Korean J Physiol Pharmacol
Vol 8: 133–140, June, 2004

Activation of Vestibular Neurons Projecting to Autonomic Brain Stem Nuclei Following Acute Hypotension in Rats

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The purpose of the present study was to elucidate the possible involvement of the medial vestibular nucleus (MVN) and inferior vestibular nucleus (IVN) following acute hypotension in the vestibulo-autonomic reflex through vestibulosolitary or vestibuloventrolateral projections. Acute hypotension-induced cFos expression was assessed in combination with retrograde cholera toxin B subunit (CTb) tract tracing. After injection of CTb into the solitary region, CTb-labeled neurons were located prominently around the lateral borders of the caudal MVN and medial border of the IVN. The superior vestibular nucleus also had a scattered distribution of CTb-labeled neurons. After injection of CTb toxin into the unilateral VLM, the distributions of CTb-labeled neurons in the MVN and IVN were similar to that observed after injection into the solitary region, although there were fewer CTb-labeled neurons. In the caudal MVN, about 38% and 13% of CTb-labeled neurons were double-labeled for cFos after injection of CTb into the solitary region and the VLM, respectively. In the IVN, 14% and 7% of CTb-labeled neurons were double-labeled for cFos after injection of CTb into the solitary region and the VLM, respectively. Therefore, the present study suggests that acute arterial hypotension may result in activation of vestibulosolitary pathways that mediate behavioral and visceral reflexes, and vestibuloventrolateral medullary pathways that indirectly mediate vestibulosympathetic responses.

Key Words: Hypotension, Nucleus tractus solitarius, Medial vestibular nucleus, c-Fos, Cholera toxin B

INTRODUCTION

The vestibular system can be affected by cardiovascular abnormalities. Vertigo is the most common symptom (> 75%) in patients suffering from vertebrobasilar insufficiency (Kikuchi et al, 1993), and some patients with dizziness, vertigo, or postural imbalance have hyper- or hypotension (Nario et al, 1997). The hyperviscosity syndrome in polycythemia and Waldenstroms disease is accompanied by vestibular symptoms such as dizziness, spontaneous nystagmus, and postural instability (Baer et al, 1985; Andrews et al, 1988; Pujol et al, 1993). Experimental studies have demonstrated that change of blood flow in the vertebrobasilar artery can alter the duration of nystagmus and change directional predominance in the vestibulo-ocular reflex (Matsunaga et al, 1979; Yamamoto et al, 1985).

We recently demonstrated that systemic hypotension induced by infusion of a vasodilator can induce expression of cFos protein in the medial (MVN), inferior (IVN), and superior (SVN) vestibular nuclei (Kim et al, 2003). In particular, the expression of cFos protein in response to hypotension was more pronounced in the caudal aspect of the MVN. cFos protein, an immediate early gene product, is a readily inducible, sensitive, and broadly applicable marker when neurons are excited by extracellular stimuli (Morgan & Curran, 1991). Furthermore, electrophysiological studies have shown that dynamic and resting firing rates of vestibular neurons in the MVN increase in response to systemic hemorrhage (Park et al, 2001). However, physiological significance of the changes in neuronal excitability in the vestibular nuclei by systemic hypotension is yet to be determined.

The vestibular system also appears to have important effects on autonomic functions, including arterial blood pressure, respiratory rate, and gastrointestinal motility (Balaban, 1999). Motion sickness is a clinical symptom that illustrates the interaction between the vestibular and autonomic systems (Money, 1970). Such interactions are reflexive and well organized, and are sometimes called vestibulo-autonomic or vestibulosympathetic reflexes. Lesion studies have demonstrated that the IVN and caudal MVN are crucial for mediating vestibulo-autonomic reflexes (Yates et al, 1993a, b, 1994). Activation of otolith organ receptors, which are sensitive to gravitational force, produces significant and selective increases in cFos expression in the MVN and IVN (Kaufman et al, 1992). Therefore, it has been suggested that activation of the MVN and IVN by acute hypotension may involve the vestibulo-

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ABBREVIATIONS: MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus; VLM, ventrolateral medulla, CTb, cholera toxin B

autonomic reflex.

The neural pathways responsible for vestibulo-autonomic reflexes are not yet fully understood. One area of the brain stem that mediates this reflex is the solitary region, including the nucleus tractus solitarius, vagal motor nucleus, and nucleus intercalatus. These regions receive afferent projections from the MVN and IVN (Balaban & Beryozkin, 1994; Yates et al, 1994; Steinbacher & Yates, 1996; Porter & Balaban, 1997) as well as primary input from internal visceral afferents (Berger, 1979; Kalia & Mesulam, 1980; Altschuler et al, 1989), and relay signals to neurons that regulate autonomic functions (Feldman, 1986; Loewy, 1990).

The ventrolateral medulla (VLM) is divided into rostral, intermediate, and caudal regions (Paxinos & Watson, 1997). Stimulation of the rostral VLM increases arterial blood pressure, whereas stimulation of the caudal VLM induces the opposite cardiovascular effects (Blessing & Reis, 1982). Moreover, the caudal VLM exerts inhibitory control over rostral VLM neurons via gamma-aminobutyric acid-producing (GABAergic) projection (Chalmers, 1992). Involvement of the VLM in cardiovascular regulation may be mediated by connections between the VLM and preganglionic neurons located in the thoracic intermediolateral nucleus (Zagon & Smith, 1993; Hardy et al, 1998; Pyner & Coote, 1998), mainly in the T1-T3 segments. The VLM receives afferent input from the MVN and IVN through direct or indirect projections from relay nuclei in the brain stem (Yates et al, 1991, 1993a, b, 1995). It is generally accepted that the VLM is the final regulator of vestibulosympathetic reflexes in response to vestibular stimulation (Yates, 1996).

Cholera toxin B (CTb) is transported to neuronal cell bodies via axons in a retrograde direction, and can be detected throughout the cytoplasm in proximal and distal dendrites and axons by means of immunohistochemical staining (Ericson & Blomqvist, 1988; Llewellyn-Smith, 1992). CTb is not degraded after it reaches the neuronal cell body, and can readily be detected even several weeks after injection. CTb immunohistochemical staining is highly sensitive and made it possible to visualize pathways that cannot be observed by WGA-HRP or Fluorogold (Luppi et al, 1990; Peyron et al, 1998). The combination of both CTb and cFos immunohistochemistry is a powerful tool for probing the functional significance of autonomic pathways identified in the brain (Chan & Sawchenko, 1994; Horiuchi et al, 1999). In this study, immunohistochemistry was employed to determine whether the MVN and IVN that are activated by acute hypotension were involve in the vestibulo-autonomic reflex through vestibulosolitary or vestibuloventrolateral neural projections.

METHODS

Injection of retrogade tracer

Sprague-Dawley rats weighing 250~350 g were anaesthetized with intraperitoneal injection of chloral hydrate (Merck, Germany). Twelve rats were injected with CTb subunit into the soliatry region (n=6) and VML (n=6). The head was positioned in stereotaxic apparatus (Narishige Instruments, Tokyo, Japan) with head tilted about 30° nose-down and maintained at a constant body temperature of 37°C. Skin was incised, and underlying muscle layers were dissected to expose the occipital bone, atlas, and atlano-occipital membrane. The lower portion of the occipital bone was removed by using microdental drill and rongeurs. The medulla was exposed by removing the atlano-occipital membrane.

A glass micropipette with tip diameter of 10~50 um was filled with 2% CTb (List Biological Labs, USA) dissolved in 50 mM phosphate-buffered saline (PBS). The micropipette was inserted unilaterally into the solitary region (0.5~1 mm rostal to the calamus scriptorius, 0.5~0.7 mm lateral to midline, and 0.6~0.9 mm below the dorsal surface) and VLM (0.7~1.5 mm rostal to the calamus scriptorius, 1.4~1.7 mm lateral to midline, and 2.2~2.5 mm below the dorsal surface) with a steretoxic manipulator under a surgical microscopy. About 10 min after the micropipette insertion, a total of 60~100 nl of 2% CTb was slowly injected by a pressure delivery system (WPI, Sarasota, FL, USA). Following the injection, the micropipette was left in situ for 10 min and withdrawn in order to avoid CTb leakage. After the injection, the craniotomy was packed with Gelfoam and the incision line was sutured. Animals were injected with antibiotic (Gentamicine, 1 mg, i.m.) immediately after surgery. Animals were kept for 4 days before hemorrhage procedures, since retrograde transport proceeds for more than several days.

Induction of acute hypotension

Four days after the CTb injection, all the animals were re-anesthetized with chloral hydrate. The bilateral femoral arteries were then exposed, dissected under a surgical microscope, and polyethylene catheter (PE 50) that contained heparinized saline was inserted into the artery. One catheter was connected to a pressure transducer (Astro-Med, West Warwick, RI, USA), that was connected to a physiograph (Model 7400; Astro-Med, West Warwick, RI, USA), to record arterial blood pressure. The analogue data from the physiograph were stored in a personal computer via a CED 1401 digital recording unit (Cambridge Electronic Design, Cambridge, UK). The other catheter was connected to a 5-ml heparinzed syringe that was mounted on an SP200i peristaltic pump (WPI, FL, USA), which was partly modified to withdraw the syringe.

Baseline measurements of arterial blood pressure were carried out over 1 hour after the surgery. Peristaltic pump with constant velocity of 200 ml per hour retracted the syringe to remove about 4 ml of arterial blood from the femoral artery. This velocity and volume of hemorrhage were empirically determined through several experimental trials, wherein about 50% of baseline blood pressure rapidly dropped within 2 min. Rectal temperature was monitored and maintained at 37°C throughout the experiment, using a thermoregulator and an electronic blanket (Fine Science Tools, Vancouver, BC, Canada).

Double fluorescent immunohistochemical staining

Two hours after acute hypotension, animals were put under deep anesthesia with an overdose of chloral hydrate, perfused transcardially with 100 ml of 1% paraformaldehyde in 0.1 M phosphate buffer (PB), and then perfused with 500 ml of 4% paraformaldehyde dissolved in 0.1 M PB (pH 7.4). The brain was dissected out of the skull, and

postfixed with the same fixative solution for 4 hours at room temperature. Fixed brains were immersed in 30% sucrose in PBS for 2 days at 4°C for cryoprotection, and were sectioned to 30 μm thickness on a freezing microtome (Leica, Germany). Subsequently a series of brain sections from animals was double immunoreacted to CTb and c-Fos. First, the sections were washed for 3×10 minutes in potassium phosphate buffered saline (KPBS), followed by incubation with 5% goat serum (Zymed, USA) for 20 min. After rinse of 3×10 min in KPBS, the sections were incubated overnight at 4°C with rabbit anti-cFos antibody (Oncogene, USA) diluted 1 : 2,000 in KPBS with 0.3% Triton X-100 (TX) and 1.0% bovine serum albumin (BSA). On the next day, the sections were rinsed for 3×10 min with KPBS containing 0.1% TX and 0.3% BSA (KPBS-BT), and then incubated with goat anti-rabbit IgG secondary antibody conjugated with Alexa-594 dye (Molecular Probes, USA; diluted 1 : 1,000 in KPBS-BT) for 60 min at room temperature. After rinse of 3×10 min in KPBS-BT, the sections were incubated again with goat anti-CTb (List Biological Lab, USA; 1 : 1,000 diluted) overnight. On the next day, after rinsing for 3×10 min in KPBS-BT, the sections were incubated with biotinylated anti-goat IgG (Vector, USA; 1 : 1,000 diluted in KPBS-BT), followed by another rinse of 3×10 min in KPBS-BT. The sections were then incubated with ABC-streptavidin horseradish peroxidase (Vector Elite Kit) solution for 60 min, finally incubated with strept-ALEXA 488 (Molecular Probes, USA) 1 : 1,000 for 60 min, rinsed for 3×10 minutes in KPBS-BT, and were mounted on gelatinized slides coverslipped with mount solution that has antifade agent (Biomeda, USA).

The brain tissue sections were examined under a fluorescent microscopy (Olympus X-14; Olympus, Japan), and immunofluorescent images were captured and digitized using a microscope-mounted digital camera and image-capture software (DP-50, Olympus, Japan). After uniform adjustment of the brightness and contrast of each image file, the number of CTb neuron (green), cFLI neurons (red) and double-labeled neurons (yellow) in the vestibular nuclei was quantified according to their color using image analysis software (Image Pro Plus, USA). At least three counts per nucleus were performed for each animal, and the average number of immunoreactive neurons was calculated.

RESULTS

Changes in arterial blood pressure

Before the manipulation of arterial blood pressure, baseline mean arterial blood pressure was 98.3±4.3 mmHg (n =12). An arterial hemorrhage was generated by rapidly removing a relatively large volume of blood (4 ml) from the femoral artery using a peristaltic pump, and this caused blood pressure to rapidly drop within a few seconds to a maximum of ~50% (47.3±5.3 mmHg) occurring within ~2 min. After the hemorrhage, the blood pressure rapidly returned to the baseline.

Distribution of CTb-labeled neurons in the brain stem after injection of CTb into the solitary region

Neurons retrogradely labeled with CTb were predominantly found within the solitary nucleus and spread into the vagal dorsal motor nucleus, nucleus intercalatus, and dorsal aspect of the hypoglossal nucleus. No CTb-labeled neurons were found in the nucleus prepositus, nucleus of Roller, or the MVN longitudinal fasciculus. A large population of CTb-labeled neurons were observed bilaterally in the deep cerebellar nuclei, rostral VLM, locus coeruleus, and rostral pole of the solitary nucleus, with a slight ipsilateral predominance (Fig. 1).

In general, the intensity of CTb immunoreactivity was weaker in the vestibular nuclei than in other brainstem

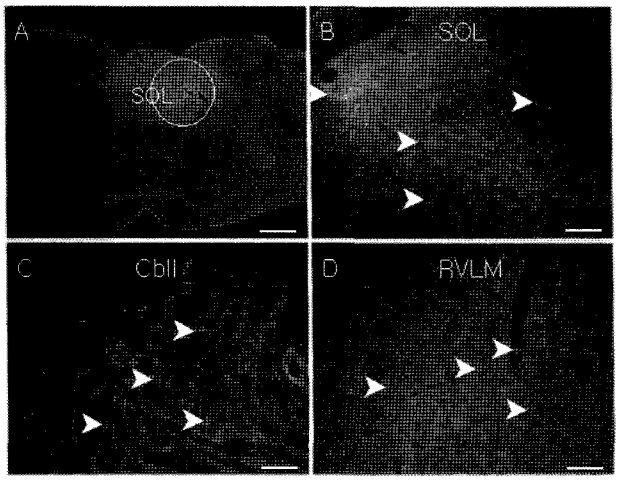

Fig. 1. Fluorescent photographs showing injection site (large circle) of the solitary region (A) and distribution of cholera-toxin B-subunit (CTb) immunoreactive neurons (arrow heads) in the brain stem and cerebellar nuclei (B-D). SOL, solitary nulceus; RVLM, rostral ventrolateral medulla; CbII, deep cerebellar nucelus. Scale bar, A, 500 μm; B-D, 100 μm.

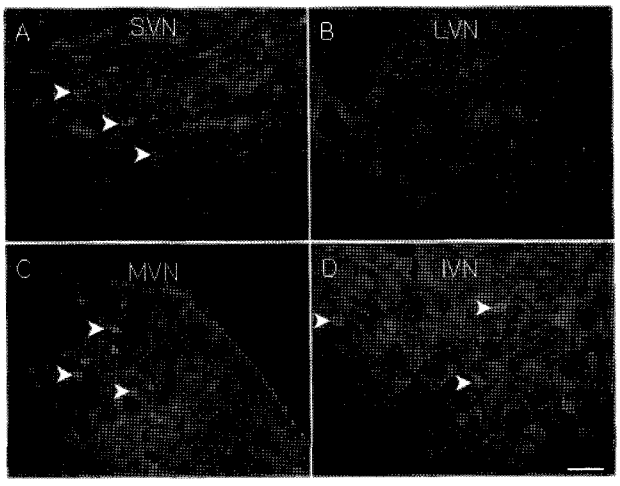

Fig. 2. Fluorescent photographs showing distribution of cholera-toxin B-subunit (CTb) immunoreactive neurons (arrow heads) in the vestibular nuclei of the brain stem 4 days after CTb injection into the solitary region. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus. Scale bar, 100 μm.

nuclei. CTb-labeled neurons were observed bilaterally in the MVN, with an ipsilateral predominance. The caudal aspect of the MVN had a greater number of CTb-labeled neurons than did the rostral MVN. In the caudal MVN, CTb-labeled neurons appeared to be arranged in a line extending ventrodorsally close to the lateral borders of the nuclei, but rarely occupied the ventral portion of the nucleus adjacent to the fourth ventricle. At the level of 11.3 mm from the bregma, the numbers of labeled cells in the MVN ipsilateral and contralateral to the injection site were 35.3±6.5 and 30.2±6.1, respectively (Fig. 2, 3).

In the IVN, CTb-labeled cells were scattered bilaterally, but with an ipsilateral predominance. As observed in the MVN, CTb-labeled neurons were observed mainly on the medial border of the nucleus. The numbers of labeled cells in the IVN ipsilateral and contralateral to the injection sites were 24.5±5.2 and 15.7±4.8, respectively (Figs. 2, 3). The SVN also exhibited scattered CTb-labeled neurons with 9.4±3.4 labeled cells ipsilateral and 7.2±2.2 contralateral to the injection sites (Figs. 2, 3). There was no evidence of retrograde transport of CTb to other regions of the vestibular nuclei.

Distribution of CTb-labeled neurons in the brain stem after injection of CTb into the VLM

CTb injection sites were located within the unilateral VLM and covered the rostrocaudal aspect of the VLM, with some spreading into the neighboring reticular nucleus. Photomicrographs of representative injection sites are shown in panel A of Fig. 4. Cases, in which there was evidence of spreading into the inferior olivary nucleus (ION), were excluded from this study.

After the injection of CTb into the rostrocaudal VLM, the toxin appeared to be transported retrogradely to the cerebellar cortex (bilaterally), ipsilateral solitary nucleus, and contralateral ION (Fig. 4). CTb-labeled neurons were observed bilaterally in the MVN, but with an ipsilateral predominance. The caudal aspect of the MVN had a greater number of CTb-labeled neurons than did the rostral MVN. In the caudal MVN, CTb-labeled neurons were clustered within the central portion and lateral borders close to the IVN and the numbers of labeled cells within 11.6 mm of the bregma were 20.6±6.2 ipsilateral and 15.2±7.2 contralateral to the injection site. In the IVN, CTb-labeled cells were bilaterally scattered with an ipsilateral predominance, and retrogradely labeled neurons were located mainly on the medial border of the IVN and the numbers of labeled cells within 11.6 mm of the bregma were 16.2± 4.5 ipsilateral and 7.4±3.3 contralateral to the injection

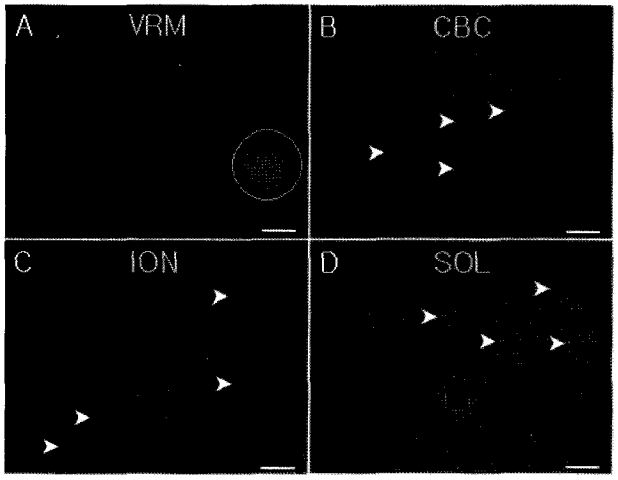

Fig. 4. Fluorescent photographs showing injection site of ventrolateral medulla (A) and distribution of cholera-toxin B-subunit (CTb) immunoreactive neurons (arrow heads) in the brain stem and cerebellar nuclei (B-D). SOL, solitary nucleus; ION, inferior olivary nucleus; CBC, deep cerebellar cortex. Scale bar, A, 500 μm; B-D, 100 μm.

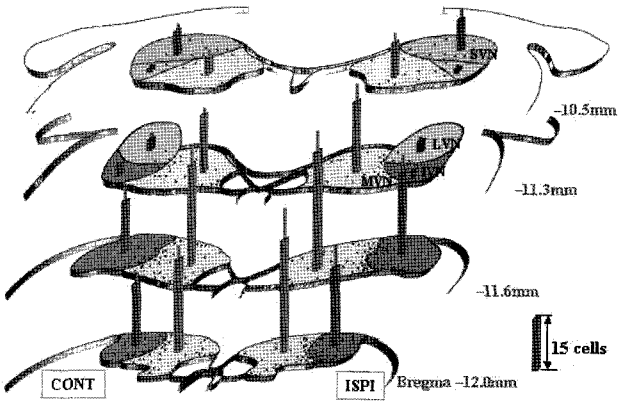

Fig. 3. Schematic illustration showing regional distribution of cholera-toxin B-subunit (CTb) labeling and number of CTb immunoreactive neurons in the vestibular nuclei at four levels of the brain stem after CTb injection into the solitary region. Values are mean±S.D. Number of rats was 6. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus; IPSI, ipsilateral side to injection site; CONT, contralateral side.

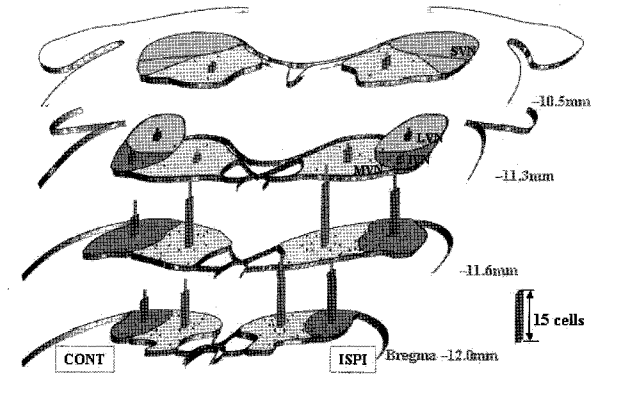

Fig. 5. Schematic illustration showing regional distribution of cholera-toxin B-subunit (CTb) labeling and number of CTb immunoreactive neurons in the vestibular nuclei at four levels of the brain stem after CTb injection into the rostral ventrolateral medulla. Values are mean±S.D. Number of rats was 6. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus; IPSI, ipsilateral side to injection site; CONT, contralateral side.

site. There was no evidence of retrograde transport of CTb to other regions of the vestibular nuclei (Fig. 5).

Distribution of cFos-immunopositive neurons in the vestibular nuclei after acute hemorrhage

The anesthesia and surgery required to record arterial blood pressure without hemorrhage had a minimal effect on the expression of cFos protein in the vestibular nuclei (n=3). By contrast, acute hemorrhage resulted in a significant symmetrical increase of cFos protein expression in the MVN and IVN. In the caudal MVN, cFos-immunopositive neurons were distributed evenly throughout the nucleus, and there were more cFos-immunopositive neurons than in the rostral MVN. There were 130.5±25.2 labeled cells in the unilateral side of the MVN (n=12), which was the highest level of hemorrhage induced cFos expression in the vestibular nuclei. There were 74.5±10.3 cFos-immunopositive cells in the unilateral IVN. cFos-immunoreactivity was also observed in the SVN (37.2±6.5 unilaterally 10.5 mm from the bregma). By contrast, hemorrhage did not increase cFos expression in the lateral vestibular nuclei 90 min after the hemorrhage (Fig. 6).

Distribution of neurons labeled with both cFos and CTb in the vestibular nucleus after hemorrhage

In the caudal MVN, approximately 11% of cFos-immunopositive neurons that were activated by acute hemorrhage were also immunopositive toward CTb transported retrogradely from the solitary region. Conversely, approximately 38% of CTb-labeled neurons also expressed cFos. In the IVN, ablut 4% of cFos-immunopositive neurons also expressed CTb, whereas about 14% of the CTb-labeled neurons were doubly labeled for cFos. In the SVN, about 10% of the cFos-immunopositive neurons and about 27% of the CTb-labeled neurons were double-labeled, respectively (Fig. 7, 9). There were substantial numbers of double-labeled neurons in the rostal aspect of solitary nuclei, RVL, and locus coeruleus after CTb was injected into the solitary region (Fig. 8).

In the caudal MVN, approximately 2% of the cFos-immunopositive neurons that were activated after acute hemorrhage were double-labeled with CTb which was transported from the VLM, and about 13% of the CTb-labeled neurons were double-labeled. In the IVN, about 1%

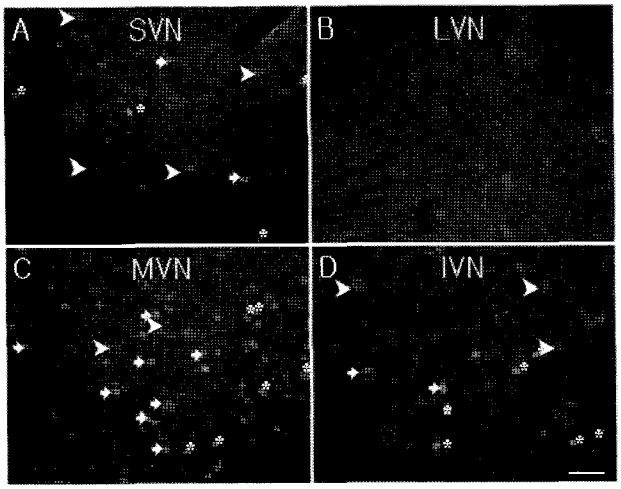

Fig. 7. Fluorescent photographs showing expression of double-labeled neurons for cholera-toxin B-subunit (CTb)/cFos proteins that were induced by acute hypotension in the vestibular nuclei 4 days after CTb injection into the solitary region. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus. Arrow head, CTb(+) neuron; arrow, double-labeled neuron; asterisks, cFos(+) neuron. Scale bar, 100 μm.

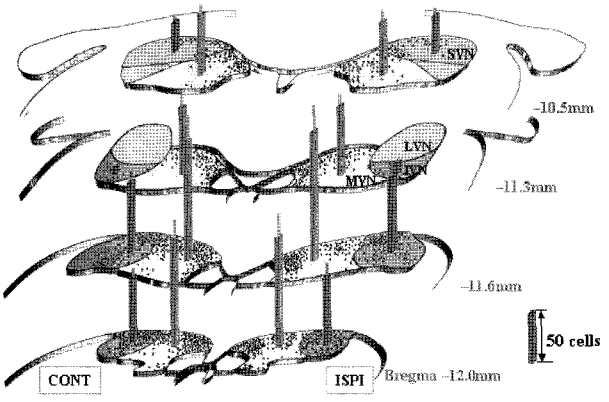

Fig. 6. Schematic illustration showing number of cFos immunoreactive neurons in the vestibular nuclei at four levels of the brain stem 2 hours after acute hemorrhage. Values are mean±S.D. Number of rats was 12. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus; IPSI, ipsilateral side to injection site; CONT, contralateral side.

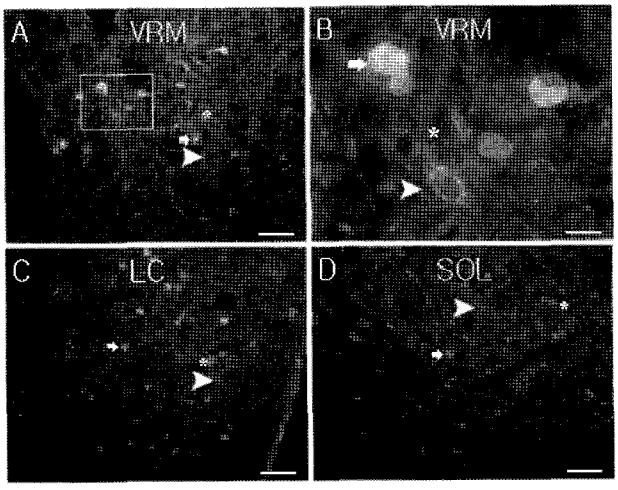

Fig. 8. Fluorescent photographs showing expression of double-labeled neurons for cholera-toxin B-subunit (CTb)/cFos proteins that were induced by acute hypotension in RVLM, SOL, and locus coeruleus (LC) 4 days after CTb injection into the solitary region. B photograph is high magnification view of rectangular area in A photograph. Arrow head, CTb(+) neuron; arrow, double-labeled neuron; asterisk, cFos(+) neuron. Scale bar, A-D, 50 μm; 15 μm.

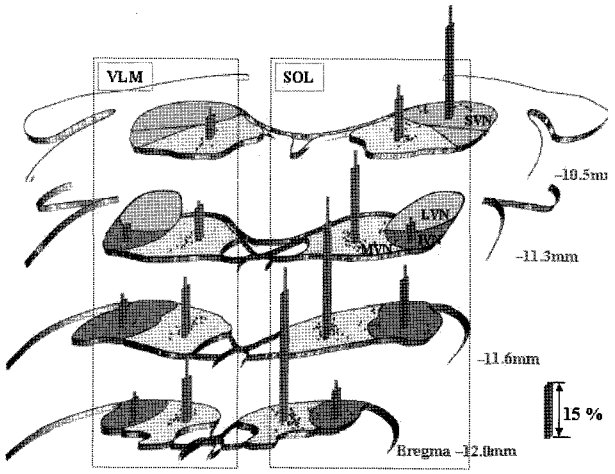

Fig. 9. Bar histographs showing percentage of double-labeled neurons (cFos(+)/CTb(+)) to cholera-toxin B-subunit (CTb(+)) neurons of the vestibular nuclei that were transported from the solitary region (SOL) and ventrolateral medulla (VLM), respectively, following acute hemorrhage. Values are mean ± S.D. Number of rats in each injection was 6. SVN, superior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus

of the cFos-immunopositive neurons were double-labeled with CTb, whereas about 7% of the CTb-labeled neurons were double-labeled after acute hemorrhage (Fig. 9).

DISCUSSION

In the present study, CTb-labeled neurons were found to be clustered around the lateral borders of the caudal MVN, but rarely occupied the ventral aspect of the nucleus after injection of CTb into the solitary region. CTb-labeled cells were scattered bilaterally in the IVN with an ipsilateral predominance. The numbers of labeled cells in caudal MVN and IVN ipsilateral to an injection site ranged from 16 to 21. The SVN also had a scattered population of retrogradely CTb-labeled neurons.

In previous studies on retrograde labeling in rat and rabbit (Balaban & Beryozkin, 1994; Ruggiero et al, 1996; Porter & Balaban, 1997), the authors observed that retrogradely labeled neurons were restricted to caudal levels of the MVN and IVN, but were not found in the lateral ventromedial nucleus or SVN after injections of a retrograde tracer into the NTS and/or dorsal motor nucleus. The numbers of labeled cells in the MVN in those studies ranged from 16 to 29 (Porter & Balaban, 1997). In the IVN, the retrogradely labeled cells had a scattered distribution, but tended to be located ventrally and laterally (Balaban & Beryozkin, 1994). The numbers of labeled cells in the IVN ranged from 12 to 36 (Porter & Balaban, 1997). These observations together with the present results suggest direct nerve fiber projections from the caudal MVN and IVN to the solitary region. These data are further supported by the observation that dense terminations of anterogradely labeled fibers from the caudal MVN and IVN were observed in the lateral ventrolateral and intermediate regions of the nucleus tractus solitarius (NTS), dorsal motor nucleus (DMX), and nucleus intercalatus near the NTS (Ruggiero et al, 1996; Porter & Balaban, 1997). However, the apparent discrepancy in our present finding that CTb-labeled neurons were present in the SVN might have been due to species differences or to spreading of the CTb tracer into other areas, such as the nucleus intercalatus or hypoglossal nucleus.

Some of the caudal and intermedial aspects of the NTS and intercalatus neurons were driven from the labyrinth at short latency from the vestibular nucleus (Yates et al, 1994). In these areas, some vestibular-driven neurons exhibit excitatory responses to cervical or abdominal vagal input. In the dorsal and dorsolateral regions of the NTS, barosensitive neurons that fluctuate it's resting activity in response to oscillations in blood pressure receive convergent vestibular input. Therefore, vestibulosolitary pathways are potential targets for vestibular effects on sympathetic, parasympathetic, and somatic motor brain stem circuits which control respiratory, cardiovascular, and gastrointestinal function through direct efferents.

In the caudal MVN, about one quarter of CTb-labeled neurons were double-labeled for cFos protein, and about 14% of CTb-labeled neurons in the MVN were double-labeled after acute hemorrhage. The NTS receives both excitatory and inhibitory inputs from the peripheral vestibular nerves through vestibulosolitary projections (Yates et al, 1994). In general, cFos protein in neurons is translocated into the nuclei as a consequence of transsynaptic depolarization (Morgan & Curran, 1991). Kim et al (2002) showed that labyrinthectomy did not increase cFos protein expression in vestibular nuclei neurons after acute hypotension which was induced by intravenous injection of a vasodilator, and suggested that activation of vestibular end-organs due to hypotensive ischemia is an essential element for the synthesis of cFos protein in the vestibular nuclei. It is, therefore, highly likely that excitatory neurons are more important than inhibitory neuorns in the MVN for triggering activation of the solitary or vagal nucleus in response to ischemic excitation of the vestibular sensory receptor by hemorrhage.

Approximately 10% of the cFos-immunopositive neurons activated by acute hemorrhage in the caudal MVN and about 4% of the cFos-immunopositive neurons in the MVN were double-labeled for CTb. Unilateral injury or stimulation of the peripheral vestibular system induces cFos protein expression in the vestibular nucleus as well as in several brain stem nuclei that have synaptic circuits within the vestibular system (Kaufman & Perachio, 1994; Cirelli et al, 1996; Kim et al, 2002). About 20% of cFos-immunopositive neurons in the MVN were double-labeled for CTb after injection of the tracer into the vestibulocerebellum following unilateral labyrinthectomy. Most of these double-labeled neurons were dorsomedial or caudally distributed, close to the ventricular surface (Kitahara et al, 1997). There are considerable amount of experimental evidence to support the view that well organized anatomical or physiological neural circuits linking the vestibular nuclei and vestibulocerebellum are involved in the regulation of vestibular and cerebellar functions (De Zeeuw & Berrebi, 1995; Baurle et al, 1997; Kitahara et al, 1997). Accordingly, the present results suggest that, when vestibular nuclei are activated, excitation of vestibulosolitary projections is less important than vestibulocerebellar projection.

In agreement with an earlier study of Stocker et al

(1997), we observed in the present study that, after injection of CTb into the RVL, retrogradely labeled cells were heavily concentrated dorsal and medial to the lateral reticular formation, solitary nucleus, and inferior olivary nucleus. Retrogradely labeled cell bodies were also located within the caudal MVN and adjacent IVN, however, the number of labeled cells in the MVN and IVN following this procedure was lower than after the injection into the NTS. Moreover, about 10% of CTb-labeled neurons in the caudal MVN and IVN were activated, and a few cFos-immunopositive neurons were double labeled with CTb. These patterns of double-labeling for cFos and CTb in the vestibular nucleus are comparable to the results of an earlier study by Horiuchi et al (1999). In the NTS, the number of neurons labeled with CTb from the rostral VLM was greater than the number of cFos-positive neurons; about 3% of the retrogradely-labeled neurons were double-labeled after acute arterial hypotension. Between 15 and 20% of cFos-positive neurons were double-labeled (Horiuchi et al, 1999).

Almost all neurons (80%) in the rostral VLM of cats respond to vestibular nerve stimulation, however, labyrinth stimulation tends to inhibit neural resting activity in a substantial population of rostral VLM neurons that receive vestibular input. Furthermore, the latency of changes of activity in these cells increases after vestibular nerve stimulation, suggesting that they receive vestibular signals through a relay comprised of several synapses (Yates et al, 1991; Steinbacher & Yates, 1996). In addition, the present study further supports the possibility that activation of the vestibular nuclei by acute hypotension or vestibular nerve stimulation might be transmitted to the RVL through indirect rather than direct synaptic pathways. Because the lateral tegmental field and lateral parabrachial nucleus are the major interneuronal sites that connect the vestibular nuclei and VLM (Yates et al, 1995, 1996; Balaban, 1996, 1998), further experiments are needed to elucidate the expression pattern of double-labeling for cFos and CTb in this experimental model.

In summary, acute arterial hypotension may result in activation of vestibulosolitary pathways that mediate behavioral and visceral reflexes, and vestibulorostral VLM pathways that indirectly mediate vestibulosympathetic responses.

ACKNOWLEDGEMENTS

This study was supported by the KOSEF through the Vestibulocochlear Research Center at Wonkwang University and Korea Health R&D Project, Ministry of Health & Welfare (02-PJ1-PG10-21402-0001)

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