ELSEVIER

European Journal of Pharmacology 454 (2002) 199–207

www.elsevier.com/locate/ejphar

Role of the vestibular nuclei in endothelin-1-induced barrel rotation in rats

Tomohiro Kozako[a], Akio Kawachi[a], Shi-Bin Cheng[b], Satoshi Kuchiiwa[b], Toshiro Motoya[a], Shiro Nakagawa[b], Katsushi Yamada[a,*]

[a] *Department of Clinical Pharmacy, Faculty of Medicine, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima 890-8520, Japan*
[b] *Department of Anatomy, Faculty of Medicine, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima 890-8520, Japan*

Received 25 September 2002; accepted 1 October 2002

Abstract

The fourth or lateral ventricular injection of endothelin-1 resulted in a dose-dependent increase in the barrel rotation and produced marked induction of c-Fos-positive cells in the vestibular nuclei. The doses of the former injection were lower and had shorter mean latent periods compared with the later injection. c-Fos expression after endothelin-1 injection was prevented by the pretreatment with the endothelin ET_A receptor antagonist, cyclo(D-alpha-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123), the glutamate NMDA receptor antagonist, dizocilpine maleate (MK-801), or the L-type Ca^{2+} channel antagonist, verapamil, in addition to the incidence of the rotational behavior. There was a significant difference in c-Fos expression between the right and left medial vestibular nuclei, and the number of c-Fos-labeled neurons in the medial vestibular nucleus was markedly increased on the opposite side of the rotational direction. These results suggest that the elicitation of the barrel rotation may be mediated by endothelin ET_A receptors, glutamate NMDA receptors, and L-type Ca^{2+} channels. The changes in the receptor and channel systems induced by endothelin-1 injections appeared to exert crucial influences on the vestibular nuclei and then on the maintenance of equilibrium. The direction of the barrel rotation has a deep connection with the imbalance of neuronal activity in the left and right medial vestibular nuclei.

Keywords: Barrel rotation; c-Fos; Endothelin-1; Vestibular nucleus; (Rat)

1. Introduction

Endothelin-1 is generally considered the most potent and long-lasting vasoconstrictor (Yanagisawa et al., 1988). The activities of endothelin peptides are mediated by two types of heterotrimeric G protein-coupled endothelin receptors (ET_A and ET_B), which are located in blood vessels, heart, lung, intestine, kidney, and brain. (Hoyer et al., 1989; Bax and Saxena, 1994; Becker et al., 1996; Endoh et al., 1998). Injection of endothelin-1 into the lateral ventricle or the lateral-caudal periaqueductal gray of rats has been shown to produce a characteristic rotation along the long axis of the body (barrel rotation) and other behaviors including ataxia, head tilt, and nystagmus (Gross et al., 1992; Maione et al., 1993). Barrel rotation is also induced by the central administration of somatostatin, vasopressin, or dynorphin-A (Cohn and Cohn, 1975; Dokas et al., 1983; Kruse et al., 1977; Yamada and Furukawa, 1981; Katz, 1980). The rotational behavior appeared to be associated with an asymmetric increase in stretch muscle tone and the direction of rotation is clockwise or counterclockwise with some rats showing rotation towards the same side while others rotate towards both sides. Recently, we reported that the endothelin ET_A receptor antagonist, cyclo(D-alpha-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123), prevented endothelin-1-induced barrel rotation but the vasopressin V_1 receptor antagonists, $d(CH_2)_5[Tyr(Me)^2]$vasopressin and 1-{1-[4-(3-acetylamino-propoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone, did not affect. In addition, both vasopressin V_1 receptor antagonists also prevented vasopressin-induced barrel rotation but BQ-123 did not inhibit it. Thus, trigger receptors for the induction of barrel rotation following the injection of endothelin-1 or vasopressin may be different (Kawachi et al., 1998). However, the glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine maleate (MK-801), prevented the barrel rotation induced by endothelin-1 or dynorphin-A

* Corresponding author. Tel.: +81-99-275-5543; fax: +81-99-265-5293.
E-mail address: yamada@m3.kufm.kagoshima-u.ac.jp (K. Yamada).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02498-6

(Gross et al., 1994; Chew et al., 1994; Shukla et al., 1997). Moreover, the barrel rotation induced by the central administration of endothelin-1 is mediated by voltage-gated calcium channels, because the L-type Ca^{2+} channel antagonist, nimodipine, prevented it (Gross et al., 1994). Therefore, NMDA receptors and L-type Ca^{2+} channels appear to be concerned with the induction of barrel rotation.

Severe postural and oculomotor asymmetries such as barrel rotation, head tilt, and spontaneous nystagmus are also induced by unilateral labyrinthectomy. Unilateral labyrinthectomy has been reported to cause impairment of ocular movement and postural reflex due to an imbalance between the activity of the left and right vestibular nuclei as a result of impairment of the vestibular input to the ipsilateral brainstem vestibular nuclei (Pan et al., 1998). The labyrinth has a connection with vestibular neurons that convey this information to ocular nuclei, the cerebellum, and the spinal cord to maintain body balance and posture (Walberg et al., 1958; Peterson, 1967; Schor and Miller, 1981). The vestibular nuclei complex consists of heterogenous neurons controlled by excitatory glutamatergic afferents from the ipsilateral labyrinth via glutamatergic commissural projections connected to inhibitory interneurons (Waele et al., 1990; Furuya and Koizumi, 1998). In addition, pharmacological and electrophysiological studies have shown that NMDA and non-NMDA receptors mediate the main vestibular inputs (Kurumaji et al., 1989; Doi et al., 1990; Kinney et al., 1994; Wenzel et al., 1997). Thus, glutamate is the major neurotransmitter of the vestibular afferents (Reichenberger and Dieringer, 1994; Harvey et al., 2000).

c-Fos protein, one of the immediate-early gene products, was suggested to be related to the regulation of the gene expression (Morgan and Curran, 1991; Herrera and Robertson, 1996). Various stimuli, e.g., stress, sensory stimulation, injury, and others, induce the expression of the c-*fos* gene and protein in the mammalian nervous system (Hunt et al., 1987; Séquier and Lazdunski, 1990; Hol et al., 1993; Gilron et al., 1999; Palkovits, 2000; Cheng et al., 2002). Therefore, c-Fos expression in the nervous system is regarded as a marker of functional activation and functional mapping (Dragunow and Faull, 1989; Bauters et al., 1992). In addition, c-Fos immunoreactivity has been found in the medial vestibular nucleus and inferior olive in rats after chemical unilateral labyrinthectomy by sodium arsanilate and in the rat brain after exposure to gravito-inertial force change (Saika et al., 1991; Kaufman et al., 1992; Gustave et al., 2000). The experimental model of vestibular dysfunction including barrel rotation has a possibility to be achieved by rebalancing the neuronal activity in the central vestibular system and serves as an experimental model of the human movement disorders dystonia and vertigo.

The objective of this study was to clarify the involvement of the vestibular nuclei on the barrel rotation following the injection of endothelin-1 into the fourth ventricle. We investigated whether rotational behavior correlates with neuronal activity in the vestibular nuclei using c-Fos expression together with the effects of receptor antagonists on the barrel rotation.

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 relative humidity (60%). The animals were maintained under a 12-h light–dark cycle (7:00 a.m. to 7:00 p.m.). All experimental procedures were carried out in accordance with the *Guide for Animal Experimentation* of the Faculty of Medicine of Kagoshima University.

2.2. Drugs

The drugs used were endothelin-1 (Peptide Institute, Osaka, Japan), cyclo(D-alpha-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123) (RBI, Natick, MA, USA), *N*-[*N*-[*N*-[(2,6-Dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl-D-tryptophyl]-D-norleucine monosodium (BQ-788) (RBI), dizocilpine maleate (MK-801) (RBI), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (RBI), and verapamil (RBI). BQ-123, BQ-788, MK-801, and verapamil were dissolved in saline. CNQX was dissolved in 1% dimethylsulfoxide in saline and endothelin-1 was dissolved in 0.1% acetic acid in saline.

2.3. Surgery

The rats were anaesthetized with pentobarbital sodium (40 mg/kg) and placed in a stereotaxic apparatus (Narishige Scientific Instrument Lab., Tokyo, Japan). Stainless-steel guide cannulae (Eicom, Kyoto, Japan) were implanted into the right lateral ventricle (0.2 mm posterior to the bregma, 1.5 mm lateral to the midline, and 3.5 mm ventral to the dura) and the fourth ventricle (9.8 mm posterior to the bregma, ± 0.0 mm lateral to the midline, and 6.5 mm ventral to the dura) of rats, using stereotaxic coordinates according to the atlas of Pellegrino et al. (1979). The cannulae were fixed to the skull with dental cement. Experiments were performed 5 days after surgery.

2.4. Behavioral tests

The behavioral test was conducted in a quiet room. An injection into the lateral ventricle or the fourth ventricle was carried out with a Hamilton 25-μl syringe connected by means of a polyethylene tube to a stainless-steel guide cannula, which was carefully inserted into the fixed guide cannula. Endothelin-1 (10–100 ng/rat) was injected into the lateral ventricle or the fourth ventricle, in a total volume of

10 μl over 1 min as the sole treatment or in combination with BQ-123, BQ-788, MK-801, CNQX, or verapamil. Endothelin-1 (50 ng/rat) was administered 10 min after treatment with BQ-123, BQ-788, MK-801, CNQX, and verapamil, also in a total volume of 10 μl over 1 min. After the injection of drugs, two observers recorded the incidence and latent period of the barrel rotation. After the experiments, the positioning of the cannula was checked histologically.

2.5. c-Fos immunocytochemistry

Two hours following the injection of endothelin-1 (50 ng/rat), the animals were perfused transcardially with 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 containing 0.2% picric acid under deep anesthesia (pentobarbital sodium 40 mg/kg, i.p.). The brains were removed and postfixed in the perfusion fixative overnight and then left in 20% sucrose. Serial 40-μm thick coronal sections were cut with a freezing microtome and immersed in 0.1 M phosphate-buffered saline (PBS). The immunocytochemical detection of c-Fos was performed with the peroxidase–antiperoxidase (PAP) detection system. To insure penetration of antibodies, the sections were preincubated in a solution of 0.3% Triton X-100 in PBS after blocking of endogenous peroxidase. Free-floating sections were then incubated for 3 days at 4 °C with rabbit anti-c-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:5000 in PBS containing 0.3% Triton X-100. The sections were incubated in a 1:2000 dilution of goat anti-rabbit immunoglobulin G (EY Laboratories, San Mateo, CA, USA) for 2 days at 4 °C and in a 1:2000 dilution of peroxidase–antiperoxidase (DAKO, Carpinteria, CA, USA) for 1 day at 4 °C. The primary, secondary, and third antibodies were diluted to appropriate concentrations in 0.3% bovine serum albumin and 1% normal goat serum. Between incubation steps, sections were thoroughly washed with PBS. For visualization of peroxidase, the sections were treated for 15 min at room temperature with 0.02% diaminobenzidine in 0.003% hydrogen peroxide. After final rinses in 0.05 M Tris–HCl buffer, pH 7.6, the sections were mounted onto gelatin–chrome alum-coated glass slides, air dried, dehydrated with ethanol, cleared in xylene, and coverslipped. For the detailed examination of the vestibular nuclei, another series of sections (not processed by the immunocytochemical procedures) were stained with thionine for cytoarchitectural study.

A semi-quantitative analysis was performed using the NIH image software (Version 1.60) and a Power Macintosh (8600/250). The specificity of the immune reaction was assessed by absorption of the primary antiserum with an excess of c-Fos and by use of this absorbed antiserum in place of the primary antiserum as described above. Immunostaining controls were carried out by substitution of the primary antiserum with normal rabbit serum and absorbed primary antiserum. In these experiments, no immunoreactivity was found in the above-detected sections.

2.6. 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 the injections of drugs into the lateral ventricle and fourth ventricle, 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 the barrel rotation latency was evaluated using the unpaired *t*-test (Stat View system, Abacus Concept, Berkeley, CA, USA). The mean number of c-Fos-immunoreactive cells per area was statistically analyzed by means of an analysis of variance (ANOVA) completed with Fisher's protected least significant difference (PLSD) analysis and two-tailed Student's *t*-test (Stat View system).

3. Results

3.1. Induction of the barrel rotation by endothelin-1: injections into the lateral ventricle versus the fourth ventricle

The effects of endothelin-1 on the incidence of barrel rotation are illustrated in Fig. 1. Injections of endothelin-1 (30–100 ng/rat) into the lateral ventricle resulted in a dose-dependent increase in the barrel rotation. Following the injection of saline, 30, or 50 ng endothelin-1, no barrel rotation was observed, while 50% of rats showed barrel rotation following the 60 ng dose. Of the rats receiving doses of 70 and 100 ng endothelin-1, 100% showed barrel rotation ($P < 0.01$) (Fig. 1A). On the other hand, injections of endothelin-1 (10–100 ng/rat) into the fourth ventricle resulted in a dose-dependent increase in barrel rotation. Following the injection of saline or 10 ng endothelin-1, no barrel rotation was observed, while 50% of rats showed the barrel rotation following the 30 ng dose. Of the rats receiving doses of 50, 70, and 100 ng endothelin-1, 85%, 100%, and 100% showed the barrel rotation, respectively ($P < 0.01$) (Fig. 1B). The mean latent periods until the barrel rotation after injections of endothelin-1 into the lateral ventricle and the fourth ventricle were 117.1 ± 17.6 and 40.8 ± 2.2 s, respectively ($P < 0.01$) (Fig. 1C).

3.2. Effects of various antagonists on the barrel rotation induced by injections of endothelin-1 into the fourth ventricle

As shown in Table 1, the barrel rotation induced by the fourth ventricular injection of endothelin-1 (50 ng/rat) was dose-dependently inhibited by the pretreatment with the endothelin ET_A receptor antagonist, BQ-123 (0.5–10 μg/rat), but not by the endothelin ET_B receptor antagonist, BQ-788 (1 μg/rat). In addition, the rotational behavior was dose-

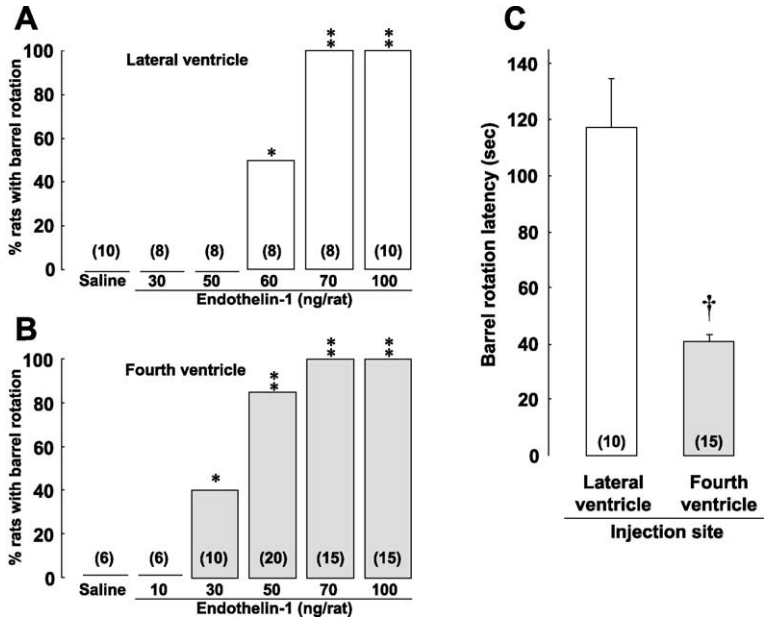

Fig. 1. Induction of barrel rotation by injection of endothelin-1 (10–100 ng/rat) into the lateral ventricle or the fourth ventricle in rats. Response is expressed as a percentage of the number of rats treated with drugs that showed rotation and were statistically evaluated by means of 2 × 2 contingency tables and with Fisher's exact probability test (A, B). Barrel rotation latency (s) is expressed as a mean ± S.E. and was statistically evaluated with the unpaired *t*-test (C). The number of rats in each group is indicated in parentheses. $^{*}P<0.05$, $^{**}P<0.01$, significant difference from saline group. $^{\dagger}P<0.01$, significant difference from the lateral ventricle injection group.

dependently inhibited by the pretreatment with the glutamate NMDA receptor antagonist, MK-801 (1–20 μg/rat), but not by the glutamate non-NMDA receptor antagonist, CNQX (1 μg/rat). Moreover, the pretreatment with the L-type Ca^{2+} channel antagonist, verapamil (1–5 μg/rat), showed a decrease in the barrel rotation induced by endothelin-1 in a dose-dependent manner.

Table 1
Effects of endothelin, glutamate receptor, and L-type calcium channel antagonists on the barrel rotation induced by injection of endothelin-1 (50 ng/rat) at the fourth ventricle in rats

	Dose (μg/rat)	Number	% Rats with barrel rotation
Saline		6	100
BQ-123	0.1	6	100
	0.5	9	56.6[a]
	1	9	33.3[a]
	10	6	0[b]
BQ-788	1	6	100
MK-801	0.1	6	100
	1	6	66.7[a]
	10	10	50[a]
	20	6	0[b]
CNQX	1	6	100
Verapamil	0.1	6	100
	1	6	33.3[a]
	5	6	0[b]

Response is expressed as a percentage of the number of rats treated with drugs that showed rotation.
Statistical analysis was performed using Fisher's exact probability test.
[a] Statistically different from the saline group at $P<0.05$.
[b] Statistically different from the saline group at $P<0.01$.

3.3. c-Fos expression in the rat vestibular nuclei

Two hours after a single injection of saline into the fourth ventricle, we observed only a few c-Fos-immunoreactive neurons in the vestibular nuclei (the medial, spinal, superior, and lateral vestibular nuclei), as shown in Fig. 2 (A1–A4). On the other hand, abundant c-Fos-immunoreactive nerve cells were observed in each vestibular nucleus after injection of endothelin-1. In the medial vestibular nucleus, we found the highest density of c-Fos-immunoreactive neurons. c-Fos-labeled neurons in the other vestibular nucleus showed a moderate expression in comparison with the saline-injected group Fig. 2 (B1–B4). Massive c-Fos-immunoreactive nerve cells were presented in the endothelin-1 group compared with the saline group in each vestibular nucleus.

Fig. 3 shows semi-quantitative evaluation plots of c-Fos-positive cells for each of the vestibular nuclei in rats treated with saline or endothelin-1. In each vestibular nucleus, a marked difference in the number of c-Fos-labeled neurons was observed between both treated groups ($P<0.01$). In particular, a significant difference in the c-Fos expression was shown in the medial vestibular nucleus ($P<0.0005$).

3.4. Effects of various antagonists on the c-Fos expression induced by endothelin-1 in the vestibular nuclei

Table 2 shows semi-quantitative evaluations of the effects of pretreatment with BQ-123 (10 μg/rat), MK-801 (20 μg/rat), and verapamil (5 μg/rat) on the number of the c-Fos-immunoreactive cell counts at the vestibular nuclei after

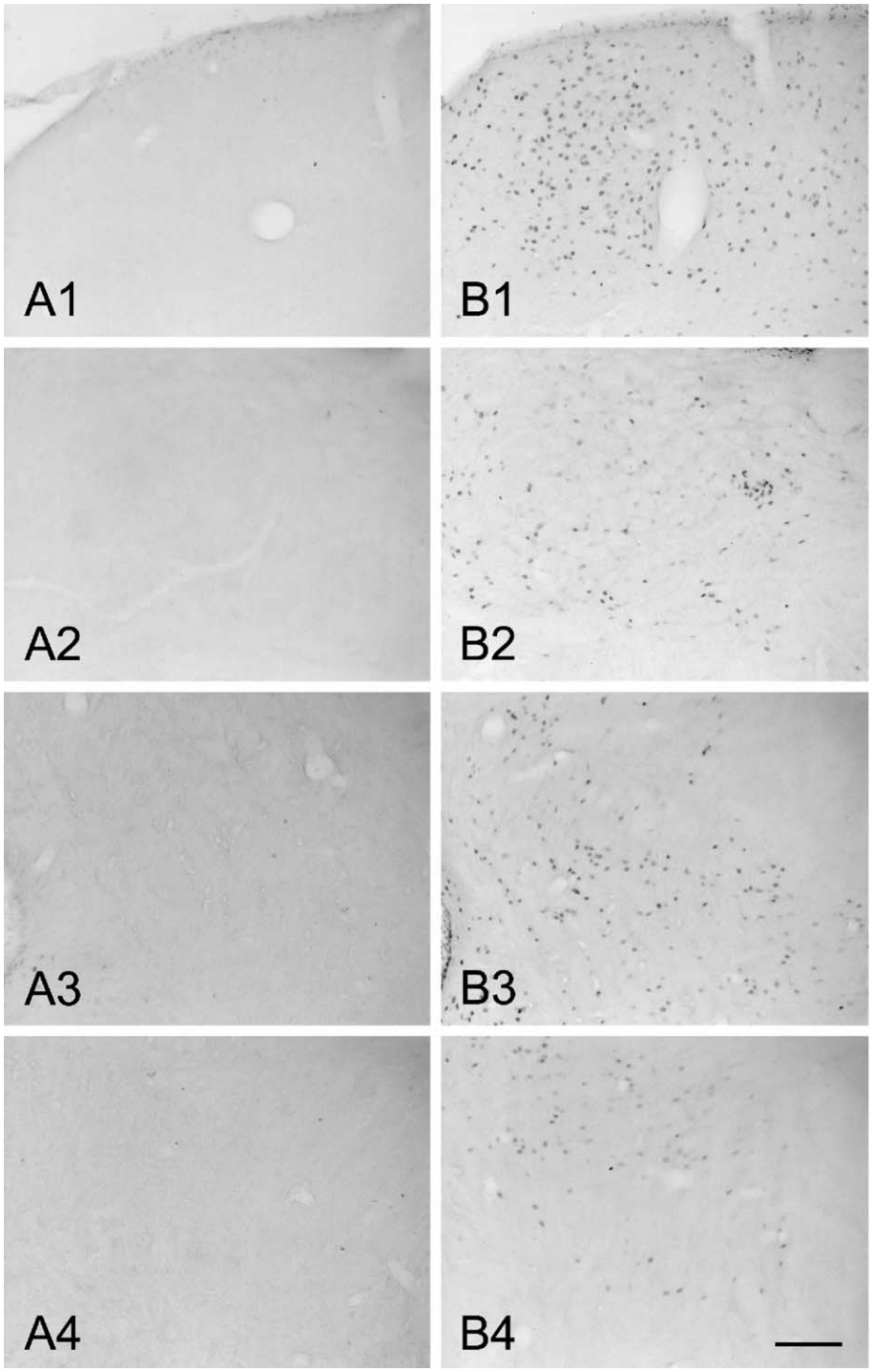

Fig. 2. c-Fos immunoreactivity in the rat vestibular nuclei (A1, B1: the medial vestibular nucleus; A2, B2: the spinal vestibular nucleus; A3, B3: the superior vestibular nucleus; A3, B3: the lateral vestibular nucleus) after the fourth ventricular injection of endothelin-1 (50 ng/rat). Photomicrograph montages of representative 40-μm brain sections in the vestibular nuclei from rats killed 2 h after the injections of saline (A1–A4) or endothelin-1 (B1–B4) (scale bar: 100 μm). Strong c-Fos-positive cells were observed in the endothelin-1-treated rats.

the injection of endothelin-1(50 ng/rat) into the fourth ventricle in rats. Control rats, receiving the pretreatment with saline prior to injection of endothelin-1, showed abundant c-Fos-positive cells in the medial, spinal, superior, and lateral vestibular nuclei. In comparison with the endothelin-1 injection alone, there was no significant difference in c-Fos-immunoreactive cell counts. In rats pretreated with BQ-123, MK-801, and verapamil, c-Fos expression after the injection of endothelin-1 was not nearly observed in the rat medial, spinal, superior, and lateral vestibular nuclei

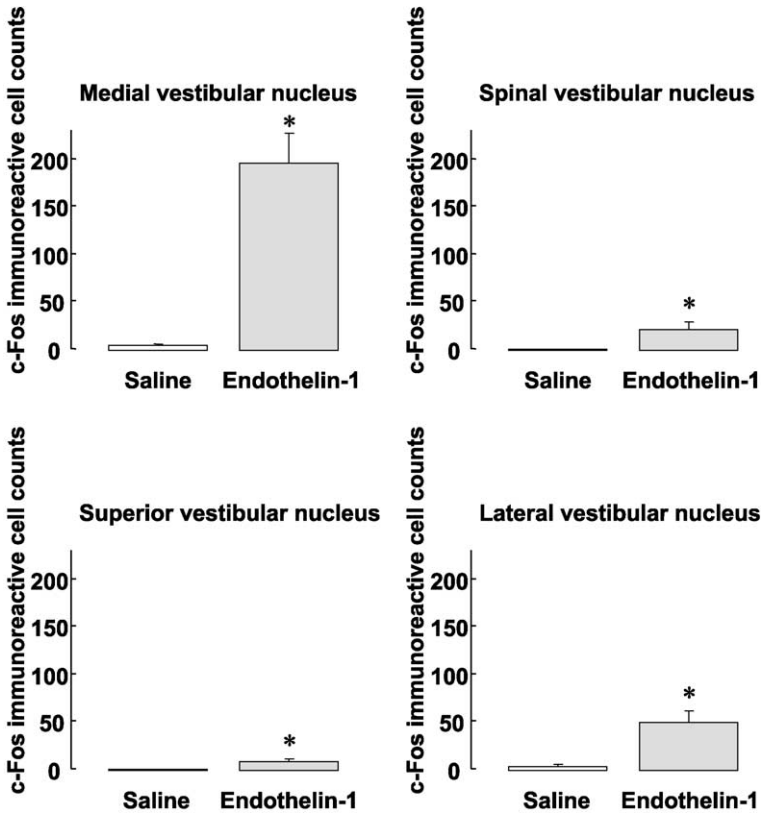

Fig. 3. Effect of endothelin-1 (50 ng/rat) on the number of c-Fos-positive cells in the medial vestibular nucleus, spinal vestibular nucleus, superior vestibular nucleus, and lateral vestibular nucleus. *$P<0.01$, significant difference from the saline-treated group. Statistical analysis was performed using a one-way ANOVA followed by Fisher's PLSD test.

($P<0.01$). c-Fos expression pretreated with those antagonists was almost equivalent to the level of the saline-injected group. c-Fos-immunoreactive cell counts were almost totally absent in rats after an injection of BQ-123, MK-801, and verapamil without injection of endothelin-1 (data not shown).

Table 2
Effects of pretreatment with BQ-123 (10 μg/rat), MK-801 (20 μg/rat) and verapamil (5 μg/rat) on the c-Fos expression at the vestibular nuclei induced by injection of endothelin-1 (50 ng/rat) in the fourth ventricle in rats

Vestibular nuclei	Endothelin-1			
	Saline (4)	BQ-123 (4)	MK-801 (4)	Verapamil (4)
Medial vestibular nucleus	197.4 ± 31.0	4.3 ± 1.2[a]	10.0 ± 2.4[a]	7.3 ± 2.2[a]
Spinal vestibular nucleus	22.1 ± 7.8	1.0 ± 0.6[a]	1.0 ± 0.4[a]	1.2 ± 0.4[a]
Superior vestibular nucleus	10.0 ± 1.7	1.3 ± 0.5[a]	1.3 ± 0.5[a]	0.7 ± 0.3[a]
Lateral vestibular nucleus	51.1 ± 11.3	2.8 ± 0.7[a]	3.0 ± 1.1[a]	1.3 ± 0.4[a]

Values represent the means ± S.E. of c-Fos-positive cells counted in the entire area of the structure for the number of rats in parentheses.
Statistical analysis was performed using a one-way analysis of variance followed by post hoc multicomparison test (Fisher's PLSD).
[a] Statistically different from saline + endothelin-1-treated group at $P<0.01$.

3.5. The difference in the c-Fos expression between the right and left vestibular nuclei

The relation between the direction of the barrel rotation and expression of c-Fos-positive cells are illustrated in Fig. 4. In the rats with the counterclockwise rotation, the right medial vestibular nucleus was found to have significantly higher numbers of c-Fos-immunoreactive cells compared with the left (the medial vestibular nucleus: 22.5 ± 11.1 cells/section in the left versus 120.8 ± 15.5 cells/section in the right, $P<0.01$; Fig. 4A). In contrast, in the rats with the clockwise rotation, the left medial vestibular nucleus were found to have significantly higher numbers of c-Fos-immunoreactive cells compared with the right (the medial vestibular nucleus: 126.2 ± 38.2 cells/section in the left versus 62.0 ± 24.0 cells/section in the right, $P<0.01$; Fig. 4B). The number of c-Fos-labeled neurons in the medial vestibular nucleus was markedly increased on the opposite side of the rotational direction. However, no significant changes were observed upon the c-Fos-immunoreactive cell counts between the left and right at the spinal, superior, and lateral vestibular nuclei in the rats with the clockwise or counter-

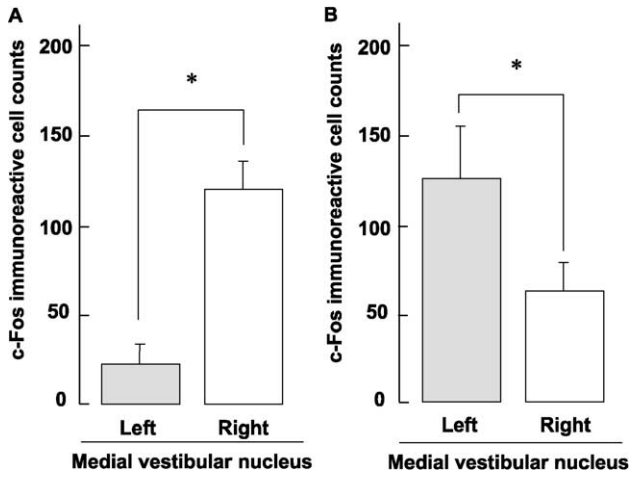

Fig. 4. The direction of the barrel rotation and the imbalance of the neuronal activity between the right and left vestibular nuclei. (A) Counterclockwise rotation group. (B) Clockwise rotation group. Three rats were used for each group and the values are expressed as means ± S.E. *$P < 0.01$, significant difference between left and right vestibular nuclei. Statistical analysis was performed using the two-tailed Student's *t*-test.

clockwise rotation (the clockwise rotation: the spinal vestibular nucleus, 7.3 ± 2.8 cells/section in the left versus 14.5 ± 4.6 cells/section in the right; the superior vestibular nucleus, 5.5 ± 1.2 cells/section versus 6.8 ± 2.6 cells/section; the lateral vestibular nucleus, 19.0 ± 7.2 cells/section versus 20.0 ± 10.8 cells/section; the counterclockwise rotation: the spinal vestibular nucleus, 4.1 ± 2.1 cells/section in the left versus 3.3 ± 2.2 cells/section in the right; the superior vestibular nucleus, 3.1 ± 1.3 cells/section versus 1.6 ± 0.6 cells/section; the lateral vestibular nucleus, 11.0 ± 5.0 cells/section versus 23.0 ± 7.9 cells/section).

4. Discussion

In the present study, the glutamate NMDA receptor antagonist, MK-801, and the L-type Ca^{2+} channel antagonist, verapamil, completely prevented the incidence of the barrel rotation induced by endothelin-1 injection into the fourth ventricle, suggesting the involvement of glutamate NMDA receptors, and L-type Ca^{2+} channels, which are the two main routes for extracellular Ca^{2+} to enter into neural cells. These findings support those of previous studies showing that the barrel rotation induced by endothelin-1 injection into the lateral ventricle was blocked by the glutamate NMDA antagonist, D,L-2-amino-5-phosphonovalerate, and the L-type Ca^{2+} channel antagonist, nimodipine (Gross et al., 1993, 1994; Maione et al., 1993). We previously reported that the endothelin ET_A receptor antagonist, BQ-123, prevented endothelin-1-induced barrel rotation but not vasopressin V_1 receptor antagonists. In addition, vasopressin V_1 receptor antagonists prevented vasopressin-induced barrel rotation but not BQ-123 (Kawachi et al., 1998). Thus, trigger receptors for the induction of barrel rotation following injections of vasopressin and endothelin-1 may be different. Furthermore, we demonstrated that MK-801 and the L-type Ca^{2+} channel antagonists, nifedipine and verapamil, prevented the barrel rotation induced by the angiotensin AT_1 receptor antagonist, losartan, suggesting that glutamate NMDA receptors and L-type Ca^{2+} channels are involved in the barrel rotation induced by losartan (unpublished data). Some studies suggested that the cerebral stimulatory effects of the injection of endothelin-1 or dynorphin-A including the barrel rotation are mediated by glutamate NMDA receptors (Gross et al., 1993; Maione et al., 1993; Shukla et al., 1997). Thus, the present results showing MK-801 and verapamil prevented the barrel rotation induced by endothelin-1 indicate glutamate NMDA receptors and L-type Ca^{2+} channels are essential for the induction of rotational behavior.

BQ-123 also prevented the incidence of the barrel rotation induced by endothelin-1 injection into the fourth ventricle but not the endothelin ET_B receptor antagonist, BQ-788, which showed the involvement of endothelin ET_A receptor in mediating the action of endothelin-1 on rotational behavior. The present findings support those that showed the barrel rotation induced by endothelin-1 was blocked by the endothelin ET_A receptor antagonist, FR139317 (Gross et al., 1994).

Some studies reported a physiologic common point to both endothelin-1 and vasopressin that hypothermia occurred in rats following intracerebroventricular injection of endothelin-1 or vasopressin (Gross and Weaver, 1993; Diamant and De Wied, 1993). Moreover, Diamant and De Wied (1993) found hypothermia to coincide with the occurrence of barrel rotation and suggested that these effects may be related.

The present findings demonstrated that the mean latent period to the barrel rotation after the injection of endothelin-1 into the fourth ventricle was markedly shorter than those into the lateral ventricle. In the fourth ventricle injection, the doses inducing barrel rotation were lower compared with the lateral ventricle injections. Despite the different route of administration, the rotational behavior was identical between both groups. These observations provide important clues to explore the core region of barrel rotation, suggesting that neural cells neighboring the fourth ventricle are involved in the rotational behavior.

The brainstem vestibular complex coordinates a wide variety of input–output connections, and these connections afford the vestibular nuclei a major role in the maintenance of balance and equilibrium (Rubertone et al., 1995). We found that abundant c-Fos-immunoreactive cells are expressed in the vestibular nuclei that lie adjacent to the fourth ventricle after the induction of barrel rotation following endothelin-1 injection. In addition, we observed that the increased c-Fos expression induced by endothelin-1 was significantly attenuated by the pretreatment with BQ-123, MK-801, or verapamil, in addition to the incidence of rotational behavior.

One important aspect of the present study was the different patterns of c-Fos expression at the vestibular nuclei following the injection of endothelin-1 into the fourth ventricle. In this study, we have shown that marked c-Fos expression occurred in the medial vestibular nucleus contralateral to the rotational direction, suggesting that the barrel rotation is evoked toward the opposite side that shows relatively high c-Fos immunoreactivity. The greater c-Fos-positive cells in the contralateral medial vestibular nucleus can be explained by the increased neuronal activity in the contralateral medial vestibular nucleus resulting from increased excitatory input via commissural connections from the ipsilateral medial vestibular nucleus. These results suggest that the direction of the barrel rotation has a deep connection with the imbalance of the neuronal activity in the medial vestibular nucleus. Pan et al. (1998) argued that the barrel rotation was induced by unilateral labyrinthectomy in rats, and suggested that the rotational behavior was attribute to the vestibular system. Several studies have demonstrated that unilateral labyrinthectomy induced c-Fos expression in the medial vestibular nucleus contralateral to the unilateral labyrinthectomy side and rats rotated to the unilateral labyrinthectomy side (Kaufman et al., 1992; Duflo et al., 1999; Kitahara et al., 2000; Fukushima et al., 2001). Furthermore, Takeshita et al. (1999) proposed that most medial vestibular nucleus neurons either exhibited an increase or a decrease in firing during tilting ipsi- or contralateral to the recording side, respectively (α type), or the opposite responses (β type), and also strongly suggested that glutamate was involved in the neurotransmission of otolith information to medial vestibular nucleus neurons. The present results are consistent with these previous findings. The present findings suggest that the activation of the vestibular nuclei induced by endothelin-1 elicited the barrel rotation, but the cause of the different c-Fos immunoreactivity produced between the right and left vestibular nuclei is unclear. It was also possible that the rotational behavior activated the vestibular nuclei. Nevertheless, our recent experiments showed that unilateral lesions of the vestibular nuclei (radiofrequency lesion: 70 °C, 45 s) induced the barrel rotation, and that the rotational behavior was evoked toward the lesion side, suggesting that the impairment of transmission caused by equilibrium paralysis in the medial vestibular nucleus induced the rotational behavior.

In conclusion, the present findings provide new and important information on the functional significance of vestibular neurons in the barrel rotation induced by endothelin-1. It is suggested that the barrel rotation could be useful for the investigation of the neurotransmitter system in the control of vestibular neurons that convey this information to the ocular nuclei, the cerebellum, and the spinal cord to maintain body balance and posture. These findings suggest that endothelin ET_A receptors, glutamate NMDA receptors, and L-type Ca^{2+} channels in the vestibular nuclei are involved in the triggering of endothelin-1-induced barrel rotation, and that the medial vestibular nucleus are attributed to the direction of the rotational behavior. Barrel rotation may be impairment of ocular movement and postural reflex due to an imbalance between the activity of the left and right vestibular nuclei as a result of impairment of the vestibular input to the brainstem vestibular nuclei.

References

Bauters, C., de Groote, P., Adamantidis, M., Delcayre, C., Hamon, M., Lablanche, J.M., Bertrand, M.E., Dupuis, B., Swynghedauw, B., 1992. Proto-oncogene expression in rabbit aorta after wall injury. First marker of the cellular process leading to restenosis after angioplasty? Eur. Heart J. 13, 556–559.

Bax, W.A., Saxena, P.R., 1994. The current endothelin receptor classification: time for reconsideration? Trends Pharmacol. Sci. 15, 379–386.

Becker, K., Erdbrügger, W., Heinroth-Hoffmann, I., Michel, M.C., Brodde, O.E., 1996. Endothelin-induced inositol phosphate formation in rat kidney. Studies on receptor subtypes, G-proteins and regulation during ontogenesis. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 572–578.

Cheng, S.B., Kuchiiwa, S., Nagatomo, I., Akasaki, Y., Uchida, M., Tominaga, M., Hashiguchi, W., Kuchiiwa, T., Nakagawa, S., 2002. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin treatment induces c-Fos expression in the forebrain of the Long-Evans rat. Brain Res. 931, 176–180.

Chew, B.H., Weaver, D.F., Balaban, C.D., Gross, P.M., 1994. NMDA mediated metabolic activation of the cerebellar cortex in behaving rats by the neuropeptide endothelin-1. Brain Res. 647, 345–352.

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

Diamant, M., De Wied, D., 1993. Simultaneous occurrence of barrel rotation and hypothermia in rats following central injection of AVP. Ann. N.Y. Acad. Sci. 689, 582–588.

Doi, K., Tsumoto, T., Matsunaga, T., 1990. Actions of excitatory amino acid antagonists on synaptic inputs to the rat medial vestibular nucleus: an electrophysiological study in vitro. Exp. Brain Res. 82, 254–262.

Dokas, L.A., Zwiers, H., Coy, D.H., Gispen, W.H., 1983. Somatostatin and analogs inhibit endogenous synaptic plasma membrane protein phosphorylation in vitro. Eur. J. Pharmacol. 88, 185–193.

Dragunow, M., Faull, R., 1989. The use of c-Fos as a metabolic maker in neuronal pathway tracing. J. Neurosci. Methods 29, 261–265.

Duflo, S.G.D., Gestreau, C.B.T., Lacour, M., 1999. Fos expression in the cat brainstem after unilateral vestibular neurectomy. Brain Res. 824, 1–17.

Endoh, M., Fujita, S., Yang, H.T., Talukder, M.A., Maruya, J., Norota, I., 1998. Endothelin: receptor subtypes, signal transduction, regulation of Ca^{2+} transients and contractility in rabbit ventricular myocardium. Life Sci. 62, 1485–1489.

Fukushima, M., Kitahara, T., Takeda, N., Saika, T., Uno, A., Kubo, T., 2001. Role of cholinergic mossy fibers in medial vestibular and prepositus hypoglossal nuclei in vestibular compensation. Neuroscience 102, 159–166.

Furuya, N., Koizumi, T., 1998. Neurotransmitters of vestibular commissural inhibition in the cat. Acta Oto-Laryngol. 118, 64–71.

Gilron, I., Quirion, R., Coderre, T.J., 1999. Pre- versus postformalin effects of ketamine or large-dose alfentanil in the rat: discordance between pain behavior and spinal Fos-like immunoreactivity. Anesth. Analg. 89, 128–135.

Gross, P.M., Weaver, D.F., 1993. A new experimental model of epilepsy based on the intraventricular injection of endothelin. J. Cardiovasc. Pharmacol. 22 (Suppl. 8), 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., Beninger, R.J., Shaver, S.W., Wainman, D.S., Espinosa, F.J.,

Weaver, D.F., 1993. Metabolic and neuroanatomical correlates of barrel-rolling and oculoclonic convulsions induced by intraventricular endothelin-1: a novel peptidergic signaling mechanism in visuovestibular and oculomotor regulation? Exp. Brain Res. 95, 397–408.

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.

Gustave, D.D.S., Gestreau, C., Tighilet, B., Lacour, M., 2000. Fos expression in the rat brain after exposure to gravito-inertial force changes. Brain Res. 861, 333–344.

Harvey, S.C., Li, X., Skolnick, P., Kirst, H.A., 2000. The antibacterial and NMDA receptor activating properties of aminoglycosides are dissociable. Eur. J. Pharmacol. 387, 1–7.

Herrera, D.G., Robertson, H.A., 1996. Activation of c-Fos in the brain. Prog. Neurobiol. 50, 83–107.

Hol, E.M., Hermens, W.T., Verhaagen, J., Gispen, W.H., Bär, P.R., 1993. Alpha MSH but not Org 2766 induces expression of c-Fos in cultured rat spinal cord cells. NeuroReport 4, 651–654.

Hoyer, D., Waeber, C., Palacios, J.M., 1989. [^{125}I]endothelin-1 binding sites: autoradiographic studies in the brain and periphery of various species including humans. J. Cardiovasc. Pharmacol. 13, S162–S165.

Hunt, S.P., Pini, A., Evan, G., 1987. Induction of c-Fos-like protein in spinal cord neurons following sensory stimulation. Nature 328, 632–634.

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

Kaufman, G.D., Anderson, J.H., Beitz, A.J., 1992. Brainstem Fos expression following acute unilateral labyrinthectomy in the rat. NeuroReport 3, 829–832.

Kawachi, A., Miyashita, M., Motoya, T., Yamada, K., 1998. The angiotensin AT_1 receptor antagonist, losartan, induces barrel rotation in the rat. Eur. J. Pharmacol. 363, 103–106.

Kinney, G.A., Peterson, B.W., Slater, N.T., 1994. The synaptic activation of NMDA receptors in the rat medial vestibular nucleus. J. Neurophysiol. 72, 1588–1595.

Kitahara, T., Fukushima, M., Takeda, N., Saika, T., Kubo, T., 2000. Effects of pre-flocculectomy on Fos expression and NMDA receptor-mediated neural circuits in the central vestibular system after unilateral labyrinthectomy. Acta Oto-Laryngol. 120, 866–871.

Kruse, H., Van Wimersma Greidanus, T.B., De Wied, D., 1977. Barrel rotation induced by vasopressin and related peptides in rats. Pharmacol. Biochem. Behav. 7, 311–313.

Kurumaji, A., Nehls, D.G., Park, C.K., McCulloch, J., 1989. Effects of NMDA antagonists, MK-801 and CPP, upon local cerebral glucose use. Brain Res. 496, 268–284.

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.

Morgan, J.I., Curran, T., 1991. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes Fos and Jun. Annu. Rev. Neurosci. 14, 421–451.

Palkovits, M., 2000. Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. Eur. J. Pharmacol. 405, 161–166.

Pan, J.B., O'Neill, A.B., Hancock, A.A., Sullivan, J.P., Brioni, J.D., 1998. Histaminergic ligands attenuate barrel rotation in rats following unilateral labyrinthectomy. Methods Find. Exp. Clin. Pharmacol. 20, 771–777.

Pellegrino, L.J., Pellegrino, A.S., Cushmann, A.J., 1979. A stereotaxic atlas of the rat brain, Second Ed. Plenum Press, New York.

Peterson, B.W., 1967. Effect of tilting on the activity of neurons in the vestibular nuclei of the cat. Brain Res. 6, 606–609.

Reichenberger, I., Dieringer, N., 1994. Size-related colocalization of glycine and glutamate immunoreactivity in frog and rat vestibular afferents. J. Comp. Neurol. 349, 603–614.

Rubertone, J.A., Mehler, W.R., Voogd, J., 1995. The Vestibular Nuclear Complex, The Rat Neurons System, 2nd ed. Academic Press, California, pp. 773–796.

Saika, T., Kiyama, H., Senba, E., Tohyama, M., 1991. Expression of c-Fos-like protein following unilateral labyrinthectomy. Neurosci. Res. 16, S36.

Schor, R.H., Miller, A.D., 1981. Vestibular reflexes in neck and forelimb muscles evoked by roll and tilt. J. Neurophysiol. 46, 167–178.

Séquier, J.M., Lazdunski, M., 1990. Mast cell degranulating peptide induces the expression of the c-Fos proto-oncogene in hippocampus. Eur. J. Pharmacol. 180, 179–181.

Shukla, V.K., Prasad, J.A., Lemaire, S., 1997. Nonopioid motor effects of dynorphin A and related peptides: structure dependence and role of the *N*-methyl-D-aspartate receptor. J. Pharmacol. Exp. Ther. 283, 604–610.

Takeshita, S., Sasa, M., Ishihara, K., Matsubayashi, H., Yajin, K., Okada, M., Izumi, R., Arita, K., Kurisu, K., 1999. Cholinergic and glutamatergic transmission in medial vestibular nucleus neurons responding to lateral roll tilt in rats. Brain Res. 840, 99–105.

Waele, C.D., Vibert, N., Baudrimont, M., Vidal, P.P., 1990. NMDA receptors contribute to the resting discharge of vestibular neurons in the normal and hemilabyrinthectomized guinea pig. Exp. Brain Res. 81, 125–133.

Walberg, F., Bowsher, D., Brodal, A., 1958. The termination of primary vestibular fibers in the vestibular nuclei of the cat. J. Comp. Neurol. 110, 391–419.

Wenzel, A., Fritschy, J.M., Mohler, H., Benke, D., 1997. NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins. J. Neurochem. 68, 469–478.

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

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.