Role of thromboxane receptors in the dipsogenic response to central angiotensin II

CHAGRIYA KITIYAKARA,1 WILLIAM J. WELCH,1 JOSEPH G. VERBALIS,2 AND CHRISTOPHER S. WILCOX1
1Division of Nephrology and Hypertension, and Center for Hypertension and Renal Disease Research; and 2Division of Endocrinology and Metabolism, Georgetown University, Washington, District of Columbia 20007

Received 12 June 2001; accepted in final form 2 November 2001

Kitiyakara, Chagriya, William J. Welch, Joseph G. Verbalis, and Christopher S. Wilcox. Role of thromboxane receptors in the dipsogenic response to central angiotensin II. Am J Physiol Regulatory Integrative Comp Physiol 282: R865–R869, 2002; 10.1152/ajpregu.00328.2001.—Central angiotensin II (ANG II) regulates thirst. Because thromboxane A2-prostaglandin H2 (TP) receptors are expressed in the brain and mediate some of the effects of ANG II in the vasculature, we investigated the hypothesis that TP receptors mediate the drinking response to intracerebroventricular (icv) injections of ANG II. Pretreatment with the specific TP-receptor antagonist ifetroban (Ifet) decreased water intake with 50 ng/kg icv ANG II (ANG II + Veh, 7.2 ± 0.7 ml vs. ANG II + Ifet, 2.8 ± 0.8 ml; n = 5 rats; P < 0.001) but had no effect on water intake induced by hypertonic saline (NaCl + Veh, 8.4 ± 1.1 ml vs. NaCl + Ifet, 8.9 ± 1.8 ml; n = 5 rats; P = not significant). Administration of 0.6 μg/kg icv of the TP-receptor agonist U-46,619 did not induce drinking when given alone but did increase the dipsogenic response to a near-threshold dose of 15 ng/kg icv ANG II (ANG II + Veh, 1.1 ± 0.7 ml vs. ANG II + U-46,619, 4.5 ± 0.9 ml; n = 5 rats; P < 0.01). We conclude that central TP receptors contribute to the dipsogenic response to ANG II.

Thus TP receptors are expressed in the brain and are implicated in the central regulation of blood pressure and pituitary function.

TP receptors are also implicated in the vasoconstrictive effects of ANG II (17, 18, 32) and the maintenance of hypertension in several models including the spontaneous hypertensive rat (30) and the Goldblatt 2 kidney, 1 clip (30) and aortic coarctation (16) rat models of renovascular hypertension. Because brain ANG II regulates thirst (8, 12, 24), we tested the hypothesis that TP receptors mediate the dipsogenic response to centrally administered ANG II. To test whether any effects are specific for ANG II, we also tested the role of TP receptors in the drinking response to hypertonic saline, which is independent of the renin-angiotensin system (24).

METHODS

Animal Preparation

These studies were reviewed by the Georgetown University Medical Center Animal Care and Use Committee and were performed according to the Guide for the Care and Use of Laboratory Animals of the NIH and the guidelines of the Animal Welfare Act. Male Sprague-Dawley rats (body wt 300–420 g) were fed a normal chow diet (Rat Chow, Ralston Purina, St. Louis, MO) with a sodium content of 0.3 g/100 g. Rats were habituated to their cages for 5 days. They were maintained in a climate-controlled environment with a 12:12-h light-dark cycle. Studies were undertaken between 10 AM and 2 PM. Under anesthesia with pentobarbital sodium (50 mg/kg ip; Abbot Labs, North Chicago, IL) a polyethylene (PE)-10 catheter connected to a PE-50 catheter was placed in the femoral vein, threaded subcutaneously, and exteriorized at the nape of the neck. The catheter was flushed and filled with 0.154 M NaCl containing heparin (500 IU/ml) and was flame-sealed. A cannula was inserted into the lateral intracerebral ventricle as described previously (10). The vascular catheter was flushed daily. The animals were allowed 4 days to recover from surgery. Studies were performed in conscious, unrestrained rats that were allowed to move freely around their cages. For icv injections, the obturator was removed and connected to a microsyringe by a PE-10 cathe-
The system was filled with the test solutions before the study. The drinking response of each rat was tested 1–2 days after the completion of the experiment by the response to 50 ng/kg icv ANG II. A drinking response of at least 5 ml over 30 min was required as evidence of a properly positioned icv cannula and an intact central ANG II behavioral pathway. Rats that did not achieve this response were killed and their data was discarded.

The aim of series 1 was to test the hypothesis that TP receptors mediate the dipsogenic response to icv ANG II. Food was removed from cages during the drinking studies. Rats received a 0.25 ml iv bolus of ifetroban (1 mg/kg) followed by an infusion at 1 mg·kg⁻¹·h⁻¹ of equivalent bolus and infusion of vehicle (Veh, 0.154 M NaCl). After 90 min, rats received a 2-μl icv injection of ifetroban (100 μg/kg) or equivalent Veh. Pilot studies indicated that it was necessary to infuse ifetroban intravenously and administer it centrally to ensure blockade of drinking responses to ANG II. Ietroban is a specific competitive antagonist of TP receptors (19). Our preliminary studies showed that this protocol for administration of ifetroban produced a consistent effect on the drinking response to icv ANG II. These doses of iv and icv ifetroban were selected after our prior studies (9) showed that they produced maximal blockade of the pressor response to U-46,619 given to conscious rats. Fifteen minutes after the icv injection of ifetroban or Veh, rats received a 2-μl icv injection of ANG II (50 ng/kg). The subsequent water intake was recorded every 15 min for 45 min. Drinking usually occurred promptly and was complete within 30 min. Two days were allowed between studies (study order was randomized).

In series 2 we tested the hypothesis that any role for TP receptors in the drinking response was specific for ANG II. Animals were prepared as in series 1. They received ifetroban (1 mg/kg iv and 1 mg·kg⁻¹·h⁻¹ iv) or equivalent Veh, and 90 min later they received a 2-μl injection of ifetroban (100 μg/kg iv) or equivalent Veh. All animals received an iv infusion of 1.5 ml of 2 M saline (0.55 g NaCl/kg) over 15 min. This dose was selected from pilot studies to produce a comparable drinking response to icv ANG II (50 ng/kg). Water intake was recorded every 15 min for 45 min. Two days were allowed between studies (study order was randomized).

Series 3 tested the hypothesis that the activation of central TP receptors enhances the dipsogenic response to a threshold dose of icv ANG II. In a pilot study, we determined that icv administration of 2 μl of the specific TP-receptor agonist U-46,619 (0.6 μg/kg; 1 μg = 2.86 nmol; see Ref. 10) did not stimulate drinking. When given to conscious rats, this icv dose of U-46,619 increases blood pressure by ~20%, which is equivalent to the increase in the blood pressure protocol by icv ANG II at 50 ng/kg (9, 31). Rats were randomized to receive one of four schedules of icv drug administration (2-μl volumes of each concentration): 1) a near-threshold dose of ANG II (15 ng/kg) + Veh; 2) ANG II (15 ng/kg) + U-46,619 (0.6 μg/kg); 3) a dipsogenic dose of ANG II (50 ng/kg) + Veh; or 4) ANG II (50 ng/kg) + U-46,619 (0.6 μg/kg). U-46,619 or Veh was given 2 min before ANG II. The cumulative water intake was recorded every 15 min for 45 min. Two days were allowed between studies.

Drugs

Ietroban (BMS-180,291; mol wt 724) was a gift from Martin Ogletree of Bristol Meyers-Squibb (Princeton, NJ). It was shipped in ethanol. Stock solutions were prepared by addition of Tris-HCl (Fisher Scientific, Fair Lawn, NJ), dried in a stream of air, dissolved in 0.154 M NaCl, and stored at −20°C. All drug doses were administered as milligrams per kilogram of body weight.

Statistical Analysis

Data were assessed by repeated-measures ANOVA. Where appropriate, a post hoc Student’s t-test was undertaken to assess differences between specific pretreatment protocols. Data are displayed as means ± SE values. Statistical significance was taken at P < 0.05.

RESULTS

Drinking Response to ANG II

For series 1, the administration of icv ANG II (50 ng/kg) stimulated drinking significantly (Fig. 1). The drinking response was complete by 30 min. The cumulative drinking response was decreased (P < 0.001) at both 15 and 30 min by ifetroban: the cumulative water intake after 30 min for ANG II + Veh was 7.2 ± 0.7 ml versus ANG II + Ifet, which was 2.8 ± 0.8 ml (n = 5 rats; P = not significant).

Drinking Response to Hypertonic Saline

For series 2, the iv infusion of hypertonic saline stimulated a drinking response comparable to that induced by icv ANG II (50 ng/kg) at 15 and 30 min (Fig. 2). In contrast to the ANG II-induced drinking response, ifetroban had no significant effects on the drinking response to hypertonic saline: the cumulative water intake after 30 min for NaCl + Veh was 8.4 ± 1.1 versus NaCl + Ifet, which was 8.9 ± 1.8 ml (n = 5 rats; P = not significant).

Interactions of ANG II and U-46,619 on Drinking

For series 3, an icv dose of ANG II (15 ng/kg) was selected that barely stimulated drinking when given
The central role of TP receptors in mediating the dipsogenic effects of ANG II remains unclear. Although isoprostanes, which are metabolites of arachidonic acid generated by phospholipases, have been detected in the cerebrospinal fluid (22), their origin and physiological significance are not yet fully understood. ANG II can generate isoprostanes (21), which are also produced by astrocytes (29) and cerebral microvascular endothelial cells (27), and the hypothalamus (14) can generate TxA2 (27), whose metabolism by cyclooxygenase yields PGH2, which is a ligand for the TP receptor (4). Moreover, ANG II can sensitize the drinking response to ANG II but does not itself initiate drinking. Similarly, in the vasculature, TP-receptor agonists at subpressor doses increase ANG II-induced vasoconstriction but require much higher doses to raise blood pressure themselves (25); ANG II-induced vasoconstriction appears to be specific for ANG II and U-46,619. Cyclooxygenase inhibition enhances drinking to icv ANG II (20) by blockade of PGE2 generation (20). In the present studies, the highly selective TP-receptor antagonist ifetroban (19) blocked much of the drinking response to icv ANG II. Thus different cyclooxygenase products may have opposing effects on ANG II-induced drinking; e.g., inhibition by PGE2 and stimulation by TP-receptor ligands. The effects of TP-receptor blockade appear to be specific to the drinking response induced by ANG II, because ifetroban had no significant effect on the drinking response to hypertonic saline. Intracellular dehydration with hypertonic saline induces a drinking response by activating pathways that are independent of ANG II (1, 23, 24). The absence of any effect of ifetroban on osmotically induced drinking indicates that its inhibition of fluid intake with ANG II cannot be ascribed to nonspecific effects such as malaise.

The drinking response to icv ANG II at either the near-threshold (15 ng/kg) or the effective (50 ng/kg) dose was comparable to the responses found in a previous study (12). Central TP-receptor activation elicited a drinking response to a near-threshold icv dose of ANG II that was comparable to that produced by a higher dose of ANG II (50 ng/kg). An icv injection of U-46,619 alone at a dose that increases mean arterial pressure by ~20% (10) did not stimulate drinking. These findings suggest that TP-receptor activation sensitizes the drinking response to ANG II but does not itself initiate drinking. Similarly, in the vasculature, TP-receptor agonists at subpressor doses increase ANG II-induced vasoconstriction but require much higher doses to raise blood pressure themselves (25); the mechanism of this apparent transactivation is not yet understood. Increases in intracellular Ca2+ mediate vasoconstriction induced by ANG II and U-46,619.

**DISCUSSION**

The novel findings of the present study are that TP receptors modulate the central dipsogenic effects of ANG II. This effect appears specific for ANG II-induced drinking, because ifetroban had no effects on the drinking response to hypertonic saline. Further evidence of interaction between central ANG II and TP receptors derived from the finding that a TP-receptor agonist sensitized the drinking response to a near-threshold dose of icv ANG II.

Astrocytes (29), cerebral microvascular endothelial cells (27), and the hypothalamus (14) can generate TxA2. Acting on AT1 receptors, ANG II releases TxA2 and other PGs from astrocytes (29) and cerebral microvascular endothelial cells (27). ANG II also can stimulate phospholipases (29) that release arachidonic acid, whose metabolism by cyclooxygenase yields PGH2, which is a ligand for the TP receptor (4). Moreover, ANG II can generate isoprostanes (21), which are also ligands for TP receptors (22). Although isoprostanes have been detected in the cerebrospinal fluid (22), their central role remains unclear.
There are differences in the signaling pathways activated by each agent (2).

It is not established why it was found necessary in pilot studies to administer ifetroban by icv and iv routes to consistently block the effects of icv ANG II. Ifetroban is a very lipid-soluble compound (19); therefore, after icv administration, it will diffuse into the systemic circulation. Adequate brain concentrations may require the maintenance of drug in the blood stream to limit the diffusion loss.

In conclusion, the central dipsogenic pathways that are activated by ANG II are reinforced by activation of TP receptors. This effect appears specific because TP receptors do not mediate drinking in response to hyperosmolality. Further studies will be needed to elucidate the cellular mechanism responsible for this interaction and the physiological role of brain TP receptors in fluid-volume homeostasis and long-term blood pressure regulation.

**Perspectives**

How might TP receptors modulate neural pathways that interact with ANG II receptors? The dipsogenic response to ANG II depends on activation of N-methyl-d-aspartate-type glutamate receptors (33) that are activated by TP-receptor agonists (13). Conversely, drinking is inhibited by GABA receptors (7) that are blocked by TP-receptor agonists (26). Thus a coordinated resetting by TP-receptor activation of the excitatory glutamate pathways and the inhibitory GABA pathways could provide a plausible mechanism whereby U-46,619 might enhance the dipsogenic response to ANG II. It is interesting that ANG II and TP-receptor agonists given centrally increase both blood pressure and release of AVP and ACTH (1, 6, 8, 10, 23, 31). On the other hand, activation of ANG II receptors stimulates drinking, whereas the present study shows that activation of TP receptors alone does not. This indicates that ANG II and TP receptors share common central pathways yet also have some discreet regulation of the drinking response. The present studies implicate the interaction of TP-receptor ligands with ANG II in the central control of fluid intake. The physiological importance of this finding will require further study.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grants DK-36079, DK-49870, and DK-38094) and by funds from the George E. Schreiner Chair of Digestive and Kidney Diseases (Grants DK-36079, DK-49870, and DK-38094) and by funds from the George E. Schreiner Chair of Digestive and Kidney Diseases (Grants DK-36079, DK-49870, and DK-38094).

**REFERENCES**


