Renal Effects of Specific Antagonists of Arginine Vasopressin in Dogs

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ABSTRACT—To investigate the effects on renal hemodynamics of specific antagonists of arginine vasopressin (AVP), CGP 29325 (d(CH2)5-D-Tyr(Et)VAVP), which has both anti-vasopressor and anti-antidiuretic activities against AVP, and CGP 25838E (d(CH2)5-Tyr(Me)AVP), which has only anti-vasopressor activity, were administered to normally hydrated anesthetized dogs, and the effects on renal function were examined. The pressor response and constriction of renal and mesenteric arteries induced by AVP were dose-dependently blocked by intravenous CGP 25838E. Following intrarenal arterial administration (i.r.a.) of CGP 29325 at 3 μg/min, water diuresis occurred and urine osmolality (Uosm) decreased to less than 250 mOsm/kg. Renal blood flow (RBF), glomerular filtration rate (GFR), and urinary sodium excretion (UNaV) remained unchanged. A higher dose (10 μg/min, i.r.a.) of CGP 29325 further decreased Uosm to about 110 mOsm/kg. Although arterial blood pressure (BP), GFR and UNaV remained unchanged, RBF decreased from the control value 3.7 ± 0.35 to 2.4 ± 0.40 ml/g·min. CGP 25838E (10 μg/min, i.r.a.) had no effect on renal hemodynamics and urine formation. When administered into the mesenteric artery, CGP 25838E (10 μg/min) increased mesenteric blood flow (MBF) from 199 ± 34 to 240 ± 40 ml/min without any alteration in blood pressure. We tentatively conclude that CGP 29325, at a lower dose, exerted anti-antidiuretic effects through a specific inhibition of V2 receptors, while the higher dose of CGP 29325 altered RBF, through yet to be determined mechanisms. The vasoconstrictive activity of AVP may contribute to the regulation of mesenteric circulation, but not to renal hemodynamics, in anesthetized dogs.

Specific antagonists against antidiuretic and vasopressor responses to arginine vasopressin (AVP) developed by Manning et al. (1, 2) are proving to be useful tools for investigating the physiological and biochemical aspects of the actions of AVP. At least two types of vasopressin receptors, V₁ and V₂, have been well-characterized (3), the former mediates vasopressor activity, while the latter mediates antidiuretic activity. In vitro experiments, a V₂ antagonist has been shown to specifically inhibit the AVP-dependent adenylate cyclase of human and animal kidneys in a competitive manner (4). A V₁ receptor antagonist inhibits AVP induced smooth muscle contraction of the rat uterus (2). Antagonists which have an in vitro high specificity for V₁ and/or V₂ receptors may be effective agents for treating

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the syndrome of inappropriate antidiuretic hormone secretion (5) or for some types of hypertension (6).

Azzawi Al-Omar and Shirley reported that AVP decreased renal blood flow (RBF) in rats with hereditary diabetes insipidus and that the AVP antagonist had no effect on renal hemodynamics (7). Gellai reported a fall in glomerular filtration rate (GFR) with no change in RBF following the administration of V1 and V2 antagonist (8). Although endogenous AVP might have a significant role in the regulation in renal hemodynamics, the in vivo effects of these antagonists on renal hemodynamics remain controversial (7, 8). In the present work, an antagonist for both vasopressor (V1) and antidiuretic (V2) receptors of AVP, CGP 29325 (1), and an antagonist for the vasopressor (V1) receptors, CGP 25838E (2), were given into the renal arteries of anesthetized dogs and changes in renal hemodynamics and urine formation were observed. We also examined the physiological significance of AVP on renal hemodynamics.

MATERIALS AND METHODS

Experiments were performed on mongrel dogs of both sexes weighing from 15 to 25 kg, and they were subjected to restriction of water and food for 24 hr before the experiment. They were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated artificially with a Harvard respirator (Model 607, U.S.A.). Blood pressure (BP) was monitored by a pressure transducer (P23ID, Nihon Kohden, Japan) connected to a catheter placed in the abdominal aorta via the right femoral artery. Effects of AVP antagonists on renal function

The right brachial vein and artery were cannulated for constant infusion of inulin solution (2–3 ml/min) and arterial blood sampling, respectively. A polyethylene catheter was inserted into the left ureter following exposure of the left kidney through a retroperitoneal flank incision for urine collection, as described (9). All visible nerves and connect-
Specific AVP Antagonists and Renal Function

mU/kg. Intravenous administration of each dose of AVP was not started until the BP, RBF and MBF reverted to preinfusion levels, with more than a 20-min interval. Effects of AVP on BP, RBF and MBF were observed before and after CGP 25838E administration (10 µg, i.v.). Renal and mesenteric vascular resistances were calculated by dividing the mean arterial BP by RBF and MBF, respectively. AVP administrations were repeated in the same animal following an additional 100 µg (i.v.) of CGP 25838E. In separate experiments, to examine the roles of endogenous AVP on mesenteric vascular tone in anesthetized dogs, CGP 25838E was infused directly into the mesenteric artery. Mesenteric arteries were exposed in 6 dogs as described above, and flow probes were placed. After stabilization, CGP 25838E in a dose of 10 µg/min was infused into the mesenteric artery for 20 min and MBF and BP were measured.

The V₁ antagonist CGP 25838E (d(CH₂)₅-Tyr(Me)AVP) and the V₁V₂ antagonist CGP 29325 (d(CH₂)₅-D-Tyr(Et)VAVP) were kind gifts from Prof. K.G. Hofbauer, Ciba Geigy, Basel, Switzerland.

Analytical procedures

Sodium and potassium were measured by flame photometry (205D, Hitachi, Japan). Osmolality of urine was measured by freezing point depression using a Fiske Osmometer (Fiske, U.S.A.). Inulin concentrations in plasma and urine were determined by colorimetry according to Walser et al. (10). Results presented are means ± S.E.M. Data were analyzed by randomized block analysis of variance combined with the least significant difference test.

RESULTS

Effects of CGP 29325 and CGP 25838E on renal function

Following intrarenal arterial administration (i.r.a.) of CGP 29325 (3 µg/min) for 20 min, BP, RBF and GFR did not change throughout the experiments (Fig. 1). UF increased from the control value of 8.45 ± 2.21 to 20.9 ± 3.70 µl/g·min and urine osmolality (Uₘₐₛ) decreased to less than 250 mOsm/kg, 20 min after drug infusion. Water diuresis persisted for about 30 min, and then Uₘₐₛ gradually reverted to 458 mOsm. Urinary sodium excretion (Uₙa,V) decreased transiently, but this change was not statistically significant.

At the higher dose of CGP 29325 (10 µg/min) (Fig. 2), RBF gradually decreased from the control value of 3.7 ± 0.46 to 2.4 ± 0.40 ml/g·min and remained low for more than 2 hr after administration of the drug. Despite the decrease in RBF, the BP and GFR remained unchanged. The diuretic response was much higher than that observed when 3 µg/min of the antagonist was given, and UF increased to 70.3 µl/g·min, which is about 5 times the control level, Uₘₐₛ also showed a remarkable fall and reached 110 mOsm/kg. Marked water diuresis persisted throughout
the experiment. \( U_{NaV} \) decreased slightly, but not statistically. The hematocrit value did not change throughout the experiment.

On the other hand, CGP 25838E, a \( V_1 \) antagonists (10 \( \mu \)g/min, i.r.a.) did not affect BP, renal hemodynamics and urine formation (Fig. 3).

Effects of CGP 25838E on vasoconstrictor responses to AVP

Following intravenous administration of AVP in a dose of 1, 5 and 20 mU/kg, BP was increased by 3.6 ± 2.3, 11.0 ± 2.9 and 17.7 ± 3.8 mmHg, respectively. As shown in Fig. 4, the pressor response of AVP was remarkably suppressed by CGP 25838E in a dose-dependent manner. Intravenous administration of AVP increased mesenteric vascular resistance (MVR) in a dose-dependent manner, but was much less potent in the renal vasculature. AVP induced increases in renal vascular resist-

![Fig. 2. Effects of intrarenal arterial infusion of CGP 29325 (10 \( \mu \)g/min) on renal function. BP, blood pressure; RBF, renal blood flow; GFR, glomerular filtration rate; \( U_{NaV} \), urinary excretion of sodium; \( U_{osm} \), urine osmolality; i.r.a., intrarenal administration. *P < 0.05.](image1)

![Fig. 3. Effects of intrarenal arterial infusion of CGP 25838E (10 \( \mu \)g/min) on renal function. BP, blood pressure; RBF, renal blood flow; GFR, glomerular filtration rate; \( U_{NaV} \), urinary excretion of sodium; \( U_{osm} \), urine osmolality; i.r.a., intrarenal administration.](image2)

![Fig. 4. Pressor responses to arginine vasopressin (AVP) before and after intravenous administration of CGP 25838E. ○○ control (without CGP 25838E), ■■ CGP 28838E, 10 \( \mu \)g. i.v. ■■ CGP 25838E, 100 \( \mu \)g, i.v.](image3)
Specific AVP Antagonists and Renal Function

Fig. 5. Increase in renal vascular resistance (RVR) and mesenteric vascular resistance (MVR) by arginine vasopressin (AVP) following intravenous administration of CGP 25838E. O-O control (without CGP 25838E), •-• CGP 28838E, 10 μg, i.v. ■-■ CGP 25838E, 100 μg, i.v.

Fig. 6 Effect of intramesenteric arterial infusion of CGP 25838E (10 μg/min) on mesenteric blood flow (MBF). i.m.a.: intramesenteric administration. *P < 0.05.

When administered into the mesenteric artery at a rate of 10 μg/min, CGP 25838E significantly increased MBF with no change in blood pressure (Fig. 6).

DISCUSSION

As shown in Figs. 1 and 2, following the administration of CGP 29325, there was a latent period before U_{Osm} reached the lowest level, in agreement with the report of Stassen et al. (4). The increased plasma AVP levels seen in the case of surgical stress (11, 12) may have enhanced the potency. CGP 29325 in a dose of 3 μg/ml caused a marked water diuresis with no change in BP, RBF, GFR, osmolar clearance and U_{Na,V}. It was therefore considered that CGP 29325 exerts its anti-antidiuretic effect by decreasing the water permeability of the collecting duct, through V₂-receptor blockade, without affecting renal hemodynamics or sodium handling.

When a higher dose (10 μg/min) of the antagonist was infused, the water diuresis was further augmented. Although BP and GFR remained unchanged, RBF decreased significantly even after cessation of the infusion. The possibility that this reduction of RBF was induced by the V₁ agonistic action of CGP 29325 is less likely, because of the relative insensitivity of the renal vasculature to V₁ agonism (Fig. 5) and the extremely low V₁ agonistic activity of this antagonist reported by Manning et al. (1). Although the mechanism by which the higher dose of CGP 29325 induced renal vasoconstriction is not clear, alteration in the renin-angiotensin system may contribute to the vascular effects since AVP was reported to inhibit renin secretion (13). The V₂ receptor antagonism induced by CGP 29325 may have stimulated renin secretion and induced renal vasoconstriction, indirectly. Schwartz and coworkers (14) have shown, in anesthetized water loaded dogs, that the selective antidiuretic agonist DDAVP suppressed plasma renin activity (PRA) and that the selective vasoconstrictor agonist (PheOrnOT) and the V₁ antagonist (CGP 25838E) did not affect PRA. Thus, they concluded that the inhibition of renin secretion might be mediated by antidiuretic activity. Malayan and Reid (15) reported a similar observation, using another selective antidiuretic analogue (dTDAVP) in water loaded dogs. On the contrary, semipurified renin (1 ml of 18,000 ng Angiotensin I/ml/hr) administered intravenously increased BP by 6 mmHg, but did not alter RBF and GFR (16). Contribution of the renin angioten-
sin system to the altered renal hemodynamics requires further study.

The physiological significance of V1 receptors in the renal circulation is controversial (7, 8, 17). The present results demonstrated that renal hemodynamics do not respond to V1 blockade in anesthetized dogs, thereby suggesting a minor role for the vascular action of AVP in the renal vasculature. However, Azzawi Al-Omar and Shirley reported that AVP at any dose given decreased total RBF and outer cortical blood flow, determined using radioactive microspheres in rats with hereditary diabetes insipidus (7). They used an AVP analogue that inhibits AVP actions at vascular receptor sites, decreased renal vascular resistance and increased RBF in Long Evans rats but not in rats with hereditary diabetes insipidus (7). Gellai et al. reported that long-term treatment with physiological doses of AVP alters body fluid volume in association with increases in GFR and RBF, although acute intravenous administration did not alter the renal hemodynamics (17). However, Gellai noted that GFR fell substantially with no change in RBF when V1 and V2 antagonists were given together to conscious rats, although the V1 antagonists alone had no effects on RBF and GFR (8). The discrepancy between our results and those of others may be explained by the use of different animal species, antagonists, and experimental conditions.

As shown in Fig. 6, MBF was increased by 20% with no change in BP following the intramesenteric administration of CGP 25838E, hence, there was a striking difference in the response of RBF to the same antagonist (i.r.a.) (Fig. 3). This remarkable difference in response to a V1 antagonist between mesenteric and renal vascular beds suggested that the physiological contribution of the vasoconstrictive action of AVP may differ with the organ. Furthermore, the renal vasculature was far less sensitive to exogenous AVP than was the mesenteric vascular bed (Fig. 5). There are reports suggesting that AVP stimulates the release of vasodilatory prostaglandins (PGs) in the kidney (18, 19). Yared et al. (20) noted that in water deprived rats pretreated with indomethacin, an inhibitor of PGs synthesis, exogenous AVP produced vasoconstricting effects on renal and extrarenal beds, to much the same extent. They suggested that the release of PGs may be involved in the relative insensitivity of the renal artery to AVP.

The antivasopressor effects of the antagonist CGP 25838E to exogenously administered AVP at pharmacological doses were potent and long lasting, a finding consistent with evidence obtained in in vitro experiments (1, 2). The long duration of action reflects the long half life of the drugs.

In conclusion, we found that a low dose (i.r.a.) of CGP 29325 specifically antagonizes the V2 mediated antidiuretic action of AVP with no change in renal hemodynamics. The RBF altered by a higher dose of the drug does not seem to be related to either V1 or V2 receptors. Specific inhibition of renal V1 receptors has no appreciable effects on renal circulation or urine formation, in marked contrast to the apparent effects on mesenteric hemodynamics.

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