ELSEVIER

European Journal of Pharmacology 347 (1998) 205–209

ejp

Short communication

Vasopressin mediates the inhibitory effect of central angiotensin II on cerebrospinal fluid formation

Adam Chodobski *, Joanna Szmydynger-Chodobska, Conrad E. Johanson

Program in Neurosurgery, Department of Clinical Neurosciences, Brown University / Rhode Island Hospital, Aldrich Building, Room 403, 593 Eddy Street, Providence, RI 02903, USA

Received 11 March 1998; accepted 13 March 1998

Abstract

Angiotensin II infused at low doses into the cerebral ventricles decreases cerebrospinal fluid (CSF) production. Since central angiotensin II also activates the sympathetic nervous system and promotes vasopressin release, the roles of these two factors in mediating the inhibitory effect of angiotensin II on CSF formation were studied. CSF production was measured in rats by the ventriculocisternal perfusion method. During central angiotensin II infusion (5 pg min^{-1}), the following adrenoceptor antagonists were administered intravenously (i.v.): phentolamine (α_1/α_2, 2 mg/kg per h), prazosin (α_1, 1 mg/kg per h), and propranolol (β, 1 mg/kg per h). None of these agents affected the inhibitory effect of angiotensin II on CSF formation. In comparison, in animals administered i.v., the vasopressin V_1 receptor antagonist, $d(CH_2)_5$Tyr(Me)Arg-vasopressin (10 μg/kg per h), the angiotensin II-induced decrease in CSF production was abolished. Our observations indicate, therefore, that vasopressin mediates the inhibitory effect of central angiotensin II on CSF formation. © 1998 Elsevier Science B.V.

Keywords: Angiotensin II; Sympathetic nervous system; Vasopressin; Cerebrospinal fluid formation; Choroid plexus; (Rat)

1. Introduction

Angiotensin II, a putative neurotransmitter or neuromodulator within the central nervous system, has recently been demonstrated to inhibit cerebrospinal fluid (CSF) formation in rabbits and rats (Chodobski et al., 1992, 1994). The decrease in CSF production was observed with low angiotensin II doses administered into the cerebral ventricles and was angiotensin AT_1 receptor-dependent. These angiotensin II actions may involve the direct effect of the peptide on the choroid plexus, the major source of CSF (Johanson, 1995), and/or may be mediated by other neurohormonal regulatory systems. There is an ample body of evidence indicating that centrally-released angiotensin II modulates the activity of the sympathetic nervous system and regulates plasma vasopressin levels (Wright and Harding, 1992; Andersson et al., 1995; Culman et al., 1995). Choroidal tissue receives sympathetic innervation that originates from the superior cervical ganglia (Edvinsson et al., 1974). The use of adrenoceptor agonists and antagonists revealed that the sympathetic nervous system exerts both α- and β-adrenoceptor-mediated inhibitory effect on CSF formation (Lindvall et al., 1979). Blood-borne vasopressin is not only a potent constrictor of the choroidal vascular bed (Faraci et al., 1990; Segal et al., 1992), but it also inhibits ion transport activity in the in vitro choroid plexus (Johanson et al., 1990). These two vasopressin actions are vasopressin V_1 receptor-dependent and result in the reduction in CSF production (Faraci et al., 1990). Thus, it is possible that the CSF formation-lowering effect of centrally-released angiotensin II is mediated by the sympathetic nervous system and/or vasopressin. The aim of the present study was to test the above hypothesis by using specific adrenoceptor and vasopressin receptor antagonists.

2. Materials and methods

The experimental procedures were similar to those previously described (Chodobski et al., 1994). In brief, male Sprague–Dawley rats (Charles River Breeding Laborato-

* Corresponding author. Tel.: +1-401-444-4285; fax: +1-401-444-8727; e-mail: adam_chodobski@brown.edu

ries, Wilmington, MA) weighing 250–350 g were used. Animals were initially anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). A tracheostomy was performed and catheters were inserted into the femoral artery and vein for measurement of arterial blood pressure, collection of arterial blood samples, and intravenous (i.v.) administration of drugs and solutions. The animals were artificially ventilated after being paralyzed with D-tubocurarine (1 mg/kg, i.v.). Arterial O_2 tension was maintained at 100–120 mm Hg by adjusting the inspired O_2 content, and arterial CO_2 tension was kept at 33–38 mmHg by adjusting the tidal volume and respiratory rate. Rectal temperature was kept at ~37°C.

CSF formation rate was measured by the ventriculocisternal perfusion method. The animals were mounted in a stereotaxic frame and two stainless steel cannulas (27-gauge) were introduced into both lateral cerebral ventricles. Artificial CSF, containing Blue Dextran 2000 (Sigma, St. Louis, MO) at a concentration of 5 mg/ml, was infused through these cannulas at a rate of 2 μl/min for each ventricle while intraventricular pressures were continuously monitored by means of T connectors inserted into the infusion lines. A stainless steel cannula (27-gauge) was inserted into the cisterna magna to enable CSF collection. CSF samples were collected at 20-min intervals and the concentration of Blue Dextran in the samples was determined colorimetrically by measuring absorbance at 620 nm.

Nine experimental groups were compared with the separate control group. Angiotensin II (Peninsula Labs., Belmont, CA) was administered intracerebroventricularly (i.c.v.) at a rate of 5 pg/min. This rate of i.c.v. angiotensin II infusion has previously been shown in rats to significantly inhibit CSF formation (Chodobski et al., 1994). To determine the role played by the sympathetic nervous system in mediating the inhibitory effect of angiotensin II on CSF production, the following adrenoceptor antagonists were given by i.v. infusion: the α_1/α_2-adrenoceptor antagonist, phentolamine (Regitine; Ciba, Summit, NJ), the α_1-adrenoceptor antagonist, prazosin (Sigma), and the β-adrenoceptor antagonist, propranolol (Inderal; Wyeth-Ayerst Labs., Philadelphia, PA). Phentolamine was administered at 2 mg/kg per h, and prazosin and propranolol were each infused at 1 mg/kg per h. The efficacy of adrenoceptor blockade was determined in preliminary experiments ($n = 2$ for each antagonist) in which hemodynamic responses to a specific adrenoceptor agonist were measured both immediately before and 1 h after adrenoceptor antagonist administration. Norepinephrine (1.5 ng/kg, i.v.) increased mean arterial blood pressure, by 25–30 and 2–4 mmHg, before and after phentolamine administration, respectively. Prazosin completely eliminated the pressor response (an increase in mean arterial blood pressure, by ~30 mmHg) following i.v. injection of phenylephrine at 3 μg/kg per h. An increase in heart rate, by 70–90 beats min^{-1}, and a decrease in mean arterial blood pressure, by ~35 mmHg, following isoproterenol (0.1 μg/kg per h, i.v.) was abolished by propranolol. The possible vasopressin involvement was examined by using the specific vasopressin V_1 receptor antagonist, 1-(β-mercapto-β,β-cyclopentamethylene propionic acid),2-(*O*-methyl)tyrosine-Arg-vasopressin [$d(CH_2)_5$Tyr(Me)Arg-vasopressin] (Peninsula Labs.). Vasopressin V_1 receptor antagonist was injected i.v. at a dose of 10 μg/kg per h, which in preliminary experiments ($n = 2$), totally eliminated the pressor response (an increase in mean arterial blood pressure, by 30–40 mmHg) to vasopressin (1 ng/kg, i.v.) 1 h after antagonist administration. The effect of adrenoceptor and vasopressin receptor antagonists on basal CSF production was assessed.

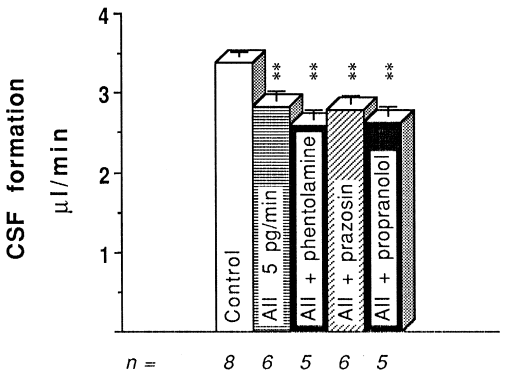

Fig. 1. Effect of α- and β-adrenoceptor blockade on the angiotensin II-mediated inhibition of CSF formation. Angiotensin II was infused i.c.v. at 5 pg/min while the adrenoceptor antagonists were given i.v. at 2 mg/kg per h (phentolamine) and 1 mg/kg per h (prazosin and propranolol). $**P < 0.01$ compared to control; AII is angiotensin II and *n* denotes the number of rats in each group.

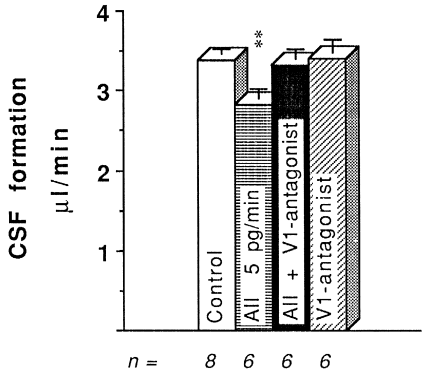

Fig. 2. Effect of the vasopressin V_1 receptor antagonist, $d(CH_2)_5$Tyr(Me)Arg-vasopressin, on the angiotensin II-mediated inhibition of CSF production. Angiotensin II was infused i.c.v. at 5 pg/min while $d(CH_2)_5$Tyr(Me)Arg-vasopressin was administered i.v. at 10 μg/kg per h. $**P < 0.01$ compared to control; AII is angiotensin II and V_1-antagonist is vasopressin V_1 receptor antagonist, respectively, and *n* denotes the number of rats in each group.

Results are presented as means ± S.E.M. The number of animals used in control and experimental groups is shown in Figs. 1 and 2. For statistical analysis of data, one-way analysis of variance was used. The Dunnett's test was then employed to make multiple comparisons with the control value. $P < 0.05$ was considered statistically significant.

3. Results

The CSF formation rate determined in control animals at 3 h of the ventriculocisternal perfusion was 3.38 ± 0.07 l μl/min. During i.c.v. administration of angiotensin II at 5 pg/min, CSF production was significantly ($P < 0.01$) decreased to 2.8 ± 20.13 μl/min (Figs. 1 and 2). Neither the α- nor β-adrenoceptor antagonists affected the inhibitory effect of angiotensin II on CSF formation (Fig. 1). Thus, this angiotensin II action was not mediated by increased activity of the sympathetic nervous system. None of the adrenoceptor antagonists used influenced basal CSF formation, which was 3.55 ± 0.12, 3.47 ± 0.06 and 3.39 ± 0.14 μl/min ($n = 5$ for each group) in rats administered phentolamine, prazosin, and propranolol, respectively. Fig. 2 shows the results of experiments with vasopressin V_1 receptor blockade. While d$(CH_2)_5$Tyr(Me)Arg-vasopressin by itself did not affect CSF formation, in animals administered the V_1 receptor antagonist, the decrease in CSF production during i.c.v. angiotensin II infusion was abrogated. d$(CH_2)_5$Tyr(Me)Arg-vasopressin has a small intrinsic antidiuretic activity of 0.31 IU/mg (László et al., 1991). However, this antidiuretic activity did not affect the animals' fluid balance during the course of the experiments, as no statistically significant changes in plasma osmolality were found in rats that were only administered vasopressin V_1 receptor antagonist (296 ± 2 and 292 ± 2 mosmol/kg before and after a 3-h observation period, respectively). Similarly, plasma osmolality was not altered during concomitant i.v. administration of d$(CH_2)_5$Tyr(Me)Arg-vasopressin and i.c.v. infusion of angiotensin II (297 ± 4 and 295 ± 3 mosmol/kg before and after a 3-h observation period, respectively). Our findings indicate, therefore, that vasopressin mediates the inhibitory effect of central angiotensin II on CSF formation.

Under control conditions, mean arterial blood pressure was 104 ± 2 mmHg. Intracerebroventricular AII infusion caused only a transient elevation of mean arterial blood pressure lasting ~ 30 min, with a maximal increase in this parameter of 20 mmHg, which occurred during insertion of cannulas into the cerebral ventricles and at the beginning of the ventriculocisternal perfusion. Administration of both the α- and β-adrenoceptor antagonists resulted in a transient fall in arterial blood pressure of variable degree; however, during the course of the experiments, arterial blood pressure stabilized at levels that were not significantly different from those observed in control animals. Also, d$(CH_2)_5$Tyr(Me)Arg-vasopressin produced no statistically significant changes in arterial blood pressure.

4. Discussion

Centrally-released angiotensin II elicits a number of behavioral and physiological responses, including activation of the sympathetic nervous system and augmentation of vasopressin release into the blood stream (Wright and Harding, 1992; Andersson et al., 1995; Culman et al., 1995). Choroid plexus epithelial cells and blood vessels are innervated by sympathetic fibers arising from the superior cervical ganglia (Edvinsson et al., 1974). The actions of the sympathetic nervous system in choroidal tissue are mediated by both α- and β-adrenoceptors and may involve an inhibition of epithelial ion transport activity and/or the reduction in choroidal blood flow (Lindvall et al., 1979). Choroidal tissue has been shown, by autoradiography and mRNA analysis, to possess vasopressin V_{1a} receptors (Phillips et al., 1988; Ostrowski et al., 1992). Physiological studies indicate that these receptors are localized to both choroidal vasculature and epithelial cells (Faraci et al., 1990; Johanson et al., 1990; Segal et al., 1992). Circulating vasopressin markedly decreases CSF production, presumably, by reducing blood supply to choroidal tissue (Faraci et al., 1990; Segal et al., 1992). However, this decrease in CSF formation may also result from a vasopressin-mediated inhibition of choroidal epithelial ion transport activity (Johanson et al., 1990).

Based on the above data, we investigated the possibility that the decrease in CSF formation observed during i.c.v. administration of low angiotensin II doses (Chodobski et al., 1992, 1994) is mediated by the sympathetic nervous system and/or vasopressin. Since neither the α- nor β-adrenoceptor antagonists affected the CSF formation-lowering effect of angiotensin II, it is unlikely that in rats, the sympathetic nervous system is involved in this angiotensin II action. Basal CSF formation was also not altered by adrenoceptor blockade. This latter finding in rats differs from that in rabbits, in which the sympathetic nervous system exerts a tonic inhibitory effect on CSF production (Lindvall et al., 1978). In both rats and rabbits, comparable i.c.v. angiotensin II doses decreased CSF formation, by 16–36% (Chodobski et al., 1992, 1994; this study). However, whereas i.c.v. angiotensin II infusion caused only a transient elevation of arterial blood pressure in rats, in rabbits, similar peptide doses resulted in a small, but long-lasting increase in blood pressure (Chodobski et al., 1992, 1994). These observations suggest, therefore, that species-related differences exist in the mechanisms regulating CSF formation.

In comparison to adrenoceptor blockade, the angiotensin II-induced decrease in CSF formation was abolished when rats were administered i.v. d$(CH_2)_5$Tyr(Me)Arg-vasopressin. This indicates that the

angiotensin II action was vasopressin V_1 receptor-mediated. d$(CH_2)_5$Tyr(Me)Arg-vasopressin is one of the most potent and selective peptide vasopressin V_1 receptor antagonists (László et al., 1991). Although this antagonist possesses a small intrinsic antidiuretic activity, we have shown that this did not affect fluid and electrolyte balance of the rats during the course of the experiments.

Faraci et al. (1990) have found that i.v. infusion of vasopressin producing plasma peptide levels of ~330 pg ml^{-1} results in a vasopressin V_1 receptor-dependent reduction in CSF formation, by 35%. Comparable plasma vasopressin levels in rats decreased CSF formation rate, by 28% (unpublished observations). Vasopressin secretion into the blood stream is promoted by central angiotensin II (Keil et al., 1975; Qadri et al., 1993). This angiotensin II action, similar to the reduction in CSF production during i.c.v. peptide administration, is abolished by angiotensin AT_1 receptor antagonists (Qadri et al., 1993; Chodobski et al., 1994). However, the circulating vasopressin is unlikely to mediate the inhibitory effect of centrally-released angiotensin II on CSF formation. While relatively high plasma vasopressin levels are needed to significantly lower CSF formation (see above), a moderate increase in plasma vasopressin concentration (up to 25–40 pg/min) has been found in rats in response to 100 ng of angiotensin II injected i.c.v. (Keil et al., 1975; Qadri et al., 1993). A 10^3-fold lower angiotensin II dose was ineffective in stimulating vasopressin release. By comparison, the angiotensin II-mediated inhibition of CSF formation was observed in rats, with i.c.v. peptide doses of 0.5–5 pg/min (Chodobski et al., 1994). Based on the above observations, we conclude that plasma vasopressin levels during i.c.v. infusion of low angiotensin II doses are likely to be considerably lower than those that would affect CSF production.

We hypothesize that the inhibitory effect of central angiotensin II on CSF formation is mediated by the vasopressin that is synthesized and/or released within the choroid plexus. In this manner, sufficient vasopressin levels to alter CSF production could be attained in choroidal tissue. Since d$(CH_2)_5$Tyr(Me)Arg-vasopressin appears to cross the blood–brain barrier (Armstead et al., 1989), it would block the vasopressin V_1 receptors on both apical and basolateral membranes of epithelial cells and on choroidal vasculature. Angiotensin II depolarizes neurons in hypothalamic paraventricular and supraoptic nuclei through the activation of angiotensin AT_1 receptors (Yang et al., 1992; Li and Ferguson, 1993). Accordingly, hypothalamic paraventricular and supraoptic nuclei have been found to give rise to neurophysin-immunoreactive neuronal fibers that innervate choroid plexus (Brownfield and Kozlowski, 1977). We have recently demonstrated that vasopressin is also synthesized by choroid plexus epithelium (Chodobski et al., 1997, 1998). Since angiotensin AT_1 receptors are present in choroidal tissue (Tsutsumi and Saavedra, 1991; Lenkei et al., 1998), angiotensin II may directly promote choroidal vasopressin release. It is thus possible that the choroid plexus-derived vasopressin plays a role in the regulation of choroidal hemodynamics and fluid secretion. Further research will be needed to validate this hypothesis.

In summary, we have demonstrated that the inhibitory effect of centrally-released angiotensin II on CSF formation is vasopressin V_1 receptor-dependent. However, the low angiotensin II doses used are unlikely to significantly increase plasma vasopressin levels. It is suggested, therefore, that the vasopressin that is released and/or produced within the choroid plexus mediates the above angiotensin II actions.

Acknowledgements

Authors wish to thank Dr. B.E. Wojcik for his critical review of the manuscript. This work was supported by NIH grant NS 27601 (C.E.J.) and research funds from the Rhode Island Hospital (A.C. and J.S.C.).

References

Andersson, B., Eriksson, S., Rundgren, M., 1995. Angiotensin and the brain. Acta Physiol. Scand. 155, 117–125.

Armstead, W.M., Mirro, R., Busija, D.W., Leffler, C.W., 1989. Vascular responses to vasopressin are tone-dependent in the cerebral circulation of the newborn pig. Circ. Res. 64, 136–144.

Brownfield, M.S., Kozlowski, G.P., 1977. The hypothalamo-choroidal tract: I. Immunohistochemical demonstration of neurophysin pathways to telencephalic choroid plexuses and cerebrospinal fluid. Cell Tissue Res. 178, 111–127.

Chodobski, A., Szmydynger-Chodobska, J., Segal, M.B., McPherson, I.A., 1992. The role of angiotensin II in regulation of cerebrospinal fluid formation in rabbits. Brain Res. 594, 40–46.

Chodobski, A., Szmydynger-Chodobska, J., Vannorsdall, M.D., Epstein, M.H., Johanson, C.E., 1994. AT_1 receptor subtype mediates the inhibitory effect of central angiotensin II on cerebrospinal fluid formation in the rat. Regul. Pept. 53, 123–129.

Chodobski, A., Loh, Y.P., Corsetti, S., Szmydynger-Chodobska, J., Johanson, C.E., Lim, Y.-P., Monfils, P.R., 1997. The presence of arginine vasopressin and its mRNA in rat choroid plexus epithelium. Mol. Brain Res. 48, 67–72.

Chodobski, A., Wojcik, B.E., Loh, Y.P., Dodd, K.A., Szmydynger-Chodobska, J., Johanson, C.E., Demers, D.M., Chun, Z.G., Limthong, N.P., 1998. Vasopressin gene expression in rat choroid plexus. In: Zingg, H.H., Bichet, D.G., Bourque, C. (Eds.), Vasopressin and Oxytocin: Synopsis of Recent Advances in Molecular, Cellular, and Clinical Research. Plenum, New York, in press.

Culman, J., Höhle, S., Qadri, F., Edling, O., Blume, A., Lebrun, C., Unger, T., 1995. Angiotensin as neuromodulator/neurotransmitter in central control of body fluid and electrolyte homeostasis. Clin. Exp. Hypertens. 17, 281–293.

Edvinsson, L., Nielsen, K.C., Owman, C., West, K.A., 1974. Adrenergic innervation of the mammalian choroid plexus. Am. J. Anat. 139, 299–307.

Faraci, F.M., Mayhan, W.G., Heistad, D.D., 1990. Effect of vasopressin on production of cerebrospinal fluid: possible role of vasopressin (V_1)-receptors. Am. J. Physiol. 258, R94–R98.

Johanson, C.E., 1995. Ventricles and cerebrospinal fluid. In: Conn, P.M.

(Ed.), Neuroscience in Medicine. Lippincott, Philadelphia, PA, pp. 171–196.

Johanson, C.E., Nashold, J.R.B., Preston, J.E., Dyas, M., Knuckey, N., 1990. Effects of vasopressin and atriopeptin on chloride transport in choroid plexus. Soc. Neurosci. Abstr. 16, 45.

Keil, L.C., Summy-Long, J., Severs, W.B., 1975. Release of vasopressin by angiotensin II. Endocrinology 96, 1063–1065.

László, F.A., László Jr., F. De Wied, D., 1991. Pharmacology and clinical perspectives of vasopressin antagonists. Pharmacol. Rev. 43, 73–108.

Lenkei, Z., Palkovits, M., Corvol, P., Llorens-Cortes, C., 1998. Distribution of angiotensin type-1 receptor messenger RNA expression in the adult rat brain. Neuroscience 82, 827–841.

Li, Z., Ferguson, A.V., 1993. Angiotensin II responsiveness of rat paraventricular and subfornical organ neurons in vitro. Neuroscience 55, 197–207.

Lindvall, M., Edvinsson, L., Owman, C., 1978. Sympathetic nervous control of cerebrospinal fluid production from the choroid plexus. Science 201, 176–178.

Lindvall, M., Edvinsson, L., Owman, C., 1979. Effect of sympathomimetic drugs and corresponding receptor antagonists on the rate of cerebrospinal fluid production. Exp. Neurol. 64, 132–145.

Ostrowski, N.L., Lolait, S.J., Bradley, D.J., O'Carroll, A.-M., Brownstein, M.J., Young III, W.S., 1992. Distribution of V_{1a} and V_2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. Endocrinology 131, 533–535.

Phillips, P.A., Abrahams, J.M., Kelly, J., Paxinos, G., Grzonka, Z., Mendelsohn, F.A.O., Johnston, C.I., 1988. Localization of vasopressin binding sites in rat brain by in vitro autoradiography using a radioiodinated V_1 receptor antagonist. Neuroscience 27, 749–761.

Qadri, F., Culman, J., Veltmar, A., Maas, K., Rascher, W., Unger, T., 1993. Angiotensin II-induced vasopressin release is mediated through alpha-1 adrenoceptors and angiotensin II AT_1 receptors in the supraoptic nucleus. J. Pharmacol. Exp. Ther. 267, 567–574.

Segal, M.B., Chodobski, A., Szmydynger-Chodobska, J., Cammish, H., 1992. Effect of arginine vasopressin on blood vessels of the perfused choroid plexus of the sheep. Prog. Brain Res. 91, 451–453.

Tsutsumi, K., Saavedra, J.M., 1991. Characterization and development of angiotensin II receptor subtypes (AT_1 and AT_2) in rat brain. Am. J. Physiol. 261, R209–R216.

Wright, J.W., Harding, J.W., 1992. Regulatory role of brain angiotensins in the control of physiological and behavioral responses. Brain Res. Rev. 17, 227–262.

Yang, C.R., Phillips, M.I., Renaud, L.P., 1992. Angiotensin II receptor activation depolarizes rat supraoptic neurons in vitro. Am. J. Physiol. 263, R1333–R1338.