Central thromboxane receptors: mRNA expression and mediation of pressor responses

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Gao, Hubin, Bo Peng, William J. Welch, and Christopher S. Wilcox. Central thromboxane receptors: mRNA expression and mediation of pressor responses. Am. J. Physiol. 272 (Regulatory Integrative Comp. Physiol. 41): R1493–R1500, 1997.—These studies tested whether activation of central thromboxane (Tx) A<sub>2</sub>/prostaglandin (PG) H<sub>2</sub> receptors raises blood pressure (BP). Messenger RNA for TxA<sub>2</sub>/PGH<sub>2</sub> receptors was detected in normal Sprague-Dawley rat brain and in rat neuronal and astroglial brain cells in culture. The mean arterial blood pressure (MAP) was recorded in conscious rats during graded administration of the TxA<sub>2</sub>/PGH<sub>2</sub> receptor agonist U-46,619 given intracerebroventricularly or intravenously. Because the pressor responses to intracerebroventricular (but not intravenous) U-46,619 were significantly greater in high-salt compared with low-salt rats, high-salt rats were used for subsequent studies. The rise in MAP with intracerebroventricular administration of U-46,619 was greater than with intravenous administration and was more sustained. A comparison of plasma radioactivity after intracerebroventricular or intravenous injection of [3H]U-46,619 demonstrated that ~35% of the drug reached the systemic circulation by 5–15 min after intracerebroventricular administration. Co-administration of a TxA<sub>2</sub>/PGH<sub>2</sub> antagonist, ifetroban, by intravenous or intracerebroventricular routes blocked the pressor responses induced by U-46,619. The half-maximal inhibition for blockade of responses was substantially lower for intracerebroventricular than for intravenous responses (intracerebroventricular: 0.03 ± 0.01 vs. intravenous: 3.1 ± 0.6 μg/kg; P < 0.001). The intravenous administration of ifetroban (10 μg/kg) caused a greater (P < 0.02) inhibition of pressor responses to U-46,619 (1 μg/kg) given intravenously (81 ± 3%) compared with U-46,619 given intracerebroventricularly (40 ± 13%). In conclusion, TxA<sub>2</sub>/PGH<sub>2</sub> receptor mRNA is expressed in neurons, glial, and brain stem of normal rats. The central administration of a TxA<sub>2</sub>/PGH<sub>2</sub> mimetic raises blood pressure by interaction with specific central and peripheral receptors. This response is augmented in rats fed a high-salt compared with a low-salt diet.

thromboxane A<sub>2</sub>; prostaglandin H<sub>2</sub>; blood pressure; brain; hypertension; thromboxane receptor; U-46,619; ifetroban

THROMBOXANE (Tx) A<sub>2</sub>/prostaglandin (PG) H<sub>2</sub> receptors can be activated by a number of agonists, including TxA<sub>2</sub>, prostaglandin endoperoxides (13), and U-46,619 (16). TxA<sub>2</sub>/PGH<sub>2</sub> receptors mediate many vascular and renal actions, including endothelium-dependent constrictor responses in hypertensive rats (22), renal (32) and systemic (7) vasoconstriction, enhanced reabsorption of chloride in the loop of Henle (31), and enhanced tubuloglomerular feedback responses in the kidney (28, 29, 31). TxA<sub>2</sub>/PGH<sub>2</sub> receptor activation has been implicated in the development of hypertension in several animal models, including genetic hypertension (18), angiotensin II-induced hypertension (12, 13, 33), and 2-kidney, 1-clipping renovascular hypertension (2).

In previous studies, systemic infusion of the TxA<sub>2</sub> mimetic U-46,619 for 14 days into conscious rats led to progressive increases in blood pressure (BP) that were abolished by anesthesia or blockade of the sympathetic nervous system with phenoxybenzamine (27). This suggests the involvement of the central and/or peripheral sympathetic nervous systems. The rise in BP was potentiated by feeding a high-salt diet. Recently, Kitanaka et al. (11) have reported the expression of mRNA for TxA<sub>2</sub>/PGH<sub>2</sub> receptors in rat astrocyte cells. The metabolite of TxA<sub>2</sub>, TxB<sub>2</sub>, is released from the rat hypothalamus in vivo (24). There is a massive release of TxB<sub>2</sub> into the cerebral circulation of goats during hypotension (4). These results suggest that TxA<sub>2</sub>/PGH<sub>2</sub> receptors can be expressed in the brain and might function in BP control. However, a previous physiological study involving central administration of a TxA<sub>2</sub>/PGH<sub>2</sub> mimetic reported pressor responses in spontaneously hypertensive rats, but not in normotensive Wistar rats fed a normal salt intake (23). The present studies were designed to investigate whether mRNA for TxA<sub>2</sub>/PGH<sub>2</sub> receptors is expressed in rat neural and glial cells and brain and to reinvestigate the BP responses to central and peripheral administration of graded doses of U-46,619 in normotensive, conscious Sprague-Dawley rats. The responses to U-46,619 were contrasted in rats fed low and high salt intakes. Because a high salt intake was found to enhance the response to intracerebroventricular administration of U-46,619, this was the model used in subsequent protocols in this study.

METHODS

Animal preparation. Male Sprague-Dawley rats (280–350 g) were maintained on a rat chow with a low (0.03 g/100 g) or high (2.4 g/100 g) Na content (Teklad, Madison, WI) with tap water to drink. A high salt intake was used in all studies after series 1 because we found that it potentiated the rise in BP during prolonged infusion of U-46,619 (27), potentiated the tubuloglomerular feedback responses to U-46,619 (30), and potentiated the response to intracerebroventricular administration of U-46,619 (series 1). Rats were anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott Labs, North Chicago, II.). With the use of a stereotaxic frame (David Kopf, Tujunga, CA), the skull was immobilized and drilled, and a 23-gauge stainless steel guide cannula was lowered 5 mm into the lateral cerebral ventricle, which was located 1.4 mm lateral and 0.9 mm posterior to the bregma. After insertion of two stainless steel anchoring screws, dental acrylic was applied to secure the guide cannula in the skull and sealed with a 30-gauge stainless steel obturator carefully placed in the lumen. Approximately 3 days after implanting the intra-
cerebroventricular cannula, rats were anesthetized with pentobarbital sodium and a PE-10 catheter, connected to a PE-50 catheter, was placed in a femoral artery and a PE-50 catheter in a femoral vein. Catheters were threaded subcutaneously and exteriorized at the nape of the neck, where they were protected by a light, flexible metal spring secured to a plate that was sutured to the skin. Catheters were flushed and filled with a solution of 500 IU/ml of heparin and 1,000 IU/ml penicillin G in 0.154 M NaCl. After recovery from anesthesia, rats were placed in individual metabolism cages with free access to food and water. The metal spring attachment and cannula were connected to a swivel to permit freedom of movement. Catheters were flushed daily.

The rats were allowed 3 days to recover from surgery. Experiments were performed on rats in the conscious and unrestrained state. The arterial line was connected to a pressure transducer to record the mean arterial pressure (MAP) and heart rate (HR) on a computer monitoring system (MacLab 4E, AD Instruments, Milford, MA). For intracerebroventricular injections, the obturator was removed and replaced with an injector inserted via the guide cannula and connected to a microsyringe via a PE-20 catheter. This whole system was filled with the test solution, and the desired volume (10 µl) was injected when required. The position of the intracerebroventricular cannula was confirmed at the end of the experiments by injection of methylene blue and postmortem examination of the brain.

Experimental protocols. The aim of the first series was to compare the effects of salt intake on the dose-response relationships for U-46,619 administered intravenously or intracerebroventricularly. Diets were identical except in salt content (Teklad, Madison, WI). Groups of rats were accommodated to a low-salt (n = 6; Na content 0.03 g/100 g) or a high-salt (n = 6; Na content 2.4 g/100 g) diet for 6–8 days before testing. They were habituated to the metabolic cages for 3 days to become accustomed to the experimental environment. Their BP was recorded continuously for 30 min or until it became stable. Baseline measurements of MAP and HR were made over 30 min, and the data were means. Thereafter, rats were given graded doses of U-46,619 (0.1, 0.3, 1, and 3 µg/kg) or vehicle intracerebroventricularly or intravenously at intervals of 20 min, or longer if required for BP to return to baseline levels. Measurements of MAP and HR were made at 1, 3, 10, and 15 min after injections. The order of intravenous and intracerebroventricular injections was randomized. At least 24 h was allowed between intravenous or intracerebroventricular testing.

The aim of the second series was to assess what fraction of U-46,619 given intracerebroventricularly gained access to the systemic circulation. Rats were fed a high-salt diet and were given [3H]U-46,619 (2,250,000 disintegrations/min (dpm); New England Nuclear Products, Boston, MA) either intracerebroventricularly or intravenously. Blood was drawn before and at 1, 3, 10, and 15 min after injections. Plasma (10 µl) samples were counted in triplicate in a liquid scintillation counter.

The aim of the third series was to contrast the effects of a TxA2/PGH2 receptor antagonist ifetroban (BMS-180,291) (16) on the central and peripheral responses to U-46,619 in rats fed a high-salt diet. A dose of 1 µg/kg of U-46,619 was chosen for central and peripheral injections because this was found to produce a reproducible pressor response of 20 mmHg. Ifetroban was given in graded doses of 0.001, 0.01, 0.1, 1, and 10 µg/kg or equivalent vehicle. BP was recorded 1 min after injections. The TxA2/PGH2 mimetic and antagonist were coadministered by the intracerebroventricular route, but for the intravenous route the antagonist was given 1 min before the agonist. The order of central and peripheral testing was randomized, and studies were undertaken on separate days.

The aim of the fourth series was to assess the relative contributions of central and peripheral TxA2/PGH2 receptors to the rises in BP produced by intracerebroventricular or intravenous injections of U-46,619 (1 µg/kg). First, the vehicle or ifetroban (10 µg/kg) was administered intravenously, and 1 min later U-46,619 was administered intracerebroventricularly. Second, the vehicle or ifetroban (0.1 µg/kg) was administered intracerebroventricularly, and 1 min later U-46,619 was administered intracerebroventricularly. The changes in MAP were recorded 1 min after administration of vehicle or U-46,619. All four studies were conducted on separate days.

The aim of the fifth series was to determine whether mRNA for TxA2/PGH2 receptors was expressed in rat brain and glomeruli and in rat neural or glial cells in culture. The kidney and brain of male Sprague-Dawley rats fed a normal salt intake were harvested. The cerebellum and cortex of the brain were excised, and total RNA was extracted from the remaining brain tissue using RNA ATAT-60 (Tel-test B, Friendswood, TX). The astroglial cells and neuronal cells were harvested from brains of Wistar rats fed a normal salt intake and were kindly provided by Dr. M. Raizada (University of Florida, Gainesville, FL). These rats were fed a chow with a sodium content of 0.3 g/100 g throughout. Hypothalamus-brain stem areas of 1-day-old rat brains were dissected, and brain cells were dissociated by trypsin. The hypothalamic block contained the paraventricular nucleus and the supraoptic, inferior, lateral, posterior, dorsal, medial, and ventral-medial nuclei. The brain stem block contained medulla oblongata and pons. Dissociated brain cells were plated onto poly-L-lysine-precoated tissue culture dishes (18 × 106 cells per 100 ml diameter dishes) in a Dulbecco’s modified Eagle’s medium containing 10% plasma-derived horse serum. Neuronal cultures were established as described previously (26). Cultures were allowed to grow for 10–15 days before their use in the experiments. Astroglial cultures were prepared from the same hypothalamic-brain stem areas of 1-day-old rats, as described previously (26). Trypsin-dissociated brain cells suspended in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum were plated in 100 ml culture dishes (18 × 106 cells per dish). Cells were grown for 6 days at 37°C in 5% CO2-95% air. They were dissociated from dishes by trypsin and replated in 100 ml culture dishes (1 × 106 cells per dish). They were allowed to grow for 10–15 days before being used in the experiment. The procedure for microdissection of glomeruli was conducted as previously reported (17).

The mRNA from the glomeruli, brain stem, and astroglial and neuronal cells was reverse transcribed (RT) with oligo(dT)18 as primer and MuLV reverse transcriptase using an RNA polymerase chain reaction (PCR) kit (Perkin Elmer, Branchburg, NJ). The primers used for PCR for the TxA2/PGH2 receptor gene product were selected from the published cDNA sequence of the rat TxA2/PGH2 receptor (1). They were nucleotides 5’-TGACGTGCTGTGAACACTGT-3’ (sense primer, position base pair (bp) 275–294) and 5’-AGCAAAGGATCCACACACCGTG-3’ (antisense primer, position bp 753–776). The reaction mixture contained 50 pmoles of each primer, 1.25 mM deoxynucleotide mixture, 2.5 U Taq DNA polymerase, 10 mM tris(hydroxymethyl)aminomethane (Tris); HCl (pH 10), 50 mM KCl, 1.5 mM MgCl2, and 0.001% (wt/vol) gelatin in a final volume of 50 µl. The PCR was carried out by the following protocol: after an initial melting temperature of 94°C for 4 min, there was 30 s of denaturation at 94°C, 45 s of annealing at 60°C, and 45 s of extension at 72°C for 40 cycles of amplification, followed by a final
extension at 72°C for 7 min. The PCR product was analyzed on a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light. The size of the products was compared with a rat cDNA probe for TxA2/PGH2 receptor kindly provided by Kazu Takeuchi (Tokoh University, Japan). To verify the authenticity of the PCR products, the amplified TxA2/PGH2 receptor cDNAs from rat brain stem were purified with MICROCON (Amicon, Beverly, MA) and sequenced with Amplitaq cycle sequencing kit (Perkin Elmer, Branchburg, NJ).

Drugs. U-46,619 (9,11-dideoxy 11α,9α-epoxymethanoprostaglandin F2α) was a gift from the Upjohn Company (Kalamazoo, MI). It was shipped in methyl acetate and stored at −20°C. Stock solutions were prepared by addition of Tris (Fisher Scientific, Fair Lawn, NJ), dried in a stream of air, dissolved in 0.154 M NaCl and stored at −20°C. The dilutions were made fresh in 0.154 M NaCl before each experiment. Ifetroban (BMS-180,291) was a gift from Martin Ogletree of the Bristol-Myers-Squibb company (Princeton, NJ). It was dissolved fresh in 0.154 M NaCl before each experiment.

Statistical analysis. Data were assessed by analysis of variance (ANOVA) with repeated measures. Where appropriate, Dunnett's tests were applied to detect post hoc differences between groups. Data are shown as means ± SE values, and statistical significance was taken at P < 0.05.

RESULTS

The MAP of rats receiving the high-salt diet did not differ between series and averaged 109 ± 9 mmHg. This value is not significantly different from the average MAP of rats receiving the low-salt diet of 115 ± 3 mmHg. Administration of vehicle by the intravenous or intracerebroventricular route did not change MAP consistently (intravenous: +1.2 ± 1.3; intracerebroventricular: +1.2 ± 0.8 mmHg).

Series 1. This series contrasted the effects of salt intake on the responses to U-46,619. Administration of U-46,619 by intravenous (Fig. 1A) or intracerebroventricular (Fig. 1B) injection produced graded, dose-dependent rises in MAP 1 min after administration, accompanied by reductions in HR ranging from 10 beats/min (bpm) at the lowest dose to 50 bpm at the highest dose (data not shown). Changes in HR were similar after intravenous or intracerebroventricular administration. As shown in Fig. 1A, the pressor responses to intravenous U-46,619 were similar in low- and high-salt rats. In contrast, as shown in Fig. 1B, the early and later rises in MAP after intracerebroventricular U-46,619 were significantly greater in high-salt rats.

Series 2. This series contrasted the responses to intravenous and intracerebroventricular administration of U-46,619 to high-salt rats. The early pressor responses 1 min after intracerebroventricular injection of higher doses of U-46,619 were greater than to intravenous administration, and this difference was significant at the highest dose level tested (Fig. 2). The time course of the pressor responses were different (Fig. 3). Whereas the MAP returned to a value close to baseline by 15 min after intravenous injection, it remained more elevated at 10 and 15 min after intracerebroventricular injection. At 15 min, the increases in MAP above baseline after intracerebroventricular injection of U-46,619 at doses of 0.3 mg/kg and above were significantly higher than after intravenous injections.

Series 3. The aim of this series was to evaluate the rate at which centrally administered [3H]U-46,619 gained access to the peripheral circulation. The plasma [3H] radioactivity after injections of 2,251,000 dpm of [3H]U-46,619 (equivalent to 0.1 ng/kg) given intravenously or intracerebroventricularly (at 10.2 ppm 30 on July 2, 2017 http://ajpregu.physiology.org/ Downloaded from) to rats fed a high-salt (•; n = 6) or a low-salt (○; n = 6) diet for 6–8 days before testing. Comparing low-salt and high-salt data: *P < 0.05; **P < 0.01.

Series 4. The results obtained for the blockade of the central and peripheral effects of U-46,619 (1 µg/kg) coadministered with graded doses of ifetroban are shown in Fig. 5. Ifetroban alone did not significantly change MAP when given intravenously (ifetroban 1 µg/kg; MAP change +0.8 ± 1.5 mmHg) or intracerebro-
The RT product from rat brain stem was eluted and purified. Its sequence had 100% concordance with the published sequence from rat kidney (1).

As shown in Fig. 8, the PCR products from mRNA extracted from kidney glomeruli of Sprague-Dawley rats fed a normal salt diet and from astroglial and neuronal cells in culture from the brains of Wistar rats fed a normal salt diet yielded strong bands in the presence, but not in the absence, of reverse transcription. The bands corresponded in size to the cDNA probe for rat TxA2/PGH2 receptor. The control lane shows the absence of RT products from the buffer used. All studies were amplified for 40 cycles.

**DISCUSSION**

The results of the present study provide the first evidence for a central pressor pathway mediated by TxA2/PGH2 receptors expressed in the brain of normotensive rats. Siren et al. (23) had reported that intracerebroventricular injection of U-46,619 induced a dose-related increase in MAP in spontaneously hypertensive rats but did not alter the BP of normotensive rats. They studied doses of U-46,619 equivalent to those in the present study where we found the threshold intracerebroventricular dose for U-46,619 to be ~0.1 µg/kg. The reason for the differences in response to intracerebroventricular U-46,619 between these studies is not clear but may relate to the rat strain. Siren et al. (23) studied Wistar rats, whereas the present study used Sprague-Dawley rats. It cannot be ascribed to the high salt intake used in the present protocols because we found that there were strong pressor responses to intracerebroventricular administration of U-46,619 in Sprague-Dawley rats given either a high- or a low-salt diet. However, the pressor responses of rats fed a high-salt diet, compared with a low-salt diet, were enhanced for intracerebroventricular but not for intravenous administration of U-46,619. This suggests that a high salt intake may potentiate brain TxA2/PGH2 receptor expression or responsiveness. We had found previously that prolonged peripheral administration of U-46,619 increased the systolic BP to a greater extent in rats fed a high-salt than a normal or low-salt diet (27). However, this effect of salt intake was seen only after 8–12 days of administration of U-46,619 and is consistent with the present finding that the early response to peripheral administration of U-46,619 is not enhanced by a high salt intake.

An analysis of the central cardiovascular effects of U-46,619 is complicated by the extreme lipid solubility of the compound (olive oil:water partition coefficient 66:34) (31) and the sensitivity of the peripheral circulation to it (threshold dose for pressor response - 10–10 M/kg). As a consequence, centrally administered U-46,619 gains rapid access to the peripheral circulation and could raise BP by peripheral actions. Indeed, examination of the dose-response relationships for centrally and peripherally administered drug assessed 1 min after administration to high-salt rats showed a similar sensitivity but an increased responsiveness to intracerebroventricular administration at the highest doses.
Fig. 3. Mean ± SE values for time course of changes in MAP after intravenous (○; n = 6) or intracerebroventricular (●; n = 6) administration of U-46,619 to rats fed a high-salt diet. Data are shown for different doses of U-46,619: A, 0.1; B, 0.3; C, 1; and D, 3 μg/kg. Comparing intravenous and intracerebroventricular data: *P < 0.05; **P < 0.01; ***P < 0.005.

However, the time course of the pressor responses differed. After intravenous administration, the MAP returned to values very close to baseline within 15 min, whereas after intracerebroventricular administration the pressor responses were more sustained (Fig. 3).

Further studies were undertaken to assess the contribution of peripheral actions of U-46,619 to the pressor response after central injection. Radiolabeled U-46,619 appeared in the peripheral circulation within 1 min after central injection in a concentration that was 20% of that after equivalent intravenous injections. Thus centrally injected U-46,619 rapidly leaves the brain and enters the peripheral circulation where it interacts with peripheral receptors to raise the BP. The MAP was increased similarly by low doses of intracerebroventricularly or intravenously administered U-46,619 1 min after injection, despite the plasma 3H concentration after intracerebroventricular administration being only 20% that after intravenous injection. This suggests that much of the early pressor response to intracerebroven-

Fig. 4. Mean ± SE values for plasma 3H activity after intravenous (○; n = 6) or intracerebroventricular (●; n = 6) administration of [3H]U-46,619 to rats fed a high-salt diet. dpm, disintegrations/min.
BRAIN TXA2 RECEPTORS AND BP

The brain contains TXA2/PGH2 receptors that, when activated, can raise BP. Indeed, the present study demonstrates expression of mRNA for TXA2/PGH2 receptors in rat neuronal and astroglial cells and brain stem.

The sequence of the RT-PCR product for TXA2/PGH2 transcripts isolated from the brain stem corresponded completely with the reported sequence for the TXA2/PGH2 receptor mRNA cloned from rat kidney (1). These findings extend the recent report of Kitanaka et al. (11), which showed the presence of TXA2 receptor mRNA in astrocytes cultured from neonatal rat brain. The finding that TXA2/PGH2 receptor mRNA is expressed in neural cells is of particular interest as it suggests that these receptors may have a potential role in modulating neural functions. Previous receptor binding studies...

ventricular administration originated from actions of U-46,619 within the brain. Moreover, at 15 min, the plasma concentration of 3H after intracerebroventricular injection was <40% of that after intravenous injection, yet the MAP was clearly higher. Thus, at this time, most of the pressor response was centrally mediated. The relative importance of central and peripheral TXA2/PGH2 receptors in the response to centrally administered U-46,619 was examined further by comparing the efficacy of intravenous ifetroban in blocking the pressor responses to intracerebroventricular and intravenous administration of U-46,619 to high-salt rats. Intravenous ifetroban, in a dose that blocked almost all of the pressor response to intravenous U-46,619, blocked less than one-half of the response to intracerebroventricular U-46,619. Likewise, centrally administered ifetroban was more effective in blocking the pressor response to intracerebroventricular than to intravenous U-46,619. Taken together, these observations suggest that the pressor responses after central or peripheral administration of U-46,619 are due to combined actions of the drug in the brain and the peripheral circulation. At least one-half of the pressor responses to intracerebroventricular U-46,619 are due to actions in the brain, and at least one-half of the pressor responses to intravenous U-46,619 are due to actions in the peripheral circulation.

Further evidence for central actions of U-46,619 came from studies that contrasted the blockade by ifetroban of the responses to U-46,619 when both agents were administered centrally or peripherally. Coadministration of ifetroban blocked >90% of the pressor responses to intravenously or intracerebroventricularly administered U-46,619. These results confirm that the cardiovascular responses to U-46,619 are receptor mediated (6, 7, 32). Of interest was the finding that the IC50 for antagonism of the pressor response by intracerebroventricular administration was some 100-fold below that for intravenous administration. This indicates that the brain contains TXA2/PGH2 receptors that, when activated, can raise BP. Indeed, the present study demonstrates expression of mRNA for TXA2/PGH2 receptors in rat neuronal and astroglial cells and brain stem.

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have shown TxA₂ receptors linked to activation of phospholipase C in human (15) and rabbit (14) astrocytes. The present studies demonstrate that brain neurons are also potential sites of action of TxA₂ or other endogenous ligands for this receptor.

In conclusion, mRNA for TxA₂/PGH₂ receptors is expressed in the brain stem and in neuronal and astroglial cells of normal rats. Studies with a specific TxA₂/PGH₂ receptor agonist demonstrate that activation of these receptors within the brain can raise BP and that salt loading enhances this response.

**Perspectives**

The mechanism of the central pressor response to U-46,619 remains to be explored. In previous studies, we found that prolonged subcutaneous infusions of U-46,619 via an osmotic minipump raised the systolic BP progressively. This hypertensive response depended on an intact sympathetic nervous system because it was abolished by α-adrenergic blockade with phentolamine (27) or prazosin (8). Other studies have shown that TxA₂/PGH₂ receptors can potentiate the release of norepinephrine or the action of the sympathetic nervous system (10) and this may contribute to the central and peripheral actions of U-46,619. It is unlikely that the pressor response relates to cerebral ischemia because ischemia is accompanied by a rise in HR (9). It is possible that the central pressor response also involves vasopressin release because prostaglandins are implicated in vasopressin regulation (3). Data relating specifically to vasopressin release with TxA₂ or PGH₂ are required.

It is not clear from these pharmacological studies whether TxA₂/PGH₂ receptors in the brain could be involved in the normal regulation of BP. Astroglial cells are a significant source of TxA₂ synthesis, whereas brain neuronal cells form less PGs (19, 20). PGD₂ is the predominant PG synthesized by endothelial cells from brain capillaries and can be metabolized to 9α,11β-prostaglandin F₂α, which is not only a vasoconstrictor but can release TxB₂ and PGE₂ from endothelial cells (25). Moreover, very large quantities of TxB₂ are released into the cerebral circulation during hypoperfusion (4, 21) and by the spinal cord after trauma (5).
Therefore, there are significant potential sources of vasoconstrictor PGs within the brain and spinal cord that could interact with TxA2/PGH2 receptors that our studies predict may be expressed on both astroglial and neuronal cells and in the brain.

We are grateful to Dr. Karen Gayle and David Dybdal for assistance in the techniques of central placement of intracerebroventricular cannulas in the rat and to Drs. Richard A. Gillis and Joe A. DiMicco for helpful discussion. U-46,619 was kindly provided by Upjohn Pharmaceutical and fetroban by Martin Ogletree of Bristol-Meyer Squibb. A cDNA probe for rat renal TxA2/PGH2 receptor was kindly provided by K. Takeuchi at the Second Department of Internal Medicine, Tohoku University, Japan.

This work was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-36079 and DK-49870) and the George E. Schreiner Chair of Nephrology. H. Gao was supported by an International Society of Nephrology Fellowship grant.

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Received 18 June 1996; accepted in final form 17 December 1996.

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