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Short communication

The angiotensin AT_1 receptor antagonist, losartan, induces barrel rotation in the rat

Akio Kawachi, Masahide Miyashita, Toshiro Motoya, Katsushi Yamada *

Department of Hospital Pharmacy, Faculty of Medicine, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima 890-8520, Japan

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Abstract

Intracerebroventricular injections of [Arg^8]vasopressin (500 ng/rat) or endothelin-1 (70 ng/rat) into the right lateral ventricle induced rotation along the long axis of the body (barrel rotation) in rats. Losartan (10–200 μg/rat), an angiotensin AT_1 receptor antagonist, also evoked barrel rotation, which was not inhibited by vasopressin and endothelin receptor antagonists. However, barrel rotation was not observed after injections of high doses of another angiotensin II receptor antagonist, [Sar^1,Ile^8]angiotensin II (100 μg/rat), or after angiotensin II (10 μg/rat). The results indicate that losartan does evoke barrel rotation which may be not mediated via vasopressin and endothelin receptors.

Keywords: Losartan; Vasopressin; Endothelin-1; Barrel rotation; (Rat)

1. Introduction

An unusual motor disturbance occurs in conscious rats following intracerebroventricular (i.c.v.) injections of somatostatin, dynorphin-A and [Arg^8]vasopressin (Cohn and Cohn, 1975; Katz, 1980; Yamada and Furukawa, 1981; Diamant et al., 1994). This behavioural syndrome includes ataxia, body swaying, oculoclonus, forepaw dystonia, tonic extension of hind limbs and tail, and, most characteristically, rotation along the long axis of the body (barrel rotation). Thus, barrel rotation may serve as an experimental model of the human movement disorder dystonia. Barrel rotation has also been reported to be induced by the injection of endothelin-1 into the lateral ventricle or the dorsolateral periaqueductal gray area (Gross et al., 1992; D'Amico et al., 1995). In addition, Chew et al. (1995) proposed that the caudate nucleus, substantia nigra pars reticulata, inferior olivary nuclei, and cerebellar cortex are integrated functionally as a stimulated circuit in the barrel rotation induced by the i.c.v. injection of endothelin-1. Recently, Yamada et al. (1997) have shown that infusion of fluorocitrate, a glial-selective metabolic inhibitor, into the cerebellum induced barrel rotation.

Diamant et al. (1994) suggested that vasopressin V_1 receptors are involved in vasopressin-induced barrel rotation, as the vasopressin V_1 receptor antagonist, $d(CH_2)_5$[Tyr(Me)^2]vasopressin, prevented vasopressin-induced barrel rotation. In addition, Gross et al. (1992, 1994) and Gross and Weaver (1993) suggested that the cerebral stimulatory effects of an i.c.v. injection of endothelin-1, including barrel rotation, are mediated by endothelin ETA receptors, NMDA receptors and voltage-gated Ca^{2+} channels. The basis for this suggestion was that the endothelin ETA receptor antagonist, FR139317, the NMDA receptor antagonist, dizocilpine maleate (MK-801), and the L-type Ca^{2+} channel antagonist, nimodipine, prevented endothelin-1-induced barrel rotation. Moreover, Maione et al. (1993) have reported that injections of the NMDA receptor antagonist, D,L-2-amino-5-phosphonovalerate (2-APV), into the dorsolateral periaqueductal gray area attenuated the barrel rotation induced by endothelin-1 injected into the dorsolateral periaqueductal gray area. However, the underlying neuronal mechanisms of barrel rotation are still not completely understood.

Losartan has been reported to be the first potent and selective non-peptide angiotensin AT_1 receptor antagonist which exerts its antihypertensive effects by selectively antagonizing the binding of angiotensin II to angiotensin AT_1 receptors (Siegl, 1993). We examined the behavioural

* Corresponding author. Tel.: +81-99-275-5543; Fax: +81-99-265-5293

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effect of losartan in rats, and found that it could induce barrel rotation. The present experiments were also performed to investigate whether vasopressin and endothelin receptors are involved in the barrel rotation induced in rats by losartan.

2. Materials and methods

2.1. Animals

Male Wistar rats (Kyudo, Kumamoto, Japan) weighing 270–300 g with ad libitum access to food and water were housed in cages (2–3 animals/cage) at a constant temperature (21 ± 2°C) and related humidity (60%). The animals were maintained under a 12-h light–dark cycle (0700–1900 h).

2.2. Drugs

The drugs used were vasopressin (Peptide Institute, Osaka, Japan), endothelin-1 (Peptide Institute), angiotensin II (Peptide Institute), [Sar^1,Ile^8]angiotensin II (Peptide Institute), losartan (2-*n*-butyl-4-chloro-1-[2′-(tetrazol-5-yl)-1,1′-biphenyl-4-ylmethyl]-1*H*-imidazole-5-methanol monopotassium salt) (Merck, Rahway, NJ, USA), d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (Peptide Institute), OPC-21268 (1-{1-[4-(3-acetylamino-propoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone) (Otsuka Pharmaceutical, Tokushima, Japan) and BQ-123 (cyclo(D-α-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (RBI, Natick, MA, USA). Vasopressin, angiotensin II, [Sar^1,Ile^8]angiotensin II, d(CH$_2$)$_5$-[Tyr(Me)^2]vasopressin, BQ-123 and losartan were dissolved in saline and endothelin-1 was dissolved in 0.1% acetic acid in saline. OPC-21268 was dissolved in 1% dimethylsulfoxide in saline. Doses are expressed in terms of the base, with the exception of losartan.

2.3. Experimental protocols

The rats were anaesthetized with pentobarbital sodium (40 mg/kg) and placed in a stereotaxic apparatus (Narishige Scientific Instrument Lab., Tokyo, Japan). Stainless guide cannulas (Eicom, Kyoto, Japan) were implanted into the right lateral ventricle of rats, using stereotaxic coordinates according to the atlas of Pellegrino et al. (1979) (0.2 mm posterior to bregma, 0.8 mm lateral to midline and 3.5 mm ventral to dura). The cannulas were fixed to the skull with dental cement. Experiments were performed 3 days after surgery.

An i.c.v. injection was carried out with a Hamilton 25-μl syringe connected by means of a polyethylene tube to a stainless-steel cannula, which was carefully inserted into the fixed guide cannula. Vasopressin (100 and 500 ng), endothelin-1 (70 ng), angiotensin II (10 μg), losartan (10–200 μg) or [Sar^1,Ile^8]angiotensin II (100 μg) was injected intracerebroventricularly, in a total volume of 10 μl over 1 min as sole treatment or in combination with d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin, OPC-21268 or BQ-123. Vasopressin (100 ng/rat), endothelin-1 (70 ng/rat) or losartan (200 μg/rat) was given 10 min (i.c.v.) after i.c.v. treatment with d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin, OPC-21268 and BQ-123, also in a total volume of 10 μl over 1 min. After the injection of drugs, the latent period and the duration of barrel rotation were recorded by two observers. After the experiments, the positioning of the cannula was checked histologically.

2.4. Statistical and data analysis

Upon conclusion of the experiments, only data obtained from rats with a correctly placed cannula were included in the analysis. Following i.c.v. injections of drugs, some rats showed typical symptoms of barrel rotation. The incidence of barrel rotation was statistically evaluated by means of 2 × 2 contingency tables and with Fisher's exact probability test.

3. Results

3.1. Induction of barrel rotation by vasopressin, endothelin-1 and losartan

The effects of vasopressin, endothelin-1 and losartan on the incidence of barrel rotation are illustrated in Fig. 1. Injections of vasopressin (500 ng/rat, i.c.v.) or endothelin-1 (70 ng/rat, i.c.v.) elicited barrel rotation in rats. Injections of losartan (10–200 μg/rat, i.c.v.) also resulted in a dose-dependent increase in barrel rotation. Following the injection of 10 μg losartan no barrel rotation was observed, while 16.7% of rats displayed barrel rotation fol-

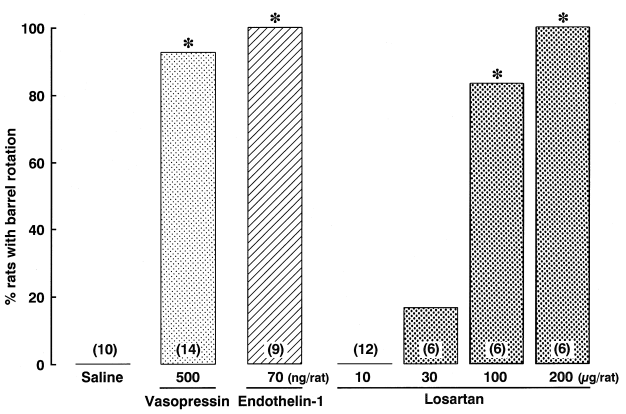

Fig. 1. Induction of barrel rotation by [Arg^8]vasopressin (500 ng/rat), endothelin-1 (70 ng/rat) and losartan (10–200 μg/rat) in rats. Response is expressed as a percentage of the number of rats treated with drugs that showed rotation (the number of rats in each group is indicated in parentheses). *: Significant difference from saline group at $P < 0.01$. Statistical analysis was done using Fisher's exact probability test.

lowing the 30-μg dose. Of the rats receiving doses of 100 and 200 μg losartan, 83.3% and 100% showed barrel rotation, respectively ($P < 0.01$). The median latent periods to barrel rotation after i.c.v. injections of vasopressin, endothelin-1 and losartan were 70 s, 84 s and 140 s, respectively, and the median durations of barrel rotation after i.c.v. injections of vasopressin, endothelin-1 and losartan were 30 s, 54 s and 133 s, respectively. No essential difference in the rotation behaviour induced by vasopressin, endothelin-1 or losartan was observed. On the other hand, barrel rotation was not observed after injections of high doses of the peptide angiotensin II receptor antagonist, [Sar^1,Ile^8]angiotensin II (100 μg/rat) or angiotensin II (10 μg/rat) (data not shown).

3.2. Effects of various receptor antagonists on vasopressin-, endothelin-1- and losartan-induced barrel rotation

As shown in Table 1, the barrel rotation induced by vasopressin (100 ng/rat) or endothelin-1 (70 ng/rat) was inhibited by pretreatment with the vasopressin V_1 receptor antagonists, d(CH_2)$_5$[Tyr(Me)^2]vasopressin (10 ng/rat) and OPC-21268 (10 μg/rat), and the endothelin ETA receptor antagonist, BQ-123 (10 μg/rat), respectively, but not by the angiotensin AT_1 receptor antagonist, losartan (10 μg/rat). On the other hand, d(CH_2)$_5$[Tyr(Me)^2] vasopressin (10 ng/rat) and OPC-21268 (10 μg/rat) were not effective on endothelin-1-induced barrel rotation, and BQ-123 (10 μg/rat) had no effects on vasopressin induced barrel rotation (data not shown). In addition, d(CH_2)$_5$[Tyr(Me)^2]vasopressin (1 μg/rat), OPC-21268 (10 μg/rat) and BQ-123 (10 μg/rat) were not effective on losartan induced barrel rotation (data not shown).

Table 1
Effects of vasopressin, endothelin and angiotensin II receptor antagonists on [Arg^8] vasopressin (100 ng/rat)- or endothelin-1 (70 ng/rat)-induced barrel rotation in rats

	Dose	Number	% Rats with barrel rotation
Vasopressin-induced barrel rotation			
Saline		22	68.2%
d(CH_2)$_5$[Tyr(Me)^2]vasopressin	10 ng/rat	6	0%[a]
OPC-21268	10 μg/rat	9	0%[a]
Losartan	10 μg/rat	6	66.7%
Endothelin-1-induced barrel rotation			
Saline		11	100%
BQ-123	10 μg/rat	13	15.4%[a]
Losartan	10 μg/rat	6	100%

Response is expressed as a percentage of the number of rats treated with drugs that showed rotation.
[a]: Significant difference from saline group at $P < 0.01$.
Statistical analysis was done using Fisher's exact probability test.

4. Discussion

The central administration of high doses of somatostatin, dynorphin-A, vasopressin and endothelin-1 results in the occurrence of a typical motor disturbance termed 'barrel rotation' (Cohn and Cohn, 1975; Katz, 1980; Yamada and Furukawa, 1981; Gross et al., 1992; Maione et al., 1993; Diamant et al., 1994). In early investigations, barrel rotation was regarded as an epileptic phenomenon, as its occurrence was inhibited by antiepileptic drugs (Abood et al., 1980). However, Ehlers et al. (1985), Grabow et al. (1989) and Gross et al. (1992) have reported that the EEG abnormalities commonly associated with epileptic disorders were not found.

In the present experiments, i.c.v. injections of vasopressin or endothelin-1 evoked barrel rotation in rats. Recently, OPC-21268 was developed as a non-peptide vasopressin V_1 receptor antagonist, which acts as a specific antagonist of vasopressin-induced vasoconstriction as shown by Yamamura et al. (1991). Both vasopressin V_1 receptor antagonists, d(CH_2)$_5$[Tyr(Me)^2]vasopressin and OPC-21268, completely prevented the incidence of the barrel rotation induced by vasopressin, indicating the involvement of vasopressin V_1 receptors in mediating the action of vasopressin on rotation behaviour. The endothelin ETA receptor antagonist, BQ-123, also prevented the incidence of barrel rotation induced by endothelin-1, showing the involvement of endothelin ETA receptors in mediating the action of endothelin-1 on rotation behaviour. Our data are in complete agreement with results of previous studies showing that the barrel rotation induced by vasopressin and endothelin-1 was blocked by d(CH_2)$_5$[Tyr(Me)^2]vasopressin and FR139317, respectively(Gross et al., 1994; Diamant et al., 1994). On the contrary, BQ-123 did not affect vasopressin-induced barrel rotation, suggesting that endothelin ETA receptors are not involved in vasopressin-induced barrel rotation. In addition, d(CH_2)$_5$[Tyr(Me)^2]vasopressin and OPC-21268 did not affect endothelin-1-induced barrel rotation, implying no involvement of vasopressin V_1 receptors in endothelin-1-induced barrel rotation. Thus, trigger receptors for the induction of barrel rotation following injections of vasopressin and endothelin-1 may be different.

As mentioned in Section 1, losartan is the first potent and selective non-peptide angiotensin AT_1 receptor antagonist which exerts its antihypertensive effects by selectively antagonizing the binding of angiotensin II to angiotensin AT_1 receptors (Siegl, 1993). In the present experiments, i.c.v. injections of losartan as well as of vasopressin and endothelin-1 were found to evoke barrel rotation. Losartan-induced barrel rotation was not inhibited by d(CH_2)$_5$[Tyr(Me)^2]vasopressin, OPC-21268 and BQ-123, suggesting that neither vasopressin V_1 nor endothelin ETA receptors are involved in losartan-induced barrel rotation. In addition, i.c.v. injections of [Sar^1,Ile^8]angiotensin II, a peptide angiotensin II receptor antagonist, or angiotensin II

did not elicit barrel rotation in rats. Moreover, our recent experimental results showed that the potent and selective non-peptide angiotensin AT_2 receptor antagonist, PD-123319, failed to evoke barrel rotation in rats (unpublished data). Accordingly, it is assumed that the barrel rotation observed following i.c.v. injections of losartan is not mediated by block or stimulation of angiotensin II receptors.

On the other hand, the injection of losartan into the dorsolateral periaqueductal gray area was reported to attenuate the barrel rotation induced by endothelin-1 injected into the dorsolateral periaqueductal gray area (D'Amico et al., 1996). However, in the present experiments, i.c.v. injections of losartan did not affect either type of barrel rotation, i.e., that induced by vasopressin or by endothelin-1 injected into the lateral ventricle. This discrepancy may be in part related to the difference in injection sites of drugs in the rat brain. Further investigations are necessary to understand the neuronal mechanisms of barrel rotation.

In conclusion, the angiotensin AT_1 receptor antagonist, losartan, could induce barrel rotation in the rat and this barrel rotation may provide a new clue for understanding the mechanisms of barrel rotation behaviour.

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References

Abood, L.G., Knapp, R., Mitchell, T., Booth, H., Schwab, L., 1980. Chemical requirements of vasopressins for barrel rotation convulsions and reversal by oxytocin. J. Neurosci. Res. 5, 191–199.

Chew, B.H., Weaver, D.F., Gross, P.M., 1995. Dose-related potent brain stimulation by the neuropeptide endothelin 1 after intraventricular administration in conscious rats. Pharmacol. Biochem. Behav. 51, 37–47.

Cohn, M.L., Cohn, M., 1975. 'Barrel rotation' induced by somatostatin in the non-lesioned rat. Brain Res. 96, 138–141.

D'Amico, M., Berrino, L., Maione, S., Pizzirusso, A., Rossi, F., 1995. Effects of L-NAME on endothelin-1-induced barrel-rolling in periaqueductal gray area of rats. Life Sci. 57, PL357–PL360.

D'Amico, M., di Filippo, C., Berrino, L., Rossi, F., 1996. Angiotensin II, via an action at AT1 receptors, may modulate the behavioural effects of ET-1 in conscious rats. Life Sci. 59, PL355–PL358.

Diamant, M., Baars, A.M., Kovács, G.L., De Wied, D., 1994. Barrel rotation induced by central arginine^8–vasopressin treatment: involvement of neurohypophyseal peptide receptors. Pharmacol. Biochem. Behav. 47, 27–32.

Ehlers, C.L., Reed, T.K., Wang, M., Lebrun, C.J., Koob, G.F., 1985. EEG effects of subcutaneous and intracerebroventricular injections of arginine vasopressin in the rat. Psychopharmacology 87, 430–433.

Grabow, T.S., Gaumann, D.M., Yaksh, T.L., 1989. Electroencephalographic and behavioral assessment of intracerebroventricular somatostatin and a substance P analogue. Brain Res. 489, 223–230.

Gross, P.M., Weaver, D.F., 1993. A new experimental model of epilepsy based on the intraventricular injection of endothelin. J. Cardiovasc. Pharmacol. 22, S282–S287.

Gross, P.M., Wainman, D.S., Espinosa, F.J., Nag, S., Weaver, D.F., 1992. Cerebral hypermetabolism produced by intraventricular endothelin-1 in rats: inhibition by nimodipine. Neuropeptides 21, 211–223.

Gross, P.M., Weaver, D.F., Ho, L.T., Pang, J.J., Edvinsson, L., 1994. FR139317, a specific ETA-receptor antagonist, inhibits cerebral activation by intraventricular endothelin-1 in conscious rats. Neuropharmacology 33, 1155–1166.

Katz, R.J., 1980. Behavioral effects of dynorphin—a novel opioid neuropeptide. Neuropharmacology 19, 801–803.

Maione, S., D'Amico, M., Berrino, L., Filippelli, A., Leyva, J., Rossi, F., 1993. Involvement of periaqueductal gray area NMDA receptors in endothelin-induced behavioural effects. Eur. J. Pharmacol. 250, 209–212.

Pellegrino, L.J., Pellegrino, A.S., Cushmann, A.J., 1979. A Stereotaxic Atlas of the Rat Brain, 2nd edn. Plenum, New York.

Siegl, P.K.S., 1993. Discovery of losartan, the first specific non-peptide angiotensin II receptor antagonist. J. Hypertens. 11, S19–S22.

Yamada, K., Furukawa, T., 1981. Barrel rotation induced by vasopressin and involvement of dopaminergic and cholinergic functions in rats. Arch. Int. Pharmacodyn. 251, 88–94.

Yamada, K., Senzaki, K., Komori, Y., Nikai, T., Sugihara, H., Nabeshima, T., 1997. Changes in extracellular nitrite and nitrate levels after inhibition of glial metabolism with fluorocitrate. Brain Res. 762, 72–78.

Yamamura, Y., Ogawa, H., Chihara, T., Kondo, K., Onogawa, T., Nakamura, S., Mori, T., Tominaga, M., Yabuuchi, Y., 1991. OPC-21268, an orally effective, nonpeptide vasopressin V1 receptor antagonist. Science 252, 572–574.