuls test).

Table 5B. Effect of Vasopressin Antagonist on Hemodynamic Responses to TRH i.c.v. in the Conscious Rat

	ΔMAP (mm Hg)	ΔResistance (%)		
		HQ	R	M
Saline	−1±4	−1±6	+5±7	+7±14
TRH before treatment	+21±4†	+9±10	+52±7*	+122±20*
TRH after treatment	+16±5*	+6±5	+39±15*	+81±31*

PMP[1]-0-methyl-Tyr[2]-[Arg[8]]vasopressin (150 μg/kg) was injected i.v. 5 minutes before TRH (8 nmol/kg i.c.v.). Values represent maximum changes after TRH administration (mean ± SEM), $n=5$. MAP, mean arterial pressure; HQ, hindquarter; R, renal; M, mesenteric.

*$p<0.05$, †$p<0.01$, statistical significance from saline i.c.v. (Student-Newman-Keuls test).

Effect of Centrally Administered TRH in Adrenal Demedullated Rats (Figures 5 and 6)

Baseline levels of hemodynamic variables before TRH administration in intact and Adm-x rats are shown in Table 6. The cardiovascular effect of TRH (8 nmol/kg i.c.v.) in Adm-x rats was compared with that in intact rats. The increases in blood pressure, heart rate, and cardiac index induced by TRH were significantly attenuated in Adm-x rats. Additional treatments with bretylium did not significantly modify these effects.

The renal vasoconstriction induced by i.c.v. TRH was significantly blocked by Adm-x. Additional treatment of the Adm-x rats with bretylium had no further effect on renal blood flow or vascular resistance but significantly attenuated the increase in hindquarter blood flow as well as the mesenteric vasoconstriction produced by i.c.v. TRH.

Effect of Renal Denervation (Figure 7)

Unilateral renal denervation had no effect on basal systemic or regional hemodynamic variables. The basal blood flow and vascular resistance were 5 ± 1 kHz and 30.5 ± 6.7 mm Hg/kHz in the denervated kidney and 6.3 ± 1.2 kHz and 34.3 ± 19.6 mm Hg/kHz in the innervated kidney, respectively. The renal vasoconstrictor response to norepinephrine (1 μg/kg i.v.) was not altered by the denervation: The maximum increase in resistance produced by norepinephrine in the intact kidney was +2,261 ± 969% (mean ± SEM) and +2,908 ± 1,044% in the denervated kidney. The TRH-induced decrease in renal blood flow and increase

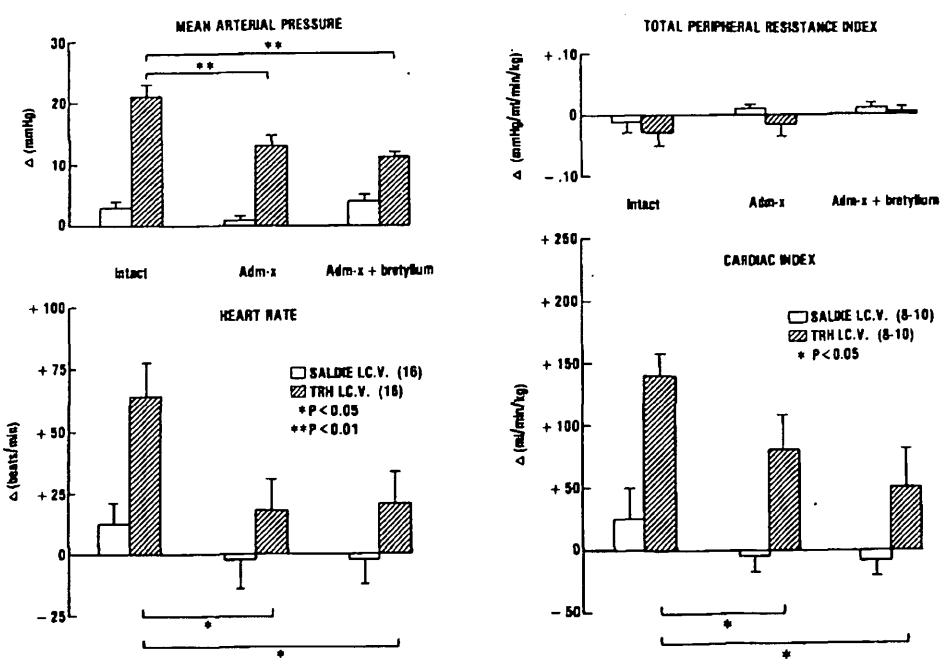

FIGURE 5. *Effect of i.c.v. TRH on gross cardiovascular variables in bilaterally adrenal demedullated (Adm-x) rats. Bretylium (30 mg/kg) injected i.v. 2 hours before i.c.v. administration of saline (10 μl) or TRH (8 nmol/kg). Values represent maximum changes within 5 minutes after saline or TRH administration (mean ± SEM). Number of rats is given in parentheses. Asterisks denote statistical significance by Student-Newman-Keuls test.*

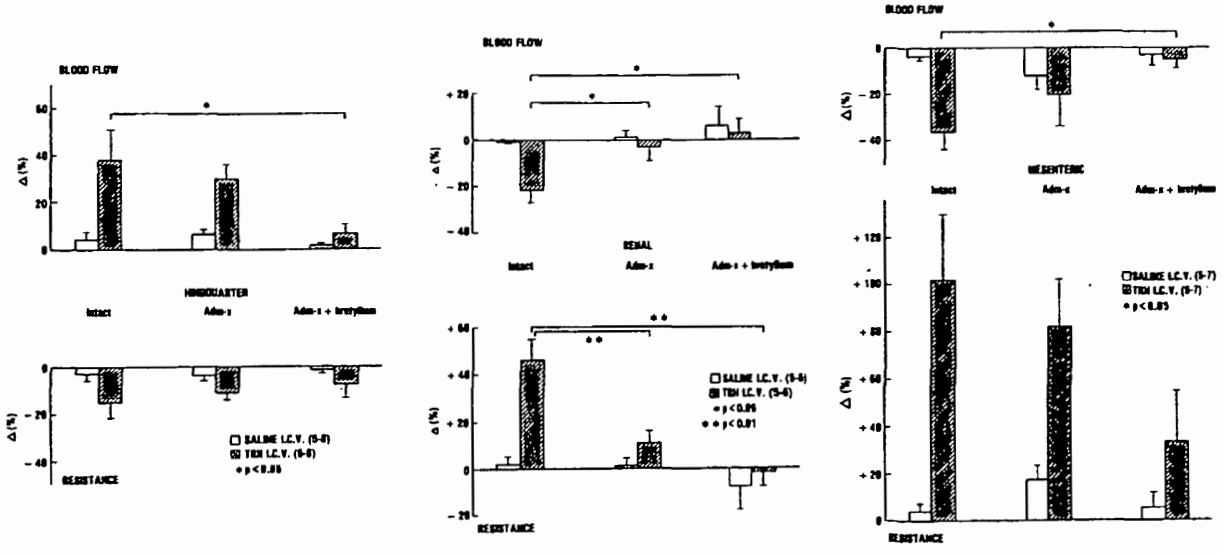

FIGURE 6. *Effect of i.c.v. TRH on regional blood flow and vascular resistance in bilaterally adrenal demedullated (Adm-x) rats. For further details, see Figure 5 legend.*

in renal vascular resistance were not influenced by the denervation.

Effects of TRH in Sinoaortic Baroreceptor–Denervated Rats (Figure 8)

The pressor and cardiac acceleration effects of i.c.v. TRH (8 nmol/kg) were significantly enhanced in SAD rats as compared with intact rats, although the baseline MAP and heart rate (140 ± 7 mm Hg and 477 ± 12 beats/min) were significantly higher than in the control group (122 ± 5 mm Hg and 375 ± 6 beats/min, $p<0.05$). The increase in hindquarter blood flow and decrease in mesenteric blood flow were also significantly potentiated in SAD rats. Also, the rise in mesenteric vascular resistance was significantly potentiated in SAD rats. There was no statistically significant difference in the renal TRH response between the SAD and intact animals, although a strong trend for potentiation of the renal response is evident.

Effect of Systemically Administered TRH

Effects of TRH on systemic and regional hemodynamic variables (Table 7). Injection of a single dose of TRH (5.5 μmol/kg) into the arterial line induced short-lasting increases in MAP, heart rate, and cardiac index with no effect on TPR. The maximum changes were reached 5–10 minutes after the TRH injection, and the effects subsided within 20 minutes. This dose of intravenous TRH also induced a similar pattern of blood flow changes as seen after 8 pmol/kg i.c.v. TRH: a brief increase in hindquarter blood flow (max +41 ± 7%) with a concomitant decrease in vascular

Table 6. Baseline Levels of Hemodynamic Variables Before Administration of TRH i.c.v. in the Conscious Rat

	n	Intact	Adm-x	Adm-x + bretylium
MAP (mm Hg)	16	118 ± 3	112 ± 2	100 ± 3†§
HR (beats/min)	16	387 ± 8	424 ± 16	423 ± 13
CI (ml/min/kg)	8–10	442 ± 18	395 ± 13	433 ± 18
TPRI (mm Hg/ml/min/kg)	8–10	0.26 ± 0.01	0.28 ± 0.01	0.24 ± 0.01‡
Blood flow (kHz)				
HQ	5–8	4.3 ± 0.7	3.0 ± 0.3	4.8 ± 0.7
R	5–6	4.6 ± 1.0	5.9 ± 1.4	6.1 ± 1.4
M	5–7	5.6 ± 0.8	3.0 ± 0.4*	3.8 ± 0.7*
Resistance (mm Hg/kHz)				
HQ	5–8	34 ± 6	38 ± 6	21 ± 3
R	5–6	38 ± 8	29 ± 7	18 ± 4
M	5–7	24 ± 4	39 ± 5	33 ± 6

MAP, mean arterial pressure; HR, heart rate in intact and adrenal demedullated rats; CI, cardiac index; TPRI, total peripheral resistance index; HQ, hindquarter; R, renal; M, mesenteric; Adm-x, adrenal demedullated.
*$p<0.05$, †$p<0.01$, statistically different from intact rats.
‡$p<0.05$, §$p<0.01$, statistically different from Adm-x rats.
Mean ± SEM; *n*, number of animals.

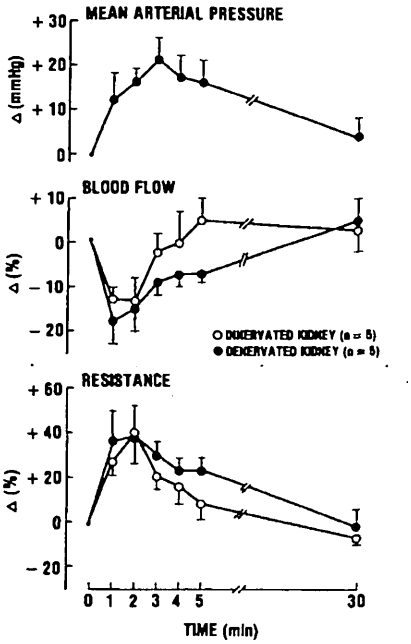

FIGURE 7. *Effect of i.c.v. TRH on renal hemodynamics in unilaterally renal denervated rats. Left kidney was denervated 1 week before experiment. Single dose of saline (results not shown) and TRH (8 nmol/kg) injected i.c.v. Values represent mean ± SEM. Number of rats is given in parentheses.*

resistance (max −19 ± 3%). Blood flow in the mesenteric and renal vascular beds decreased with a maximum drop of −24 ± 9% and −25 ± 6%, respectively. Vascular resistance in the mesenteric and renal arteries increased significantly (+41 ± 9% and +58 ± 12%, respectively). The maximum changes in blood flow and vascular resistance in all of these vascular beds became apparent 1–3 minutes after the systemic TRH administration and completely subsided after 10–20 minutes.

Effect of TRH on plasma catecholamines, vasopressin, and plasma renin activity (Table 8). In intact rats, the intra-arterial injection of TRH (5.5 μmol/kg) caused a pronounced increase in plasma epinephrine and a small increase in plasma norepinephrine. The maximum increases in plasma catecholamines were observed 5 minutes after TRH injection, and the levels were back to baseline within 30 minutes after the TRH administration.

A comparison of the hemodynamic changes and plasma levels of epinephrine after intravenous administration of increasing doses of epinephrine (0.1–1 μg/kg) is demonstrated in Figure 9. Marked pressor and vasoconstrictor responses in renal and mesenteric vascular beds were found at plasma levels of epinephrine comparable to or even lower than those produced by TRH. The hindquarter vascular resistance was not significantly altered, while blood flow to hindquarters

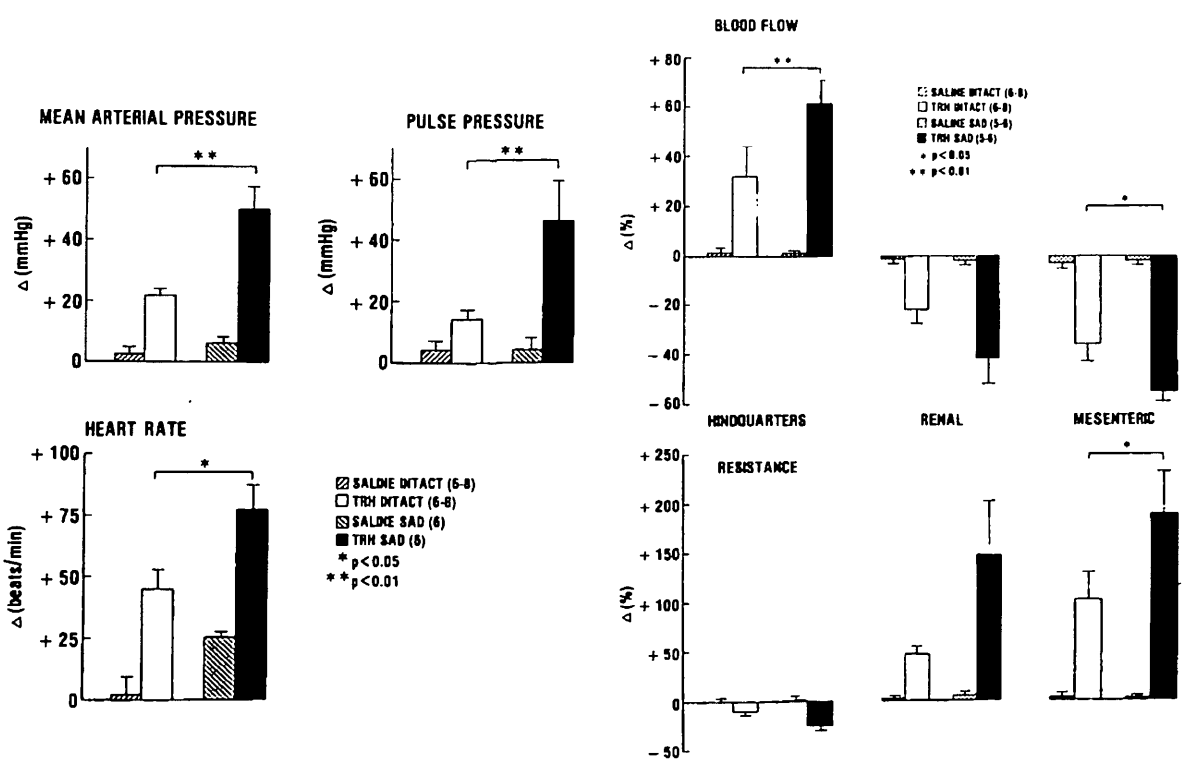

FIGURE 8. *Effect of i.c.v. TRH on cardiovascular variables in sinoaortic baroreceptor–denervated (SAD) rats. Saline (10 μl) and TRH (8 nmol/kg) were injected i.c.v. at 45-minute intervals. Values represent maximum changes within 5 minutes after injections (mean ± SEM). Number of rats is given in parentheses. Asterisks denote statistical significance by Student-Newman-Keuls test. All TRH-induced hemodynamic changes in both intact and SAD rats were significant ($p<0.05$, $p<0.01$) compared with their control group.*

Table 7. Effect of Systemically Administered TRH (5.5 μmol/kg i.a.) on Systemic and Regional Hemodynamic Variables in the Conscious Rat

	ΔMAP (mm Hg)	ΔHR (beats/min)	ΔCI (ml/min/kg)	ΔTPRI (mm Hg/ml/min/kg)
Saline	−2±2	−2±4	+9±23	+0.01±0.01
TRH	+16±2†	+48±10*	+83±7*	−0.01±0.01

	ΔBlood flow (%)			ΔResistance (%)		
	HQ	R	M	HQ	R	M
Saline	+1±1	+1±1	+1±1	+1±2	+1±2	+1±1
TRH	+41±7†	+25±6†	−24±9*	−19±3†	+58±12†	+41±9*

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; TPRI, total peripheral resistance index; HQ, hindquarter; R, renal; M, mesenteric.
*$p<0.05$, †$p<0.01$, statistically different from saline i.c.v. (Student-Newman-Keuls test).
Mean±SEM; maximum changes after TRH administration; $n=8–15$.

was increased slightly (+20±6%, $p<0.05$ versus saline).

In Adm-x rats, the basal levels of epinephrine were not detectable, while norepinephrine levels were markedly reduced (Table 6). Bretylium treatment dropped plasma norepinephrine concentration below detection level. PRA was not affected by TRH in either intact or Adm-x rats, while plasma vasopressin concentration was slightly elevated (Table 8).

Discussion

The present study confirms and extends previous reports that implicate TRH as a neurotransmitter in central cardiovascular control. Thus, TRH produced pressor and tachycardic responses when injected i.c.v. at doses that had no effect when injected systemically; in fact, more than 100,000-fold of the i.c.v. dose was necessary to produce comparable hemodynamic responses by systemic administration of TRH. These observations are in accord with previous studies in several species (see beginning of text). The present study further demonstrates that in the conscious rat, an increase in cardiac output is the primary mechanism of the pressor effect of TRH. Several mechanisms could have been involved in the increase in cardiac index by TRH. Behavioral changes after TRH administration (general excitation) could have mediated part of the increase in cardiac index. However, the pattern and time course of the behavioral responses to TRH were different from those of the vasomotor responses; increased locomotive activity, vigorous head shaking, and tremor of the paws were evident immediately after i.c.v. injections of TRH in some animals, while in others, only mild head shaking was observed 10–20 minutes after TRH injection. On the other hand, hemodynamic changes consistently became apparent within 1 minute after TRH administration and subsided in 20 minutes. Venoconstriction and an increased cardiac preload (due to behavioral excitation) as contributing factors to elevated cardiac index seem unlikely since central venous pressure remained unchanged at all times after i.c.v. TRH. Tachycardia, increase in cardiac contractility, and a fall in afterload due to decrease in TPR might all contribute to an increase in cardiac index. However, the tachycardia elicited by TRH was rather modest (10%) compared with the marked increase in effect on cardiac index (40%). Also, the TPR decreased slightly only after high doses of TRH. Thus, a positive inotropic effect on the heart might be the major determinant of the increase in cardiac index produced by TRH.

In spite of the lack of effect of TRH on TPR, substantial peripheral organ blood flow changes were observed after i.c.v. TRH; while increases in renal and mesenteric vascular resistance led to decrements in the respective organ blood flow, the blood flow to the hindquarter increased. The increase in hindquarter blood flow was not merely the result of the increase in

Table 8. Effect of Systemically Administered TRH (5.5 μmol/kg i.a.) on Plasma Levels of Catecholamines, Vasopressin, and Plasma Renin Activity in the Intact and Adrenal Demedullated (Adm-x) Conscious Rat

	Intact rats	Adm-x rats	Adm-x + bretylium
EPI (pg/ml)			
Baseline	240±40	<29‡	<29‡
Maximum	1,053±205†	<29‡	<29‡
	($n=7$)	($n=5$)	($n=5$)
NE (pg/ml)			
Baseline	259±52	73±15	<29‡
Maximum	471±125*	79±16	<29‡
	($n=7$)	($n=5$)	($n=5$)
PRA (AI ng/ml/h)			
Baseline	16±2	24±3	24±9
Maximum	21±5	44±9	41±13
	($n=6$)	($n=5$)	($n=5$)
Vasopressin (pg/ml)			
Baseline	2.1±0.3	NT	NT
Maximum	6.0±1.5†		
	($n=7$)		

Bretylium (3×10 mg/kg) was injected into the arterial line 2 hours before TRH administration. Baseline, level before TRH injections; maximum, level at 5 minutes after TRH administration; EPI, epinephrine; NE, norepinephrine; PRA, plasma renin activity; NT, not tested; AI, angiotensin I.
*$p<0.05$, †$p<0.01$ vs. baseline (Student's t test). Mean±SEM.
‡<29, levels below limit for detection, which is 29 pg/ml, or 2.9 pg/tube.

systemic arterial pressure but was also clearly contributed to by hindquarter vasodilation. In this respect, our data are in agreement with a recent report showing decreases in gastrointestinal and renal blood flow (measured by microspheres) after systemic injections of TRH in the conscious rabbit.[37] The blood flow to skeletal muscles, however, was not affected by TRH in the rabbit.[37]

TRH (i.c.v.) was previously shown to attenuate the reflex bradycardia in response to a pressor stimulus in the conscious rat,[38] and therefore, the increase in blood pressure and heart rate induced by TRH were interpreted as the result of interference in the baroreflex regulatory mechanism. In our study, however, all of the reported effects of i.c.v. TRH in intact rats were markedly potentiated in SAD rats. These results clearly indicate that TRH does not produce its hemodynamic effects by interference in the baroregulatory circuits; in fact, the moderate effects of TRH in conscious intact animals is due to the tight baroregulatory control. The difference between the studies might be the result of the primary vagal mediated response to a peripheral pressor stimulus, which is counterbalanced by the concomitant activation of the sympathetic system by TRH in intact rats. In SAD rats, however, the unbalanced sympathetic activation is the driving force for the pressor and cardiac acceleration produced by TRH. Thus, reflex bradycardia might not be a proper procedure to evaluate the effect of substances on the baroreflex mechanism. These data also imply that in rats in which the baroregulatory reflexes are deranged, e.g., spontaneous (genetic) hypertensive rats, increased activity of TRH-ergic system might lead to excessively high blood pressure. This hypothesis remains to be examined.

Several recent studies addressed the role of the various pressor systems in mediation of the hemodynamic responses to TRH. In contrast with a previous report,[39] our present findings, based on direct assays of PRA and combined pharmacologic antagonists, suggest no involvement of the renin-angiotensin system in the cardiovascular actions of TRH. The role of vasopressin was also ruled out by showing that plasma vasopressin does not reach the levels that might produce pressor responses.[40] Also, pharmacologic antagonism of vasopressin receptors failed to influence the cardiovascular actions of TRH. In this regard, our data are in accord with previous reports.[4,5,41] In contrast with the lack of evidence in support for renin-angiotensin and the vasopressinergic systems, several lines of evidence strongly support a primary role for the sympathoadrenomedullary system in mediation of TRH effects. First, plasma catecholamines were elevated at the peak of the hemodynamic responses produced by TRH. The preferential increase in plasma epinephrine found in this study is in accord with several previous reports.[12,15,16,20] Potent hemodynamic changes could also be produced by plasma epinephrine concentrations, which were comparable or even lower than those elicited by TRH (Figure 9, Yamaguchi and Kopin[42]). The pattern of blood flow changes induced by TRH is identical to that of changes produced by epinephrine. Furthermore, a marked activation of efferent renal sympathetic nerve activity by TRH was demonstrated in a recent study.[5] In preliminary studies by our laboratory, a profound increase in renal sympathetic activity was also found after central injections of TRH in anesthetized rats. Of interest, the sympathetic activation was demonstrated even at TRH doses that had no effect on blood pressure or renal blood flow and always preceded the vasomotor responses.

Second, the cardiovascular effects of i.c.v. TRH were abolished by ganglion blockade with chlorisondamine and by pharmacologic antagonists of α- or β-adrenergic receptors. The β-blocker propranolol blocked most of the increase in cardiac rate and output and completely abolished the hindquarter vasodilation.

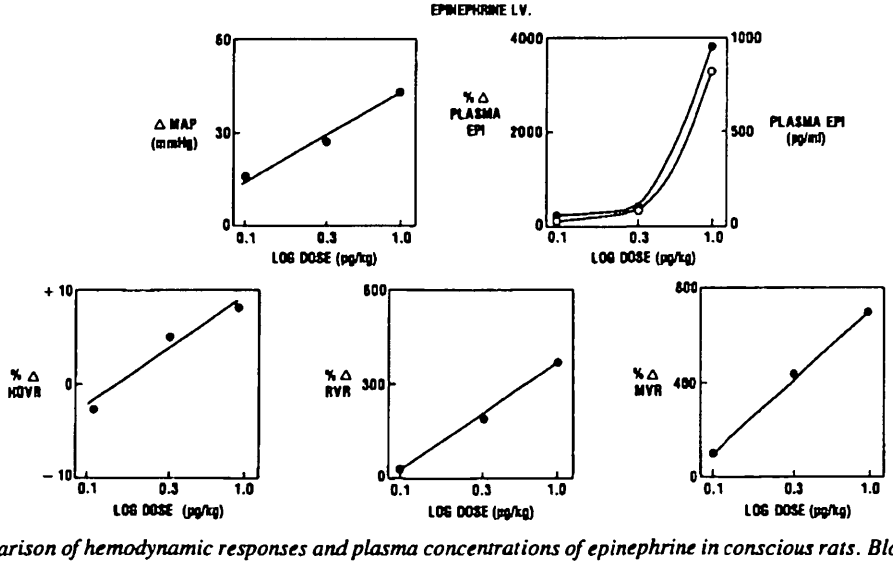

FIGURE 9. *Comparison of hemodynamic responses and plasma concentrations of epinephrine in conscious rats. Blood samples were taken at peak of cardiovascular changes.* ○, *Actual plasma levels of epinephrine;* ●, *percent changes from baseline; MAP, mean arterial pressure; HQVR, hindquarter vascular resistance; RVR, renal vascular resistance; MVR, mesenteric vascular resistance;* n = 6.

The role of β_2-adrenoceptors in mediation of TRH-induced hindquarter vasodilation has been established by blockade of the vascular β_2-adrenoceptors with ICI 188,551; this β_2-antagonist totally abolished the increase in hindquarter blood flow and reversed the decrease in hindquarter vascular resistance produced by TRH. ICI 188,551 did not alter cardiac effects of TRH. The role of β_2-adrenoceptors in hindquarter vasodilation is further supported by the finding that practolol (a selective β_1-blocker) had no effect on the TRH response in hindquarters, although it effectively blocked the cardiac effects of TRH at the peak of vasodilation. Hindquarter vessels showed a reversed response to TRH in the presence of ICI 188,551 or propranolol — namely, an increase in resistance. Thus, the sustained pressor response to TRH in propranolol-treated rats seems to result primarily from intact (and even hypersensitive) α-adrenergic–mediated vasoconstriction. This possibility is supported by the effective blockade of the increase in MAP produced by the α_1- and α_2-adrenergic blocker phentolamine. Of interest, TRH induced an increase in renal blood flow in phentolamine-treated rats. Thus, α-adrenoceptors primarily mediate the mesenteric vasoconstriction and override a vasodilation in the renal arterioles. The mediators of this effect were not further investigated in the present study. However, dopamine released from the adrenal medulla might mediate this renal vasodilation because dopamine is the only catecholamine to produce renal vasodilation. Other adrenal catecholamines acting on the β_2-adrenoceptors might also produce such an effect. The involvement of other renal vasodilator peptides, such as atrial natriuretic factor (ANF),[43] seems unlikely because preliminary results from our laboratory indicated that TRH had no effect on plasma ANF in conscious rats.

Although the levels of thyroid hormones after TRH injections were not monitored in the present study, the involvement of thyroid gland in the cardiovascular actions of TRH seems unlikely for several reasons. First, the ganglion blocker chlorisondamine effectively blocked the hemodynamic changes induced by i.c.v. TRH. Second, in agreement with a recent study,[5] our preliminary findings provided direct evidence for an increased pregangionic sympathetic nerve activity: concomitant increments of blood pressure, heart rate, and efferent sympathetic nerve activity in the urethane-anesthetized rat. Third, the time course of the cardiovascular changes by TRH was different from that reported for its endocrinologic actions in conscious rats[44]; the peak effects in systemic and regional hemodynamic variables became apparent within 5 minutes after TRH administration and subsided in 20 minutes, while the plasma levels of T_3 slowly began to increase 60 minutes after TRH. Finally, the cardiorespiratory actions of i.c.v. TRH in rats were virtually unaltered by either hypophysectomy[4,5] or thyroidectomy.[46]

Additional support for the central role of the sympathoadrenomedullary system in mediation of the central hemodynamic effects of TRH is drawn from studies conducted on Adm-x rats. Adm-x, per se, attenuated the increase in MAP, cardiac output, and renal and mesenteric vascular resistance; however, more complete blockade of TRH effect was observed after treatment of Adm-x rats with the adrenolytic compound bretylium. Residual sympathoadrenomedullary responses attributable to incomplete adrenal demedullation and sympathetic blockade by bretylium might explain the residual responses to TRH in these rats. However, plasma catecholamines were undetectable in the Adm-x-bretylium–treated rats, both before and during the peak of the residual TRH effect. Residual sympathetically mediated responses without changes in plasma catecholamines might be possible because plasma catecholamines are poor indexes for sympathetic tone.[45,46] This possibility is also supported by the data presented in this study showing lack of change in plasma norepinephrine and undetectable levels of epinephrine in Adm-x rats that still showed significant constriction of the mesenteric blood vessels, which were blocked only after addition of the sympatholytic drug bretylium. This problem, however, needs further validation by simultaneous measurements and correlations of sympathetic nerve activity to a selected organ with vasomotor responses and monitoring of the arteriovenous difference of the circulating catecholamines.

Hemodynamic responses similar to those produced by TRH in the present study have been reported earlier by electrical stimulation of various hypothalamic and mesencephalic sites in the rat brain. Folkow and Rubinstein[47] found increases in blood pressure, heart rate, and hind limb blood flow with renal and gastrointestinal vasoconstriction after stimulation of the hypothalamic defense areas in the anesthetized rat. Also, the same pattern of hemodynamic changes was recently reported by Yardley and Hilton.[48] In their study, the responses were evoked by electrical stimulation along the rostro-caudal hypothalamus and a region ventral to the fornix as well as the dorsal section of the central gray matter. Vasodilatation in hindquarters with renal and mesenteric vasoconstriction was also found after electrical stimulation of the anteroventral region of the third ventricle (AV3V) in the conscious rat.[49–51] Since TRH and TRH receptors are abundant in these hypothalamic nuclei,[7–11] thyrotropin-releasing hormonergic pathways might mediate the hemodynamic changes evoked by electrical stimulation of the hypothalamic defense area. However, discrete microinjections of TRH into the sensitive sides are needed to further test this hypothesis.

Of interest, hindquarter vasodilation evoked by central electrical stimulation was insensitive to atropine or vagotomy but was greatly reduced by the β-adrenoceptor blocker propranolol, the sympathetic blocker guanethidine, or adrenalectomy.[49,51,52] Thus, epinephrine released from the adrenal medulla as well as sudden withdrawal of on-going vasoconstrictor tone have been suggested to underlie the hind-limb vasodilation while the vasoconstrictor responses have been attributed to an activation of sympathetic

Table 9. Summary of Effect of Mediators Involved in Regional Vasomotor Responses to TRH I.c.v. in the Conscious Rat

	Sympathetic nerves		Adrenal medulla	
Vasomotor response in:	NE	EPI	NE	EPI
Hindquarter	+	+*	−	−
Receptor	β_2			
Mesenteric	+	+	−	−
Receptor	α			
Renal	−	−	+	+
Receptor			α,β_2,D	

+, positive role; −, no role; NE, norepinephrine; EPI, epinephrine.
*Based on data by Berecek and Brody.[52]

nerves.[47,49,50,52] Our present results suggest that TRH in the central nervous system produces hindquarter vasodilation by activation of sympathetic nerves and vascular β_2-receptors. Epinephrine derived from adrenal medulla was argued to also play a role in regional hemodynamic responses to AV3V stimulation in the conscious rat since adrenal demedullation greatly attenuated not only the hind limb vasodilation but also the renal and mesenteric vasoconstriction induced by AV3V stimulation.[51] Since epinephrine infusion totally restored the responses to AV3V stimulation in Adm-x rats, while blockade of catecholamine uptake to sympathetic nerves reversed the epinephrine effect, these authors concluded that adrenal epinephrine might be acting as a sympathetic neurotransmitter. However, our present data suggest a differential role of the adrenal medulla and sympathetic nerves in mediation of vascular responses to TRH (Table 9); while hindquarter and mesenteric vascular responses seem to be primarily dependent on the sympathetic nerves, the renal vessels seem to be modulated by epinephrine from the adrenal medulla. Although the preganglionic renal sympathetic nerve activity was markedly increased after TRH,[5] the renal vascular responses were not affected by complete denervation of the kidney but were abolished by adrenal demedullation. Thus, activation of the sympathetic nerves by TRH seems to not play an important role in its vasomotor responses in the kidney. These findings underscore the importance of simultaneous recordings of sympathetic nerve activity with vasomotor responses in discrete organs. Furthermore, these data call attention to the role of the adrenal medulla through circulating epinephrine as a primary modulator of renal blood flow.

In conclusion, in conjunction with neuroanatomical and biochemical data previously reported, the data presented in this study support a potential role for TRH in central regulation of peripheral organ blood flow and cardiac function through sympathetic nerves and circulating epinephrine.

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