Possible participation of prostaglandins generated in the anteroventral third ventricular region in the hypovolemia-induced vasopressin secretion of conscious rats

Ken'ichi Yamaguchi¹, Hitoshi Hama² and Kazuo Watanabe³

¹Department of Physiology, Niigata University School of Medicine, Niigata, Japan, ²Division of Medicine, Kido Hospital, Niigata, Japan and ³Department of Anatomy, Hamamatsu University School of Medicine, Hamamatsu, Japan

(Correspondence should be addressed to K Yamaguchi, R3-403 Yamanoshita-Jyūtaku, 2-3722 Akiba-Dōri, Niigata City, Niigata 950-0052, Japan)

Abstract

Objective: The aim of this study was to investigate the roles of prostaglandins (PGs) in the anteroventral third ventricular region (AV3V), a cerebral site for cardiovascular homeostasis, in hypovolemia-induced vasopressin (AVP) secretion.

Methods: We infused meclofenamate (78.3 nmol in 1 μ l), a PG synthesis inhibitor, or PGE₂ (7.1 nmol in 0.5 μ l) into the AV3V of conscious rats, examining their effects on plasma AVP and other variables in the presence or absence of hemorrhages. The hemorrhages (about 14% of blood volume) were conducted successively by taking femoral arterial blood over a 30-s period at 10-min intervals.

Results: The first hemorrhage increased plasma AVP in blood samples obtained 10 min later, without affecting plasma angiotensin II (ANG II), arterial pressure and heart rate. The second hemorrhage after 10 min raised plasma AVP further and, remarkably, augmented plasma ANG II, and reduced arterial pressure. The AVP responses to both the first and second hemorrhages were attenuated by meclofenamate infusion into the AV3V performed 35 min before the first hemorrhage. The meclofenamate infusion did not alter the response of ANG II, while that of arterial pressure was potentiated and heart rate was decreased after the second hemorrhage. These effects of meclofenamate on plasma AVP and the cardiovascular parameters were not found when the drug was infused into the nucleus accumbens, the region slightly distant from the AV3V, or the lateral cerebral ventricle. In the normovolemic state, meclofenamate administered into the three brain regions did not affect any of the variables monitored. In contrast, application of PGE₂ into the AV3V enhanced plasma AVP, heart rate and arterial pressure after 5 and 15 min. Histological examination indicated that infusion sites of meclofenamate in the AV3V were close to those of PGE₂ in several cases and included areas such as the organum vasculosum of the lamina terminalis, periventricular hypothalamic nucleus, and the median and medial preoptic nuclei.

Conclusion: These results suggest that PGs synthesized in and/or near the AV3V may be involved in the regulation of AVP release and cardiovascular function in the hypovolemic state.

European Journal of Endocrinology 138 206–215

Introduction

The prostaglandin (PG) biosynthesis inhibitors meclofenamate (1) and indomethacin when administered into the lateral cerebral ventricle in rats have been reported to block rises in plasma vasopressin (AVP) in response to hemorrhage (2). AVP secretion elicited by angiotensin II (ANG II), the formation of which in the systemic blood is enhanced by hypovolemic stimuli (3, 4) and the sites of action of which are placed in cerebral areas (5), is similarly prevented by intraventricular administration of these PG inhibitors (6–8). These reports on the effects of PG inhibitors together with reports that bleeding in rats stimulated production of PGE₂ and PGD₂ in the brain regions related to pressure–volume regulation

(9), and that these PGs have the ability to facilitate AVP release through central actions (2, 7, 10–12), seem to suggest participation of endogenous PGs, generated in the periventricular region, in hypovolemia-induced AVP secretion. However, the cerebral sites where PGs may be responsible for this postulated role have not yet been located.

The circumventricular tissue includes the area generally called the anteroventral third ventricular region (AV3V), comprising structures such as the organum vasculosum laminae terminalis, periventricular preoptic nucleus, and the median and medial preoptic nuclei (13, 14). Neurons in the AV3V receive afferent input from the brainstem regions such as the A1 cell group and the parabrachial nucleus which are

implicated in transmission of peripheral pressure–volume information (14–17). The AV3V neurons send efferent fibers to the AVP neurosecretory cells in the hypothalamic paraventricular and supraoptic nuclei, and play pivotal roles in regulating AVP release and cardiovascular function (14, 18–22). In addition to these neuroanatomical features, it is known that the AV3V is equipped with immunoreactive material like cyclooxygenase (23), a PG synthetase, and a high density of PGE₂-binding sites (24). Furthermore, it has been suggested that augmentation of PGE₂ production in the AV3V, which might be caused by bleeding (9), may lead to acceleration of AVP release (22). Therefore, it is conceivable that PGs synthesized in the AV3V may be involved in the hypovolemia-evoked AVP secretion. To our knowledge, however, experimental data supporting this view directly have not as yet been provided. Rather, it has been reported that electrical destruction of the AV3V failed to affect the plasma AVP response to bleeding in rats, suggesting a negligible role in the hypovolemia-induced AVP release of substances produced in this region (25).

The purpose of the present study was to investigate the roles of PGs in the AV3V in hypovolemia-induced AVP secretion. The effects of local infusion of PGE₂ or meclofenamate into the AV3V of rats on plasma AVP, ANG II, osmolality, sodium, potassium, chloride, heart rate and arterial pressure in the presence or absence of hemorrhage were examined. Among prostaglandins related to AVP release (2, 7, 10–12) we chose PGE₂ as the stimulator to be tested, because high concentrations of PGE₂ receptors exist in the AV3V as mentioned above (24) and activation of local PGE₂ formation in this region has been shown to be induced by a stimulus (i.v. injection of interleukin-1 β) (26). The influence of meclofenamate infusion into the nucleus accumbens (ACC) or the lateral cerebral ventricle was also tested, because it was suspected that the drug administered into the AV3V might bring about effects through its actions on other regions, owing to diffusion into the surrounding tissue or into the ventricular system.

Materials and methods

Animals and surgery

Wistar male rats weighing 280–360 g were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and placed in a stereotaxic apparatus (Type SR-6; Narishige, Tokyo, Japan). After a scalp incision was made, the lambda and bregma were located and the skull was leveled. A hole was drilled in the skull and stainless-steel guide cannulae (0.5 mm outer diameter; Eicom, Kyoto, Japan) were lowered just above the AV3V or the ACC, or into a lateral cerebral ventricle (i.c.v.). Coordinates of the tips were 0.3 mm posterior to the bregma, on the midline and 6.6 mm below the surface of the skull for AV3V cannulae, 0.7 mm anterior to the bregma,

1.0 mm right from the midline and 5.6 mm below the surface of the skull for ACC cannulae, and 0.8 mm posterior to the bregma, 1.5 mm right from the midline and 3.7 mm below the surface of the skull for i.c.v. cannulae (27). The implanted cannulae were fixed on the skull with small screws and dental resin. After surgery, rats were injected i.m. with Penicillin G (30 000 U). The rats were kept in individual cages and food and water were available *ad libitum*.

Seven or eight days after surgery, the rats were catheterized with PE-50 tubing in the left femoral artery and right jugular vein under ether anesthesia. The tubes were filled with heparinized saline (240 U/ml), sealed, exteriorized, and secured at the back of the neck. The use of the animals in this study conformed to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Experiments

Experiments were performed 1 or 2 days after the vascular catheterization mentioned above. The rats were transferred into individual plastic boxes (8 $\times$ 8 $\times$ 25 cm) which permitted them freedom of movement. After approximately 60 min, the arterial line was connected to an MPU-0.5-290-III pressure transducer (Nihon Kohden, Tokyo, Japan) connected to a PMP-3002H Medicalcorder (Nihon Kohden). Heart rates were monitored by counting the frequencies of voltage changes produced by systolic and diastolic pressure with a pulse counter. A period of 30–60 min was allowed for the arterial pressure and heart rate to stabilize. The rats were then given one of the following treatments: (i) AV3V infusion of 1 μ l isotonic saline (0.15 mol/l) containing 0 or 78.3 nmol (25 μ g) meclofenamate sodium (Biomol Research, Plymouth Meeting, PA, USA) + non-hemorrhage; (ii) AV3V infusion of 1 μ l isotonic saline containing 0 or 78.3 nmol meclofenamate + hemorrhage; (iii) ACC infusion of 1 μ l isotonic saline containing 0 or 78.3 nmol meclofenamate + non-hemorrhage; (iv) ACC infusion of 1 μ l isotonic saline containing 0 or 78.3 nmol meclofenamate + hemorrhage; (v) i.c.v. infusion of 1 μ l isotonic saline containing 0 or 78.3 nmol meclofenamate + non-hemorrhage; (vi) i.c.v. infusion of 1 μ l isotonic saline containing 0 or 78.3 nmol meclofenamate + hemorrhage; (vii) AV3V infusion of 0.5 μ l phosphate buffered-saline solution (pH 7.2, 290 mosmol/kg H₂O) containing 0 or 7.1 nmol (2.5 μ g) PGE₂ (Ono Pharmaceutical Co., Osaka, Japan).

In treatment groups (i) to (vi), a blood sample (3.0 ml) was taken 30 min after the intracranial infusion of meclofenamate or its vehicle to measure pre-hemorrhage levels (C) of plasma variables. The sampling was conducted over a 30-s period from the femoral arterial line while returning the same volume of warm (37 $^{\circ}$ C) donor blood via the jugular venous line. After 5 min, in the groups undergoing hemorrhage (treatment groups

(ii), (iv) and (vi)), a volume of femoral arterial blood (1% of the body weight, approximately 14% of the blood volume (28)) was withdrawn over a 30-s period and discarded. Two further hemorrhages of the same volume were conducted at 10-min intervals, and the blood was retained as the second and third samples (H1 and H2). In the non-hemorrhage groups (treatments (i), (iii) and (v)), the withdrawal of femoral arterial blood was made while returning the same volume of warm donor blood.

In treatment group (vii), blood samples were taken 5 min before and 5 and 15 min after the AV3V infusion of PGE₂ or its vehicle. A 3 ml blood sample was taken over a 30-s period while returning 3 ml donor blood, as mentioned above.

The intracranial infusion of meclofenamate or PGE₂ was performed using 30-gauge stainless-steel tubing that protruded 1.0 mm beyond the end of the guide cannula and was connected to a microsyringe via polyethylene tubing. The infusion rate was 0.5 μ l/min with a Harvard pump (model 11, South Natick, MA, USA). Approximately 1 min elapsed before withdrawing the infusion tubing, so as to permit drug movement away from its tip. The doses of meclofenamate and PGE₂ were determined in view of previous studies (2, 22). The donor blood was prepared on the day of experiments by collecting into a heparinized beaker (150 U/rat) the trunk blood issuing after decapitation of normal rats anesthetized lightly with diethyl ether. The blood samples were divided into two fractions immediately after collection. A 2-ml aliquot was mixed with 100 μ l of a solution containing 125 mmol/l disodium ethylenediaminetetraacetic acid and 25 mmol/l *o*-phenanthroline for subsequent measurements of plasma AVP and ANG II. The remaining blood was used to measure plasma osmolality and electrolytes. The separation of plasma was performed by centrifugation at 2500 g at 2 °C for 15 min. The plasma samples were stored at -25 °C until use.

Histological analyses

At the end of each experiment, the rats were killed by an i.v. injection of a lethal dose of pentobarbital sodium. Trypan blue solution (2%) was administered into the AV3V, ACC or the i.c.v., using the same infusion tubing as was used for the experiments. The brain was removed. Validity of the i.c.v. infusion conducted during the experiments was confirmed by cutting the brain manually and inspecting the spread of the dye in the ventricular system. When infusion sites in the AV3V and ACC were to be examined, the brains were kept in 10% formalin solution for at least two weeks. Afterwards, serial coronal sections of approximately 40 μ m in thickness were cut with a freezing microtome. The sections were mounted on glass slides and stained with 0.1% cresyl violet. The infusion sites were judged on the tissue injury made by the tubing, using the atlas of Paxinos and Watson (27) as a reference.

Measurements and statistical analyses

Plasma AVP and ANG II were extracted with acetone and petroleum ether, and determined by radioimmunoassay. Synthetic [Arg⁸]vasopressin and [Ile⁵]ANG II (Protein Research Foundation, Osaka, Japan) were used as reference standards. Tracer-labeled ¹²⁵I-AVP and ¹²⁵I-ANG II were obtained, respectively, from Mitsubishi Kagaku (Tokyo, Japan) and New England Nuclear (Boston, MA, USA). The assay procedures have been described in detail elsewhere (3, 29). Briefly, the cross-reactions of the AVP antiserum with AVP-related peptides such as lysine vasopressin, arginine vasotocin, deamino-D-AVP and oxytocin were, respectively, 3.2 $\times 10^{-1}$ %, 8.9 $\times 10^{-3}$ %, 2.6 $\times 10^{-2}$ % and less than 5.9 $\times 10^{-6}$ %. The cross-reactions of the ANG II antiserum with ANG II-related substances such as [Sar¹, Ala⁸]ANG II, des-Asp¹-[Ile⁸]ANG II, des-Asp¹-[Ile⁵]ANG II and des-Asp¹, Arg²-[Ile⁵]ANG II were, respectively, less than 2 $\times 10^{-2}$ %, less than 2 $\times 10^{-2}$ %, 100% and 75.9%. The recoveries (means $\pm$ S.D.) of synthetic [Arg⁸]vasopressin added to a plasma pool were 57.2 $\pm$ 7.4% (n = 10) at doses of 1.8–3.6 pmol/l and 54.3 $\pm$ 6.4% at doses of 9.1–36.4 pmol/l (n = 30). The recoveries of [Ile⁵]ANG II were 95.5 $\pm$ 7.4% (n = 22) at doses of 48.1–240.4 pmol/l. The values given for plasma AVP and ANG II were corrected for losses during the extraction with mean recoveries of 55.8 and 95.5% respectively. The within- and between-assay coefficients of variation for the AVP assay were 10.1 and 13.9% respectively, and those for the ANG II assay were 6.7 and 9.3% respectively. The minimum detection limit of the assay was 0.46 pmol/liter plasma for AVP and 7.7 pmol/liter plasma for ANG II.

Plasma osmolality was estimated by freezing-point depression (Dai-ichi Kagaku OM-6040, Kyoto, Japan). Plasma sodium and potassium were determined by flame photometry (Hitachi 775-A, Tokyo, Japan), and plasma chloride by electrometric titration (Hiranuma CL-7, Tokyo, Japan). Systolic arterial pressure and heart rate were measured every 30 s, and averaged for 9- or 5-min periods preceding blood sampling.

Data were statistically analyzed by one- and two-way analyses of variance for repeated measures. A multiple-range test of Duncan was used subsequently, when appropriate, to determine significant differences within and between groups.

Results

Effects of application of meclofenamate or PGE₂ to the AV3V

Figure 1 represents infusion sites in the AV3V of meclofenamate (closed circles) and the vehicle (open circles) in rats with hemorrhage. It was confirmed histologically that the regions where meclofenamate was liberated included the organum vasculosum of the

Figure 1 Infusion sites in the anteroventral third ventricular region of meclofenamate (●, $n = 11$) and its vehicle (○, $n = 11$) in the hemorrhaged rats shown in Fig. 2, and of PGE_2 (Δ, $n = 8$) in the rats depicted in Fig. 3. Panels A, B and C represent coronal brain sections obtained at distances from the bregma of +0.2, -0.26 and -0.3 mm respectively. ac, anterior commissure; AMPO, anterior medial preoptic nucleus; AVPO, anteroventral preoptic nucleus; HDB, nucleus of the horizontal limb of the diagonal band; LPO, lateral preoptic area; MnPO, median preoptic nucleus; MPA, medial preoptic area; OX, optic chiasm; Pe, periventricular hypothalamic nucleus; Shy, septohypothalamic nucleus; VDB, nucleus of the vertical limb of the diagonal band.

lamina terminalis (OVLt) and the surrounding area, median preoptic nucleus, anterior medial preoptic nucleus, periventricular hypothalamic nucleus and the medial preoptic area.

Concentrations of plasma AVP in the pre-hemorrhage control samples (C) (Fig. 2) obtained 30 min after the AV3V infusion of meclofenamate or its vehicle did not significantly differ between any two of the four groups. Although small but significant differences were detected in the levels of arterial pressure and heart rate among the groups, meclofenamate did not exert a definite effect on these variables (Fig. 2).

When the first hemorrhage was carried out in the vehicle group, plasma AVP increased significantly after 10 min (H1 samples in Fig. 2). In the meclofenamate group, however, the AVP-enhancing effect of this hemorrhage was not observed. Arterial pressure and heart rate were not changed by the first hemorrhage either in the vehicle group or in the meclofenamate

Figure 2 Effects of meclofenamate (MCL) infusion into the AV3V on hemorrhage-induced responses of plasma AVP (PAVP) and cardiovascular parameters. MCL (78.3 nmol) or its vehicle (VEH) was infused, 30 min before obtaining the first blood sample (C), at a rate of 0.5 $\mu\text{l}/\text{min}$ for 2 min. In the non-hemorrhage groups (non-HR), all of the blood samples, C (3 ml), H1 and H2 (1% of body weight), were collected while returning the same volume of donor blood to the animal. In the hemorrhage groups (HR), blood samples H1 and H2 were taken without returning donor blood, although sample C was obtained as in the non-HR groups. Systolic arterial pressure (AP, mm Hg) and heart rate (HR, beats per min) were measured every 30 s, and averaged for 9-min periods preceding each blood sampling. Values are means $\pm$ S.E.M. Numbers of rats in each group are shown in parentheses. ^a $P < 0.05$, ^b $P < 0.01$ vs C; ^c $P < 0.01$ vs H1; ^d $P < 0.05$, ^e $P < 0.01$ vs VEH AV3V + non-HR; ^f $P < 0.05$, ^g $P < 0.01$ vs MCL AV3V + non-HR; ^h $P < 0.05$, ⁱ $P < 0.01$ vs VEH AV3V + HR.

group, and values of these variables were similar in the two groups. The second hemorrhage evoked by taking the H1 samples caused a significant drop in arterial pressure and, remarkably, augmented plasma AVP in the H2 samples obtained after 10 min (Fig. 2). The depressor response was significantly greater in the meclofenamate group than in the vehicle group. Although abrupt reduction of arterial pressure immediately following the hemorrhage was similar in the two groups, its spontaneous recuperation towards pre-hemorrhage levels was slower in the meclofenamate group than in the vehicle group. In contrast, however, the extent of the AVP rise was significantly smaller in the meclofenamate group than in the vehicle group. Heart rate was decreased in the meclofenamate group, but not in the vehicle group.

Levels of plasma ANG II, osmolality and electrolytes in these rats are summarized in Table 1. Plasma ANG II showed a tendency to increase after the first hemorrhage, and increased further after the second hemorrhage. The ANG II levels detected after the second hemorrhage were not significantly different in the groups receiving meclofenamate or its vehicle. The hemorrhage elevated plasma chloride, lowered plasma potassium, and did not change plasma osmolality and sodium. The levels of these variables measured in corresponding samples of the vehicle group and meclofenamate group were not statistically different at any time point (Table 1).

In the non-hemorrhage rats in which reduction in blood volume owing to sampling was prevented by returning donor blood, plasma AVP and ANG II, osmolality, electrolytes, heart rate and arterial pressure did not alter significantly from initial values throughout the experiments, regardless of the presence or absence of the AV3V infusion of meclofenamate (Fig. 2, Table 1).

Local infusion of PGE₂ into the AV3V resulted in remarkable augmentation of plasma AVP, heart rate and arterial pressure (Fig. 3), without affecting plasma

osmolality, sodium, potassium and chloride (data not shown). The AV3V infusion of vehicle influenced none of these variables significantly. Histological examinations indicated that the infusion sites of PGE₂ (open triangles in Fig. 1) were located in the OVLT and its neighborhood, median preoptic nucleus and the periventricular hypothalamic nucleus, and that some of these sites were close to the infusion points of meclofenamate in the hemorrhage rats (closed circles in Fig. 1).

Effects of meclofenamate application into the nucleus accumbens or the lateral cerebral ventricle

Levels of plasma AVP, osmolality, sodium, chloride and potassium detected in the pre-hemorrhage control samples, as well as those of arterial pressure and heart rate, did not differ significantly among the four groups undergoing infusion of meclofenamate or its vehicle into the ACC (Fig. 4). This was also the case when these solutions were infused into the i.c.v. (Fig. 5). The first

Table 1 Plasma angiotensin II, osmolality and electrolytes in rats depicted in Fig. 2. Values are means $\pm$ S.E.M.

Group (n)	Sample		
	C	H1	H2
Plasma angiotensin (pmol/l)			
VEH AV3V + non-HR (6)	40 $\pm$ 8	40 $\pm$ 7	23 $\pm$ 3
VEH AV3V + HR (11)	49 $\pm$ 11	77 $\pm$ 19	162 $\pm$ 32 ^{b,c,d}
MCL AV3V + non-HR (6)	34 $\pm$ 3	36 $\pm$ 5	31 $\pm$ 8
MCL AV3V + HR (11)	51 $\pm$ 8	63 $\pm$ 7 ^e	135 $\pm$ 14 ^{b,c,f}
Plasma osmolality (mosmol/kg)			
VEH AV3V + non-HR (6)	293 $\pm$ 1	293 $\pm$ 1	291 $\pm$ 1
VEH AV3V + HR (11)	293 $\pm$ 1	292 $\pm$ 1	293 $\pm$ 1
MCL AV3V + non-HR (6)	292 $\pm$ 1	291 $\pm$ 1	291 $\pm$ 1
MCL AV3V + HR (11)	291 $\pm$ 1	290 $\pm$ 1	292 $\pm$ 1
Plasma sodium (mmol/l)			
VEH AV3V + non-HR (6)	143.5 $\pm$ 0.6	143.7 $\pm$ 0.6	143.5 $\pm$ 0.4
VEH AV3V + HR (11)	143.3 $\pm$ 0.7	145.0 $\pm$ 0.8	144.4 $\pm$ 0.7
MCL AV3V + non-HR (6)	142.2 $\pm$ 0.5	142.8 $\pm$ 0.3	142.8 $\pm$ 0.4
MCL AV3V + HR (11)	143.1 $\pm$ 1.0	145.1 $\pm$ 1.0	144.0 $\pm$ 0.8
Plasma chloride (mmol/l)			
VEH AV3V + non-HR (6)	105.2 $\pm$ 0.9	106.2 $\pm$ 0.7	106.0 $\pm$ 0.5
VEH AV3V + HR (11)	105.7 $\pm$ 0.6	111.7 $\pm$ 0.9 ^{b,d}	112.4 $\pm$ 0.9 ^{b,d}
MCL AV3V + non-HR (6)	104.0 $\pm$ 0.7	105.2 $\pm$ 0.8	105.5 $\pm$ 1.0
MCL AV3V + HR (11)	104.6 $\pm$ 1.1	110.5 $\pm$ 0.9 ^{b,f}	110.4 $\pm$ 1.1 ^{b,f}
Plasma potassium (mmol/l)			
VEH AV3V + non-HR (6)	6.2 $\pm$ 0.6	5.9 $\pm$ 0.5	6.0 $\pm$ 0.7
VEH AV3V + HR (11)	6.4 $\pm$ 0.5	5.1 $\pm$ 0.3 ^a	4.9 $\pm$ 0.3 ^b
MCL AV3V + non-HR (6)	6.3 $\pm$ 0.4	6.1 $\pm$ 0.3	6.0 $\pm$ 0.3
MCL AV3V + HR (11)	5.9 $\pm$ 0.3	4.9 $\pm$ 0.2 ^{a,e}	4.7 $\pm$ 0.2 ^{a,e}

Abbreviations are the same as in Fig. 2.

^a $P < 0.05$, ^b $P < 0.01$ vs C; ^c $P < 0.01$ vs H1; ^d $P < 0.01$ vs VEH AV3V + non-HR; ^e $P < 0.05$, ^f $P < 0.01$ vs MCL AV3V + non-HR.

Figure 3 Effects of PGE_2 infusion into the AV3V on plasma AVP (PAPV) and cardiovascular parameters. At 0 min, PGE_2 (7.1 nmol) or its vehicle was infused into the AV3V at a rate of $0.5 \mu\text{l}/\text{min}$ for 1 min. Systolic arterial pressure (AP, mm Hg) and heart rate (HR, beats per min) were measured every 30 s, and averaged for 5-min periods preceding blood sampling at -5, 5 and 15 min for determination of plasma AVP and other variables. Values are means $\pm$ S.E.M. (○), vehicle group ($n=6$); (●), PGE_2 group ($n=8$). ^a $P < 0.01$ vs -5 min; ^b $P < 0.01$ vs vehicle group.

hemorrhage slightly raised plasma AVP in the H1 samples collected 10 min later, without altering arterial pressure or heart rate significantly (Figs 4 and 5). The second hemorrhage applied by taking the H1 samples brought about remarkable rises in plasma AVP accompanied by reduced arterial pressure and unaltered heart rate (H2 in Figs 4 and 5). The AVP responses to these hemorrhages were not significantly changed by meclofenamate infusion into the ACC or i.c.v. Similarly, neither the responses of arterial pressure nor those of heart rate were influenced by the infusion of meclofenamate. This inability of meclofenamate administration into the ACC or i.c.v. to modify the responses of plasma AVP and the cardiovascular parameters was different to the results found with infusion of the drug into the AV3V (Fig. 2).

Plasma osmolality, sodium and potassium were not changed significantly by the hemorrhage performed after the ACC or i.c.v. infusion of meclofenamate or its vehicle. Plasma chloride was slightly elevated following the hemorrhage. Again, in this series of experiments the levels of plasma osmolality and electrolytes in the meclofenamate groups with or without hemorrhage were not significantly different

Figure 4 Effects of meclofenamate (MCL) infusion into the ACC on hemorrhage-induced responses of plasma AVP (PAPV) and cardiovascular parameters. MCL (78.3 nmol) or its vehicle (VEH) was infused, 30 min before obtaining the first blood sample (C), at a rate of $0.5 \mu\text{l}/\text{min}$ for 2 min. In the non-hemorrhage groups (non-HR), all of the blood samples, C (3 ml), H1 and H2 (1% of body weight), were collected while returning the same volume of donor blood to the animal. In the hemorrhage groups (HR), blood samples H1 and H2 were taken without returning donor blood, although sample C was obtained as in the non-HR groups. Systolic arterial pressure (AP, mmHg) and heart rate (HR, beats per min) were measured every 30 s, and averaged for 9-min periods preceding blood sampling. Values are means $\pm$ S.E.M. Numbers of rats in each group are shown in parentheses. ^a $P < 0.05$, ^b $P < 0.01$ vs C; ^c $P < 0.01$ vs H1; ^d $P < 0.01$ vs VEH ACC+non-HR; ^e $P < 0.01$ vs MCL ACC + non-HR.

from those of the vehicle groups with or without hemorrhage (data not presented).

Histological inspection showed that the cannulae for ACC administration of meclofenamate in hemorrhage rats had been placed in the core portions of this nucleus or the surrounding shell portions; the distances from the bregma of these areas ranged from 1.0 mm to 0.48 mm, according to the atlas of Paxinos and Watson (27).

Plasma AVP, arterial pressure, heart rate, osmolality and electrolytes in non-hemorrhage rats were not influenced by ACC or i.c.v. application of meclofenamate (Figs 4 and 5). Although heart rates at H1 and H2 in rats receiving ACC infusion of meclofenamate were lower than those of rats infused with vehicle, these values were not statistically different from their own initial values at C.

Discussion

The present study was conducted in conscious rats fully recovered from surgical injuries, because factors such as

Figure 5 Effects of meclofenamate (MCL) infusion into the i.c.v. on hemorrhage-induced responses of plasma AVP (PAVP) and cardiovascular parameters. MCL (78.3 nmol) or its vehicle (VEH) was infused, 30 min before obtaining the first blood sample (C), at a rate of 0.5 μ l/min for 2 min. In the non-hemorrhage groups (non-HR), all of the blood samples, C (3 ml), H1 and H2 (1% of body weight), were collected while returning the same volume of donor blood to the animal. In the hemorrhage groups (HR), blood samples H1 and H2 were taken without returning donor blood, although sample C was obtained as in the non-HR groups. Systolic arterial pressure (AP, mmHg) and heart rate (HR, beats per min) were measured every 30 s, and averaged for 9-min periods preceding blood sampling. Values are means $\pm$ S.E.M. Numbers of rats in each group are shown in parentheses. ^a $P < 0.05$, ^b $P < 0.01$ vs C; ^c $P < 0.01$ vs H1; ^d $P < 0.01$ vs VEH i.c.v. + non-HR; ^e $P < 0.05$, ^f $P < 0.01$ vs MCL i.c.v. + non-HR; ^g $P < 0.05$ vs VEH i.c.v. + HR.

operative stress, pain and the use of anesthetics have generally been shown to affect mechanisms controlling AVP release and the clearance of this hormone from plasma. The vascular catheterization was performed not on the day when cerebral cannulae were implanted, but one or two days before the experiments. The reason was to ensure that measurement of the cardiovascular parameters was successful, because occurrence of occlusion of inserted tubing (its twisting or folding, or clotting of blood) which was possibly caused by brisk movement of rats, increased with days after the vascular catheterization.

It is known that PGE₂ production in the brain tissue increases promptly following hemorrhage (9), and that a cyclooxygenase inhibitor, meclofenamate, abolishes the PGE₂-enhancing effect of experimental manipulation (middle cerebral artery occlusion) when administered 30 min previously (1). In the present study where meclofenamate was infused into the AV3V 35 min prior to the first bleeding, we found that the drug was

effective in preventing the plasma AVP responses to the hemorrhage with or without hypotension. This result is consistent with that obtained by Brooks *et al.* (2) who employed i.c.v. injections of meclofenamate, and reveals that a relatively smaller dose of the drug can exhibit similar inhibitory potency if administered into the AV3V. Reduction of arterial pressure by the hemorrhage tended to be greater in the meclofenamate group than in the vehicle group (Fig. 2), implying that the attenuation of the AVP response may not be explained by the difference in the depressor response capable of stimulating AVP release. In view of the remarkable augmentation of brain PGs by bleeding (9) and their central actions in promoting AVP release (2, 7, 10–12, 22), it is probable that endogenous PGs generated in the brain tissue may be involved in AVP secretion in response to hypotensive and nonhypotensive hemorrhages.

Meclofenamate was effective when applied into the AV3V structures such as the OVLT and median preoptic nucleus, but not when administered into the ACC, the area slightly distant from the AV3V, or the i.c.v. Similarly, when infused into the AV3V sites near the liberation points of meclofenamate, PGE₂ significantly enhanced plasma AVP (Fig. 3), despite the lack of effect of this prostanoid when given at a double dose into the extra-AV3V tissue or i.c.v. (22). Accordingly, it seems reasonable to postulate AV3V or its neighborhood as one site of action of endogenous PGs in contributing to hemorrhage-induced AVP secretion. This idea is also supported by the facts that the AV3V contains cyclooxygenase immunoreactivity and a high density of PGE₂ binding sites (23, 24), and that synthesis of PGE₂ and PGD₂ in the rat hypothalamus increased in response to bleeding comparable to that conducted in this study (9). On the other hand, electrical destruction of the rat AV3V failed to affect the plasma AVP response to hemorrhage (25), questioning the role of AV3V-derived substances in hypovolemia-induced AVP release. It is difficult to explain the causes underlying the discrepancy between their results (25) and ours. In their study, however, the AV3V was destroyed three weeks before the experiments, and it is known that following the destruction various changes occur in the regulatory mechanisms of AVP secretion and related factors such as drinking behavior, electrolytes and blood pressure (13, 14). Accordingly, these changes might have prevented expression of a definite effect of the treatment. In agreement with our data, previous reports investigating regulation of drinking behavior and blood pressure have also suggested the existence of neural mechanisms in the AV3V engaged in responses to hypovolemia and hypotension (30, 31).

The AV3V may play a role in regulating ANG II formation in the systemic blood, as suggested by elevation of plasma renin activity following electrical ablation of the tissue (28). Because plasma ANG II may participate in hypovolemia-evoked AVP secretion (4), it was suspected that the AV3V infusion of meclofenamate

might have blunted the hemorrhage-induced AVP response through inhibition of ANG II production. However, it was observed that bleeding increased plasma ANG II in the meclofenamate group as in the vehicle group, making the suspicion unlikely.

The AV3V is richly innervated by catecholaminergic fibers that extend from the brainstem regions such as the A1 cell group and may play a role in relaying information from arterial baroreceptors and cardiopulmonary receptors to control cardiovascular functions and AVP secretion (14–17, 32). Release of dopamine and noradrenaline in the AV3V was enhanced by reduction of extracellular fluid volume, suggesting excitation of the brainstem catecholaminergic neuron (33). On the other hand, AV3V neurons project to the AVP cells in the hypothalamic supraoptic and paraventricular nuclei (14, 20, 21), and dopamine or an α -adrenergic agonist phenylephrine administered into the AV3V is able to activate those cells (34). Consequently, it is conceivable that peripheral pressure–volume information generated by hemorrhage may be conveyed from the brainstem areas to the hypothalamic nuclei via the AV3V to stimulate the AVP cells. The plasma AVP response elicited by AV3V infusion of PGE₂ suggests that this substance may function to activate the neural pathways extending from the AV3V to the AVP cells. Therefore, if endogenous PGs are responsible for this role in the hemorrhage state, excitation of the AVP neurons owing to the hypovolemic stimulus would be attenuated by inhibiting synthesis of PGs in the AV3V. This might be one mechanism underlying the inhibitory effect of AV3V meclofenamate on the hemorrhage-evoked AVP response.

As to the possible functions of PGs in activating neural pathways between the AV3V and the AVP cells, their roles as neurotransmitters could be assumed, because PGs and specific binding sites for PGE₂ have been detected in the nerve endings and synaptic membranes of the rat brain (35, 36). In the AV3V, however, cyclooxygenase-like material was found not in the pathways of nerve fibers or terminal fields, but in cell bodies and dendrites (23). Furthermore, PGs may be produced not only in neurons but also in glial cells and cerebral microvessels (37, 38). Therefore, PGs may be used for modulating or fundamentally maintaining neural activity, rather than as synaptic transmitters. In AVP secretion and other phenomena, PGs are known to interact with AVP-releasing factors such as catecholamines (39, 40) and ANG II (7, 8). Consequently, it could be speculated that PGs might exert their effects by modulating the activities of catecholaminergic and angiotensinergic neurons in the AV3V.

The AV3V infusion of PGE₂ increased not only plasma AVP but also arterial pressure and heart rate. The present dose of PGE₂ was one-half of our earlier dose which did not show an appreciable effect when administered into cerebral sites other than the AV3V (22). The pressor and tachycardiac effects of PGE₂ are in

agreement with previous observations (22, 41), and may reflect its local actions on the AV3V neural mechanisms controlling cardiovascular functions. Because the pressor and tachycardiac responses following the i.c.v. administration of PGE₂ were diminished, respectively, by α - and β -adrenergic blockers (42, 43), it seems likely that PGE₂ administered into the AV3V may have produced the cardiovascular actions by stimulating the sympathetic nervous system. Hemorrhage activates the sympathetic nervous system (44), and AV3V infusion of meclofenamate intensified the depressor and bradycardiac responses to the hemorrhage (Fig. 2). Accordingly, it is possible that endogenous PGs in the AV3V may contribute to cardiovascular regulation in the hypovolemic state through such an action mode. One mechanism for the potentiated depressor response in the meclofenamate group as compared with that in the vehicle group could be the relatively larger reduction of the circulating blood volume arising from inhibition of the AV3V-mediated sympathetic vasoconstriction (45). Moreover, it could also be postulated that meclofenamate may have provoked the effects by inactivating the paraventricular and supraoptic AVP neurons that project to the extra-pituitary areas and are responsible for sympathetic activation (46), as well as those that secrete AVP into the systemic circulation. On the other hand, negligible effects of AV3V meclofenamate on the levels of plasma AVP, heart rate and arterial pressure in the non-hemorrhage rats suggest that in the normovolemic state endogenous PGs in the AV3V may not exert a tonic influence on AVP release and the cardiovascular parameters.

In conclusion, the results of this study suggest that PGs produced in and/or near the AV3V may contribute to AVP release and cardiovascular function in the hypovolemic state. This view is primarily dependent on the effects of AV3V meclofenamate in the hemorrhaged rats. The potency of this drug to inhibit PG synthesis has been well established (1) and its effects on the hypovolemia-induced AVP response were similar to those of indomethacin when applied into the i.c.v. (2). However, examining AV3V effects of other PG inhibitors with different chemical properties might be required to strengthen the validity of this hypothesis. In addition, the mechanism of action of PGs can only be speculated upon at present. Further studies to pursue these problems are necessary if we are to understand clearly the roles of PGs in the AV3V.

Acknowledgements

We wish to express our sincere appreciation to Mr Mamoru Koike (Kido Hospital) for skilful measurements of plasma osmolality and electrolytes and to Dr Yukio Hirose (Mitsubishi-Kagaku Corporation, Tokyo) for the generous supply of iodinated AVP. We are also indebted to Mr Toshiomi Minamide and Mr Mikio Nishii (Ono

Pharmaceutical Corporation, Osaka) for the gift of PGE₂ and for technical advice. This work was supported by grants-in-aid for Scientific Research (C) (06670076 and 08670076) from the Ministry of Education, Science, Sports and Culture of Japan to K Y.

References

- Bucci MN, Black KL & Hoff JT. Arachidonic acid metabolite production following focal cerebral ischemia: time course and effect of meclofenamate. *Surgical Neurology* 1990 **33** 12–14.
- Brooks DP, Share L & Crofton JT. Role of brain prostaglandins in the control of vasopressin secretion in the conscious rat. *Endocrinology* 1986 **118** 1716–1722.
- Yamaguchi K. Effects of water deprivation on immunoreactive angiotensin II levels in plasma, cerebroventricular perfusate and hypothalamus of the rat. *Acta Endocrinologica* 1981 **97** 137–144.
- Yamaguchi K, Sakaguchi T & Kamoi K. Central role of angiotensin in the hyperosmolality- and hypovolaemia-induced vasopressin release in conscious rats. *Acta Endocrinologica* 1982 **101** 524–530.
- Reid IA. Actions of angiotensin II on the brain: mechanisms and physiologic role. *American Journal of Physiology* 1984 **246** F533–F543.
- Yamamoto M, Share L & Shade RE. Effect of ventriculo-cisternal perfusion with angiotensin II and indomethacin on the plasma vasopressin concentration. *Neuroendocrinology* 1978 **25** 166–173.
- Inoue M, Crofton JT & Share L. Interactions between the brain renin-angiotensin system and brain prostanoids in the control of vasopressin secretion. *Experimental Brain Research* 1990 **83** 131–136.
- Yamaguchi K & Hama H. Evaluation for roles of brain prostaglandins in the catecholamine-induced vasopressin secretion in conscious rats. *Brain Research* 1993 **607** 149–153.
- Cernak I, Savic J, Jovanovic M & Selakovic V. Brain PGD₂ and PGE₂ changes during posthaemorrhagic hypovolemia in rats. *Vojnosanitetski Pregled* 1994 **51** 363–375.
- Yamamoto M, Share L & Shade RE. Vasopressin release during ventriculo-cisternal perfusion with prostaglandin E₂ in the dog. *Journal of Endocrinology* 1976 **71** 325–331.
- Ishikawa S, Saito T & Yoshida S. The effect of prostaglandins on the release of arginine vasopressin from the guinea pig hypothalamo-neurohypophyseal complex in organ culture. *Endocrinology* 1981 **108** 193–198.
- Yamaguchi K & Hama H. Separation of periventricular dopaminergic and alpha-adrenergic systems from the vasopressin-secreting mechanisms activated by prostaglandin D₂. *Brain Research* 1991 **559** 261–266.
- Brody MJ & Johnson AK. Role of the anteroventral third ventricle region in fluid and electrolyte balance, arterial pressure regulation, and hypertension. In *Frontiers in Neuroendocrinology*, vol 6, pp 249–292. Eds WF Ganong & L Martini. New York: Raven Press, 1980.
- Johnson AK. The periventricular anteroventral third ventricle (AV3V): its relationship with the subfornical organ and neural systems involved in maintaining body fluid homeostasis. *Brain Research Bulletin* 1985 **15** 595–601.
- Sawchenko PE & Swanson LW. The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Research Reviews* 1982 **4** 275–325.
- Saper CB, Reis DJ & Joh T. Medullary catecholamine inputs to the anteroventrolateral third ventricular cardiovascular regulatory region in the rat. *Neuroscience Letters* 1983 **42** 285–291.
- Sawchenko PE & Swanson LW. The organization and biochemical specificity of afferent projections to the paraventricular and supraoptic nuclei. *Progress in Brain Research* 1983 **60** 19–29.
- Fink GD, Buggy J, Haywood JR, Johnson AK & Brody MJ. Hemodynamic effects of electrical stimulation of forebrain angiotensin and osmosensitive sites. *American Journal of Physiology* 1978 **235** H445–H451.
- Johnson AK, Hoffman WE & Buggy J. Attenuated pressor responses to intracranially injected stimuli and altered antidiuretic activity following preoptic-hypothalamic periventricular ablation. *Brain Research* 1978 **157** 161–166.
- Honda K, Negoro H, Dyball REJ, Higuchi T & Takano S. The osmoreceptor complex in the rat: evidence for interactions between the supraoptic and other diencephalic nuclei. *Journal of Physiology* 1990 **431** 225–241.
- McKinley MJ, McAllen RM, Mendelsohn FAO, Allen AM, Chai SY & Oldfield BJ. Circumventricular organs: neuroendocrine interfaces between the brain and the hemal milieu. *Frontiers in Neuroendocrinology* 1990 **11** 91–127.
- Yamaguchi K, Hama H & Watanabe K. Possible roles of prostaglandins in the anteroventral third ventricular region in the hyperosmolality evoked vasopressin secretion of conscious rats. *Experimental Brain Research* 1997 **113** 265–272.
- Saper CB & Breder CD. Endogenous pyrogens in the CNS: role in the febrile response. *Progress in Brain Research* 1992 **93** 419–429.
- Matsumura K, Watanabe Y, Onoe H & Hayaishi O. High density of prostaglandin E₂ binding sites in the anterior wall of the third ventricle: a possible site of hyperthermic action. *Brain Research* 1990 **533** 147–151.
- Feuerstein G, Johnson AK, Zerbe RL, Davis-Kramer R & Faden AI. Anteroventral hypothalamus and hemorrhagic shock: cardiovascular and neuroendocrine responses. *American Journal of Physiology* 1984 **246** R551–R557.
- Komaki G, Arimura A & Koves K. Effect of intravenous injection of IL-1 β on PGE₂ levels on several brain areas as determined by microdialysis. *American Journal of Physiology* 1992 **262** E246–E251.
- Paxinos G & Watson C. *The Rat Brain in Stereotaxic Coordinates*, edn 2. New York: Academic Press, 1986.
- Bealer SL & Schneider EG. Preoptic recess lesions, body fluid compartments, and the renin-aldosterone system. *American Journal of Physiology* 1983 **245** R901–R905.
- Yamaguchi K, Koike M & Hama H. Plasma vasopressin response to peripheral administration of angiotensin in conscious rats. *American Journal of Physiology* 1985 **248** R249–R256.
- McGowan MK, Brown B & Grossman SP. Lesions of the PMO or AV3V: influences on fluid intake. *Physiology and Behavior* 1988 **42** 331–342.
- Fan L, Yeston NS & McIntosh TK. Effect of dynorphin microinjection in the anterior hypothalamus (AV3V) region on the hemodynamic response to hemorrhage in the rat. *Circulatory Shock* 1993 **39** 147–152.
- Head GA, Quail AW & Woods RL. Lesions of A1 noradrenergic cells affect AVP release and heart rate during hemorrhage. *American Journal of Physiology* 1987 **253** H1012–H1017.
- Tanaka J, Hori K, Koito H & Nomura M. Enhanced monoamine release in the median preoptic area following reduced extracellular fluid volume in rats. *Neuroscience Letters* 1992 **147** 110–113.
- Yamaguchi K, Hama H & Watanabe K. Possible contribution of dopaminergic receptors in the anteroventral third ventricular region to hyperosmolality-induced vasopressin secretion in conscious rats. *European Journal of Endocrinology* 1996 **134** 243–250.
- Kataoka K, Ramwell PW & Jessup S. Prostaglandins: localization in subcellular particles of rat cerebral cortex. *Science* 1967 **157** 1187–1189.
- Malet C, Scherrer H, Saavedra JM & Dray F. Specific binding of [³H]prostaglandin E₂ to rat brain membranes and synaptosomes. *Brain Research* 1982 **236** 227–233.
- Goehrlert UG, Ng Ying Kin NMK & Wolfe LS. Biosynthesis of prostacyclin in rat cerebral microvessels and the choroid plexus. *Journal of Neurochemistry* 1981 **36** 1192–1201.

- 38 Hertting G & Seregi A. Formation and function of eicosanoids in the central nervous system. *Annals of the New York Academy of Sciences* 1989 **559** 84–89.
- 39 Roberts PJ & Hillier K. Facilitation of noradrenaline release from rat brain synaptosomes by prostaglandin E₂. *Brain Research* 1976 **112** 425–428.
- 40 Hedqvist P. Basic mechanisms of prostaglandin action on autonomic neurotransmission. *Annual Review of Pharmacology and Toxicology* 1977 **17** 259–279.
- 41 Morimoto K, Morimoto A, Nakamori T, Tan N, Minagawa T & Murakami N. Cardiovascular responses induced in free-moving rats by immune cytokines. *Journal of Physiology* 1992 **448** 307–320.
- 42 Hoffman WE & Schmid PG. Cardiovascular and antidiuretic effects of central prostaglandin E₂. *Journal of Physiology* 1979 **288** 159–169.
- 43 Otorii T, Ohkubo K & Suzuki K. Central noradrenergic neurons and the hypertensive effects of intracerebroventricularly administered prostaglandin E₂ in anaesthetized rabbits. *Prostaglandins* 1985 **29** 25–33.
- 44 Schadt JC & Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. *American Journal of Physiology* 1991 **260** H305–H318.
- 45 Bealer SL. Vascular capacitance following preoptic recess lesions. *American Journal of Physiology* 1993 **264** H560–H566.
- 46 Versteeg DHG, Petty MA, Bohus B & de Jong W. The central nervous system and hypertension: the role of catecholamines and neuropeptides. In *Handbook of Hypertension: Experimental and Genetic Models of Hypertension*, vol 4, pp 398–430. Ed W de Jong. Amsterdam: Elsevier, 1984.

Received 4 June 1997

Accepted 6 October 1997