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Distribution of Precerebellar Cholecystokininergic Neurons in the Perihypoglossal Nuclei of the Gerbil

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Shih-Hsiung Lee, Chi-Yu Tseng, Chen-Yuan Wen and Jeng-Yung Shieh (2002) Distribution of precerebellar cholecystokininergic neurons in the perihypoglossal nuclei of the gerbil. *Zoological Studies* **41**(2): 162-169. This study aimed to examine the distribution of vermis-projecting cholecystokinin-immunoreactive (CCK-ir) neurons in perihypoglossal (PH) nuclei of the gerbil. Revealed by immunohistochemistry, CCK-ir neurons were mainly distributed in the nucleus of Roller (Ro) and prepositus hypoglossal nucleus (Pr), with fewer cells being located in the nucleus intercalatus (Ic). Small CCK-ir cells made up the majority of cells in the Ro, Ic, and Pr, the remainder being medium-sized. Following multiple injections of horseradish peroxidase (HRP) into the vermal cortex, the majority of CCK-positive neurons in the Ro, Ic, and Pr were labelled. On closer analysis, about 90% of CCK-ir cells in PH nuclei were double labelled. Since the predominant cells in the subnuclei of the PH are CCK-ir, and the subnuclei Ro and Pr are known to give rise to CCK-ir mossy fibers in the vermis of the cerebellum, we conclude that CCK may serve as a putative neurotransmitter or neuromodulator in this projection. http://www.sinica.edu.tw/zool/zoolstud/41.2/162.pdf

Key words: Cholecystokinin, Double-labelling, Perihypoglossal-projecting neuron, Tracer, Rodent.

Perihypoglossal (PH) nuclei as a group are composed of the nucleus of Roller (Ro), nucleus intercalatus (Ic), and prepositus hypoglossal nucleus (Pr) which serve as a relay center for oculomotor control (Fukushima et al. 1992, Godaux et al. 1993). The retrograde degeneration method indicated that cerebellar vermis-projecting neurons originate from the PH of the cat (Torvik and Brodal 1954). Retrograde tracing methods utilizing horseradish peroxidase (HRP), and its combination with lectin, have shown that all subnuclei of the PH project to vermal lobules VI-VIII of the rat, except for the Ic which does not project to lobule VII (Päällysaho et al. 1991). Wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) retrograde transport demonstrated that the Pr and Ro, but not the Ic, participate in the perihypoglossal-vermis connection mainly in lobules VI-VII in the rat (Ohtsuki et al. 1992).

Cholecystokinin (CCK), a brain-gut peptide, is widely distributed in the central nervous system (Vanderhaegen 1975, Innis et al. 1979), but its localization in the PH of the rodent has remained controversial. In situ hybridization studies also showed the lack of CCK mRNA in the PH of the rat (Abelson and Micevych 1991, Schiffmann and Vanderhaeghen 1991, Vanderhaeghen and Schiffmann 1992). Neither CCK immunoreactive (CCK-ir) perikarya nor fibers were identified in the PH of the rat using an immunofluorescence antibody test (Lorén et al. 1979, Kiyama et al. 1983, Kubota et al. 1983, Fallon and Seroogy 1985) and peroxidase-antiperoxidase immunohistochemical staining (Lorén et al. 1979, Vanderhaeghen et al. 1980, Mantyh and Hunt 1984). Contrary to the above cited findings was the detection of CCK-ir fibers in the cerebellar cortex of the opossum (King et al. 1986) and monkey (Yamashita et al. 1990) which appear to originate from some precerebellar CCK-ir cells in the PH of the opossum

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(King and Bishop 1990). This study was undertaken to explore the distribution of CCK-ir cells in the PH of the gerbil using the more-sensitive avidin-biotin complex (ABC) method. Along with this, CCK immunohistochemistry in combination with HRP retrograde tract tracing was also used to localize the origin of the cholecystokininergic vermis-projecting neurons in the PH.

MATERIALS AND METHODS

Experimental animals

Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Animal Center of National Taiwan Univ. Hospital. A total of 14 adult gerbils of either sex weighing from 60 to 80 g was used for CCK immunohistochemistry (n = 8) and the HRP/CCK double-labelling study (n = 6).

Tissue preparation

Animals were anesthetized with 7% chloral hydrate (Ferak, 0.8 ml/100 g body wt.) via an intraperitoneal injection. To enhance the immunoreactive intensity of labelled cells, six out of 8 animals were pretreated intracerebroventricularly with a stereotaxic injection (coordinates: 5.5 mm anterior to the lambda, 1.0 mm to the right of the midline, and 4.0 mm in depth) with 60 µg colchicine (Sigma) in 3 µl of saline for 15 min. On the following day, all animals were reanesthetized, heparinized (Sigma, 1000 IU/kg body wt.), and perfused transcardially with 50 ml of warm Ringer's solution containing sodium nitrite (20 mg/kg) for 3 min, followed by 200 ml of 4% paraformaldehyde (Merck) in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 30 min, and finally with 100 ml of 10% sucrose in PBS for 20 min. The brainstem was immediately removed and stored in 30% sucrose in PBS overnight at 4 ºC. Serial transverse sections of 30 µm in thickness were cut on a cryostat (Bright 5040) and collected into 0.1 M PBS.

CCK immunohistochemistry

Free-floating sections were rinsed with several changes of 0.01 M PBS (pH 7.4). Endogenous peroxidase activity was quenched by initially treating the sections for 60 min at room temperature with PBS containing 3% hydrogen peroxide (H_2O_2) and 10% methanol. Sections were pretreated with 0.4% Triton X-100 (Baker) in PBS for 30 min at room temperature, and then blocked for 60 min at room temperature with PBS containing 2% bovine serum albumin (Sigma) and 3% normal goat serum (Vector). The following protocol is the series of steps of tissue incubation: (a) 24-h incubation with rabbit anti-CCK polyclonal antibody (Sigma) diluted 1: 6000 with 2% bovine serum albumin, 3% normal goat serum, and 0.2% Triton X-100 in PBS; (b) 1-h incubation with a 1: 200 dilution of biotinylated goat anti-rabbit IgG (Vector) prepared with 0.2% Triton X-100 in PBS; and (c) 1-h incubation with ABC (Vector) diluted 1: 100 with 0.2% Triton X-100 in PBS. Following each incubation, sections were rinsed with PBS. Sections were rewashed 3 times with 0.1 M acetate buffer (pH 6.0) and visualized with a glucose-DAB-nickel solution containing 0.001% glucose oxidase (Sigma), 0.05% 3,'3-diaminobenzidine tetrahydrochloride (DAB, Sigma), 2.5% nickel ammonium sulfate, 0.2% β-D-(+)-glucose (Sigma), and 0.04% ammonium chloride for 6 min. The color reaction was then stopped with acetate buffer. The sections were mounted on gelatinized slides, air dried, dehydrated through a series of graded alcohol, cleared with xylene, and covered with Permount. Control for nonspecific staining was carried out in parallel with stained tissues without the primary antibody. Each section was examined with a Zeiss Axiophot microscope under bright field.

HRP/CCK double-labelling method

Under chloral hydrate anesthesia, animals (n = 6) were mounted on a stereotaxic head-holder (David Kopf). A midline scalp incision was made to expose the dorsal surface of the skull. A total of 20 µl of 20% HRP (Sigma Type VI) was delivered by multiple injections into the vermis over 10 min. The injection site was controlled at a 2.0-mm depth with a Hamilton microsyringe. Two days post-operation, surviving animals were reanesthetized with chloral hydrate. The procedures for perfusion, fixation, and freeze-sectioning followed those described above. The composition of the fixative was a mixture of 2% paraformaldehyde and 0.1% glutaraldehyde in 0.l M PBS. Free-floating sections were rinsed several times in 0.1 M Tris-HCl buffer (pH 7.6), incubated for 10 min at room temperature in 0.5% cobalt chloride (Sigma), and rinsed again with Tris-HCl buffer. Following further rinsing in 0.1 M PBS (pH 7.4), sections were visualized in 0.025% DAB and 0.01% H_2O_2

in 0.1 M PBS (pH 7.4) for 10 min at room temperature. Sections were rinsed 3 times in PBS. The steps in the removal of endogenous peroxidase activity, blockade of non-specific binding, incubation of primary and secondary antibodies, and the ABC method followed those described above. The sections were rinsed with Tris-HCl buffer, and incubated for 10 min at room temperature with 0.05 M Tris-HCl buffer containing 0.05% DAB and were then visualized with 0.05 M Tris-HCl buffer containing 0.05% DAB and 0.01% H_2O_2 for 10 min at room temperature. The chromogen precipitation reaction was stopped with PBS. Then these sections were mounted on a slide and examined with a Zeiss microscope. The nomenclature and localization of the medullary nuclei and cerebellum in the gerbil are based on the stereotaxic atlas of the Mongolian gerbil brain (Loskota et al. 1974).

Data analysis

Each section of every animal was studied. Cross-sectional areas of CCK-ir and HRP double-labelled cells were assessed through an Axioplan 2 microscope connected to a digital camera and counted using Image-Pro Plus software. The size composition of these cells was then rated based on their cross-sectional areas. To avoid double counts of the same cell, cells with nuclei were enumerated on the section and then corrected with an equation (Abercrombie 1945). In addition, histograms were made with the aid of Microsoft Excel 97 software. Significant differences in distribution patterns of single- and double-labelled cells in the PH nuclei and their subnuclei were checked by use of the Wilcoxon signed rank test.

RESULTS

Distribution of CCK-immunoreactive neurons in perihypoglossal nuclei

After colchicine pretreatment, the immunostaining of CCK-positive neurons was more intense than that of the nontreated groups. CCK-ir cells were identified in all subnuclei of the PH. On the basis of cross-sectional areas, CCK-ir cells may be classified as small (≤ 175 μm^2) and medium-sized (176-452 μm^2). A modest number of small and medium-sized CCK-ir cells occurred in the caudal Ro (Fig. 1). The cells increased caudorostrally, so that a considerable number of them were observed in the middle and rostral portion of the Ro (Figs. 2, 3). Many small and medium-sized CCK-ir cells were observed in the caudal part of the Pr (Fig. 2). They were, however, rather scattered in the rostral area (Fig. 3). A fair number of small and medium-sized CCK-ir cells were distributed in the caudal and rostral parts of the Ic (Figs. 2, 3). There were no significant differences in the distribution of CCK-ir cells in the various subnuclei or in the PH, using Wilcoxon signed rank test (Fig. 4). CCK-labelled cells in the PH appeared bilaterally symmetrical. A majority of the labelled cells were found in the Ro and Pr, with fewer of them in the Ic. Figure 5 shows the ratio of CCK-ir cells in the Ro, Pr, and Ic. There are no significant differences ($p < 0.05$) in the range and mean of cells in all subnuclei. Figure 6 shows that the small CCK-ir cells make up the majority of labelled cells in the Ro, Ic, and Pr. Small CCK-ir cells of the PH are generally plentiful admixed with some medium-sized cells. No significant difference in the distribution of the cells was present in the nuclei and their subnuclei using Wilcoxon signed rank test.

Distribution of labelled precerebellar CCKergic neurons in perihypoglossal nuclei

Following multiple HRP injections into the vermis, HRP$^+$/CCK$^+$-labelled cells were localized in the various subnuclei of the PH. Figure 7 shows the occurrence of a considerable number of double-labelled cells in the Ro, Ic, and Pr. A large proportion of the CCK-ir cells in the PH was double labelled. There were no significant differences in the relative occurrence of double-labelled cells of the nuclei and their subnuclei using Wilcoxon

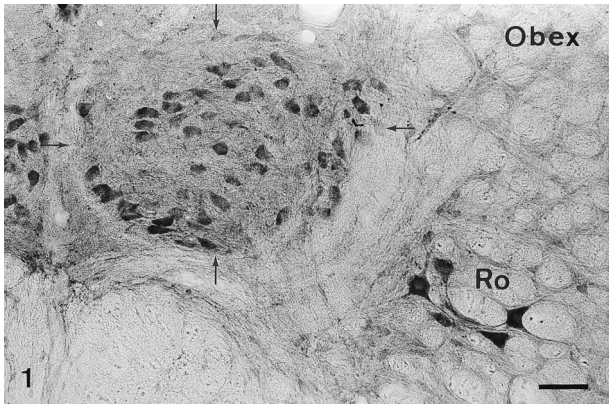

Fig. 1. Transverse section of the lower brainstem containing the nucleus of Roller (Ro) at the level of the obex. CCK-positive cells in the Ro are found ventrolateral to the hypoglossal nucleus (arrows). CCK immunohistochemistry is visualized with the glucose-DAB-nickel method. Bar = 50 μm.

signed rank test ($p < 0.05$). In the mid-level of the PH, small and medium-sized double-labelled cells were predominant in the rostral Ro, with some in the caudal Pr (Fig. 8). A few small and medium-sized double-labelled cells were present in the rostral Pr. Small and medium-sized double-labelled cells were randomly distributed in the Ic (Fig. 9). Some small and medium-sized HRP^+/CCK^+ cells occurred in the caudal Ro (Fig. 10).

DISCUSSION

Labelled CCKergic neurons

This study has shown that many neurons in the PH are CCK-positive, the majority of which are small cells, with some being medium-sized. Some medium-sized CCK-ir cells are present in the Ro. Small CCK-ir cells were mainly distributed in the Ro and Pr, but were sparsely distributed in the Ic. The distribution pattern of the CCK-ir cells is in accord with our previous study using a retrograde tracing method in which HRP was injected into different lobules of the vermis in the same species (unpubl. data). However, King and Bishop (1990), using a PAP method, reported the wide occurrence of CCK-ir cells in the PH of the opossum being numerous in the Pr, less abundant in the Ic, and negligible in number in the Ro. The discrepancy in the distribution of CCK-ir cells in the rodent PH may be attributed to species variation and/or different methods used. In our present study, the ABC procedure, the more-sensitive detecting method, was employed instead of the peroxidase-antiperoxidase method to demonstrate the distribution of CCK-ir cells.

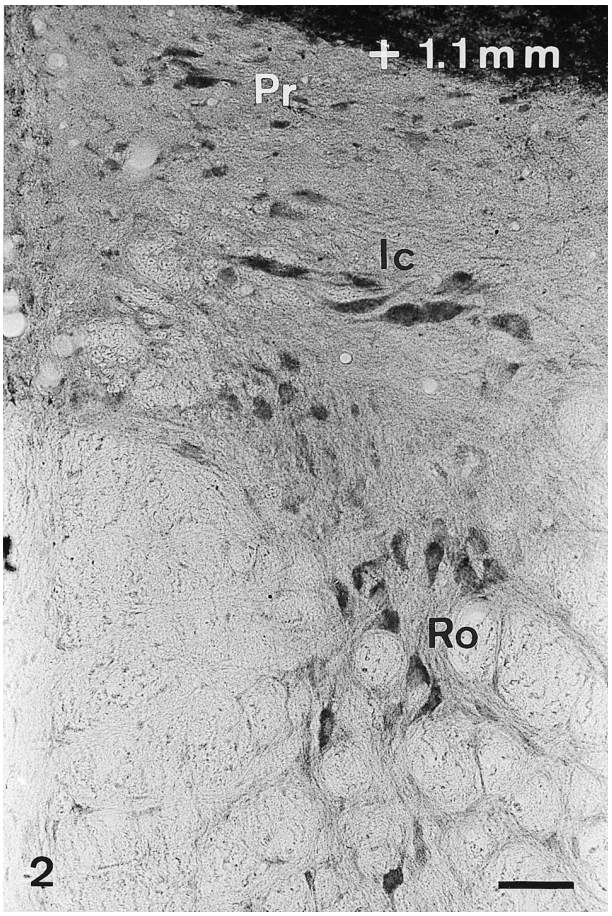

Fig. 2. Transverse section of the lower brainstem containing all 3 subnuclei of the perihypoglossal nuclei (PH) at the level of the rostral to the obex (+1.1 mm). CCK-positive cells are randomly distributed in the Pr, Ic, and Ro. Pr = prepositus hypoglossal nucleus; Ic = nucleus intercalatus. Bar = 50 μm.

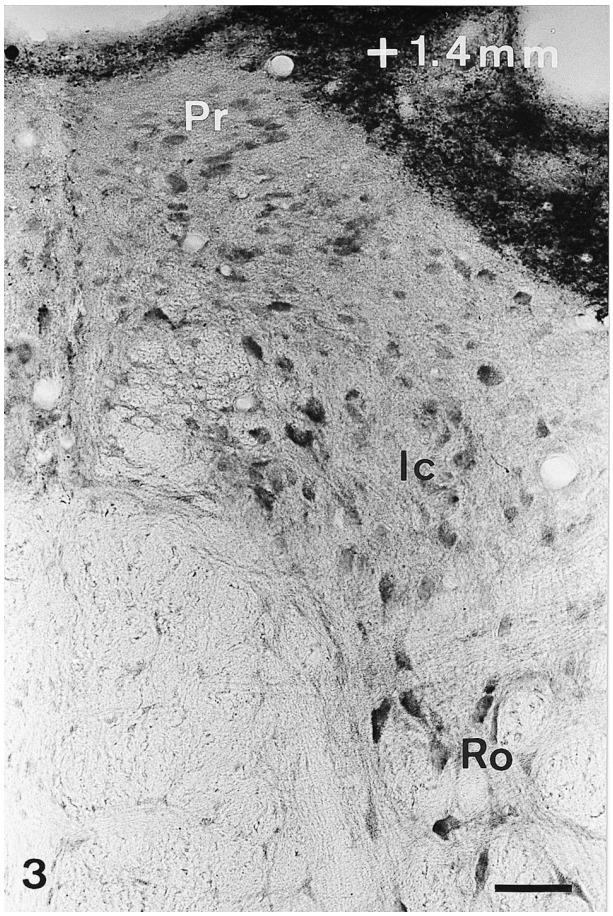

Fig. 3. Transverse section of the lower brainstem at the level of the rostral to the obex (+1.4 mm). Most of the CCK-positive cells in the Pr, Ic, and Ro are small compared to those at the caudal level. Bar = 50 μm.

Labelled precerebellar CCKergic neurons

This study shows that a large number of CCK-ir cells in the subnuclei Ro and Pr project their fibers to the cerebellar vermis. We also demonstrate that CCK-positive neurons in the Ro, Ic, and Pr are labelled following HRP injection into the vermis cortex. Multiple HRP injections into the cerebellar hemispheres and vermis have previously revealed that many CCK-ir neurons in the perihypoglossal, external cuneate, and lateral reticular nuclei send projecting fibers to the cerebellum of the opossum (King and Bishop 1990). On closer analysis, we demonstrate that the CCK-ergic neurons in the Pr and Ro are the main sources of the perihypoglossal-vermis projections. By immunocytochemistry, some other neuroactive substances have been demonstrated in the same pathway. For example, some cells in the gerbil Pr exclusively give rise to cholinergic fibers projecting to the cerebellum (Lan et al. 1995). The Pr neurons of both the rat and rabbit also send cholinergic fibers to vermal lobules IX-X (Barmack et al. 1992a b). Furthermore, many cells in the rabbit Pr send corticotropin-releasing factor containing fibers to vermal lobules VIII-IX (Errico and Barmack 1993). It was surmised that CCK and other neuroactive substances might colocalize in the perihypoglossal complex, and that CCK might be an important neuromodulator in perihypoglossal projecting neurons.

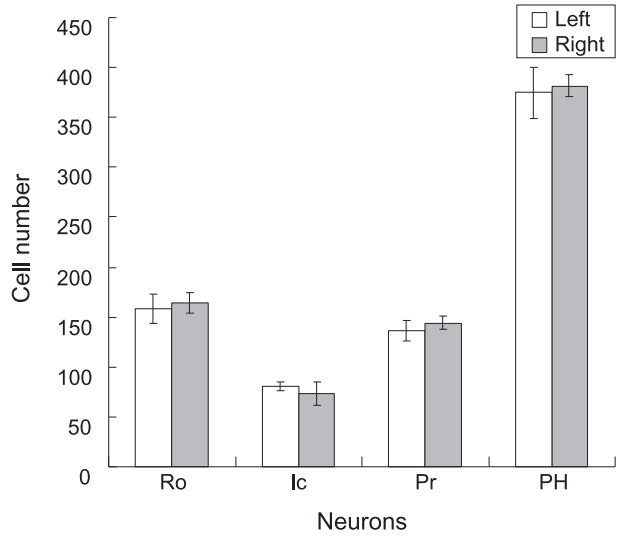

Fig. 4. Distribution pattern of CCK-ir cells in the PH (perihypoglossal) nuclei and their subnuclei (n = 6). There is no significant difference in the distribution of CCK-ir cells of the nucleus and its subnuclei using Wilcoxon signed rank test ($p < 0.05$). Cells are preponderant in the Ro and prepositus hypoglossal nucleus (Pr), and less so in the nucleus intercalatus (Ic).

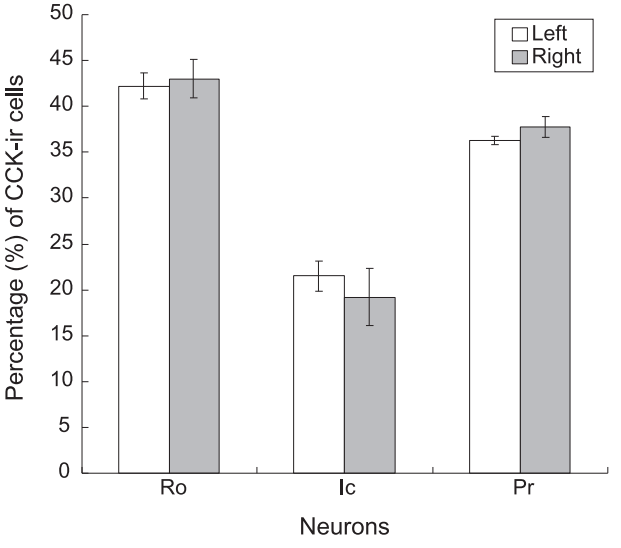

Fig. 5. Percentage of CCK-ir cells in the subnuclei of the PH. A high percentage of CCK-ir is present in the Ro and Pr. In the Ic, the frequency of CCK-ir cells is lower. There is no significant difference in the distribution of CCK-ir cells in the subnuclei using Wilcoxon signed rank test ($p < 0.05$).

Functional implication of CCKergic vermis-projecting neurons of perihypoglossal nuclei

Pr and Ro neurons are known to receive inputs from premotor areas, including the rostral interstitial nucleus of the medial longitudinal fasciculus, interstitial nucleus of Cajal, and paramedian pontine reticular formation (Cooper and Phillipson 1993, Shirashi and Nakao 1995, Helmchen et al. 1998). Anatomical and physiological evidence suggests that these areas project to the extraocular nuclei (Büttner-Ennever and Büttner 1988, Carpenter et al. 1992, Spencer and Wang 1996). The present results, taken together with the fact that the PH sends its outputs to the vermis (Päällysaho et al. 1991, Fukushima et al. 1992, Ohtsuki et al. 1992, Akaogi 1994, Nisimaru and Katayama 1995), suggest that the PH may be involved in the integration of eye movement-related signals and visual-guided responses via the perihypoglossal-vermis pathway.

CCK is not a classical transmitter, but its role in the PH-vermis pathway remains obscure. It could modulate spontaneous and excitatory amino acid-induced activity in granule cells or Purkinje cells in the opossum cerebellum (Bishop 1996).

Other reports have shown that the cerebellum contains almost no CCKergic neurons (Rehfeld et al. 1992, Daunton et al. 1998). It is reasonable to assume that CCK-ir neurons in the lower brainstem may send their fibers to the cerebellum, which may be the most important source of CCK in the cerebellum. In the present experiments, we have clearly shown that the PH sends CCKergic fibers to the vermis. Therefore, we suggest that PH nuclei may participate in some functions of the vermis including the control of gait balance and vestibular coordination. Notwithstanding the above, it may be

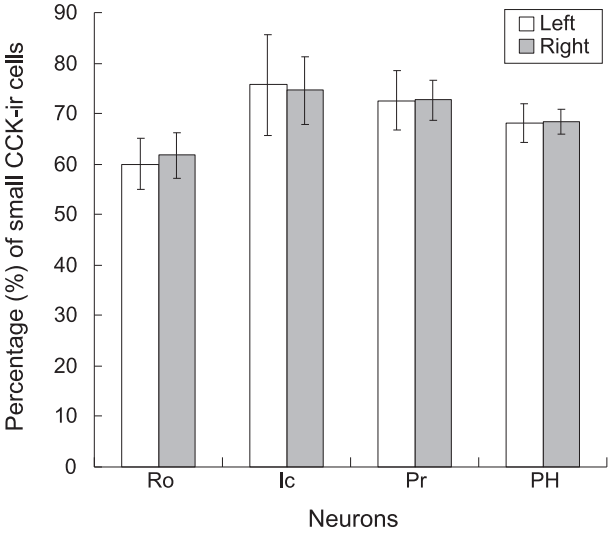

Fig. 6. Percentage of small CCK-ir cells in the PH nuclei and their subnuclei (n = 6). No significant differences in the distribution of the cells are present in the nuclei and their subnuclei using Wilcoxon signed rank test ($p < 0.05$). The small CCK-ir cells are the main constituent cells in the Ro, Ic, and Pr of the PH. The remainder consists of medium-sized cells.

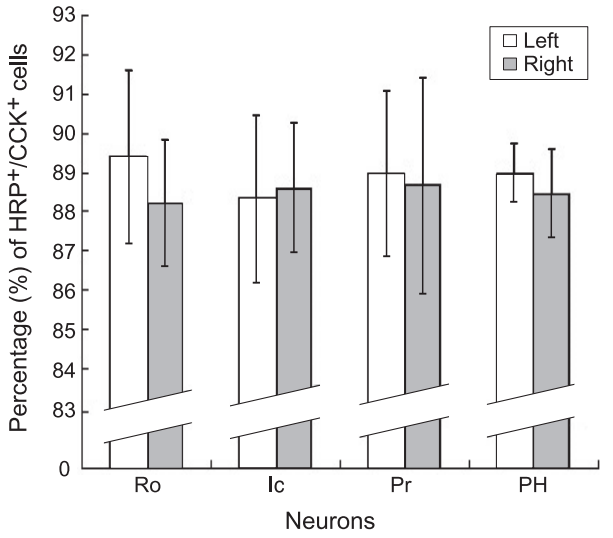

Fig. 7. Percentage of HRP$^+$/CCK$^+$ cells in the PH nuclei and their subnuclei (n = 6). There are no significant differences in the occurrence of double-labelled cells within the nuclei and their subnuclei using Wilcoxon signed rank test ($p < 0.05$). A considerably higher percentage of double-labelled cells is found in the Ro, Ic, and Pr of the PH.

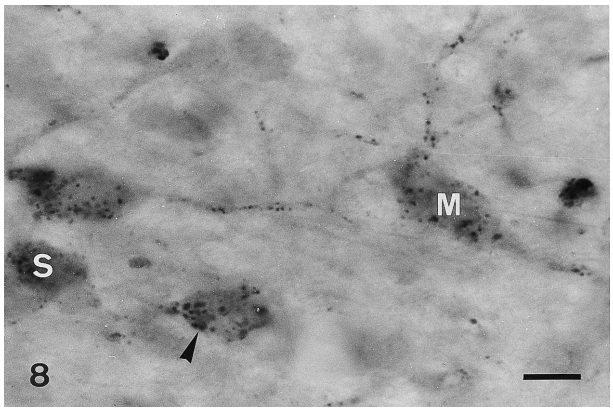

Fig. 8. HRP$^+$/CCK$^+$ double-labelled cells in the Pr showing the retrograde labelling of HRP (granules) injected into the vermis, and homogeneous CCK-like immunoreactivity. S = small cell; M = medium-sized cell. Bar = 10 µm.

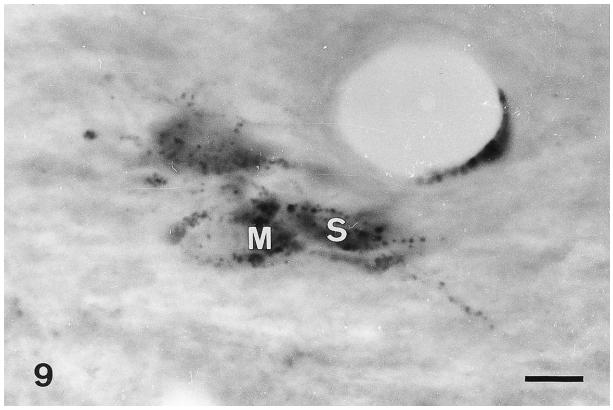

Fig. 9. HRP$^+$/CCK$^+$ double-labelled cells in the Ic. S = small cell; M = medium-sized cell. Bar = 10 µm.

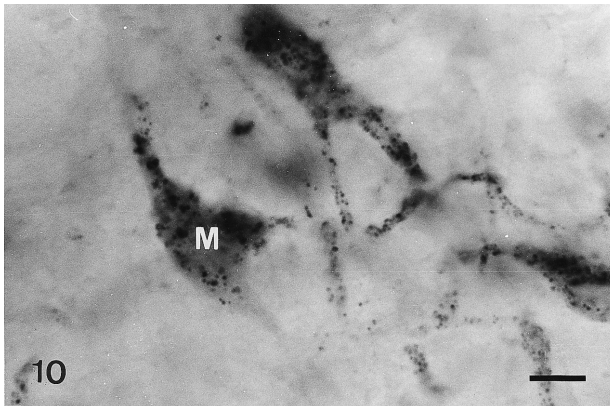

Fig. 10. HRP$^+$/CCK$^+$ double-labelled cells in the Ro. M = medium-sized cell. Bar = 10 µm.

concluded that CCKergic neurons in the PH of the gerbil are involved in the modulation of the perihypoglossal-cerebellar pathway.

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ABBREVIATIONS

ABC	avidin-biotin complex
BSA	bovine serum albumin
CCK	cholecystokinin
CCK-ir	cholecystokinin-immunoreactive
DAB	3, '3-diaminobenzidine tetrahydrochloride
GDN	glucose-DAB-nickel
HRP	horseradish peroxidase
Ic	nucleus intercalatus
NGS	normal goat serum
PAP	peroxidase-antiperoxidase
PB	phosphate buffer
PBS	phosphate buffered saline
PH	perihypoglossal nuclei
Pr	prepositus hypoglossal nucleus
Ro	nucleus of Roller
WGA-HRP	wheat germ agglutinin-conjugated horseradish peroxidase

REFERENCES

Abelson L, PE Micevych. 1991. Distribution of preprocholecystokinin mRNA in motoneurons of the rat brainstem and spinal cord. Mol. Brain Res. **10:** 327-335.

Abercrombie M. 1945. Estimation of nuclear population from microtome sections. Anat. Rec. **94:** 239-247.

Akaogi K. 1994. Afferent projections to the nodulus in the cat. II. Mossy fiber projections. Nippon Jibiinkoka Gakkai Kaiho **97:** 12-19.

Barmack NH, RW Baughman, FP Eckenstein. 1992a. Cholinergic innervation of the cerebellum of the rat by secondary vestibular afferents. Ann. NY Acad. Sci. **656:** 566-579.

Barmack NH, RW Baughman, FP Eckenstein, H Shojaku. 1992b. Secondary vestibular cholinergic projection to the cerebellum of rabbit and rat as revealed by choline acetyltransferase immunohistochemistry, retrograde and orthograde tracers. J. Comp. Neurol. **317:** 250-270.

Bishop GA. 1996. Cholecystokinin modulation of spontaneous and excitatory amino acid-induced activity in the opossum cerebellum. Neuropeptides **30:** 533-540.

Büttner-Ennever JA, U Büttner. 1988. Neuroanatomy of the oculomotor system. The reticular formation. Rev. Oculomot. Res. **2:** 119-176.

Carpenter MB, AB Periera, N Guha. 1992. Immunocytochemistry of oculomotor afferents in the squirrel monkey (*Saimiri sciureus*). J. Hirnforsch. **33:** 151-167.

Cooper JD, OT Phillipson. 1993. Central neuroanatomical organisation of the rat visuomotor system. Prog. Neurobiol. **41:** 209-279.

Daunton NG, F Tang, ML Corcoran, RA Fox, SY Man. 1998. Chronic exposure to hypergravity affects thyrotropin-releasing hormone levels in rat brainstem and cerebellum. Biol. Signal. Recept. **7:** 337-344.

Errico P, NH Barmack. 1993. Origins of cerebellar mossy and climbing fibers immunoreactive for corticotropin-releasing factor in the rabbit. J. Comp. Neurol. **336:** 307-320.

Fallon JH, KB Seroogy. 1985. The distribution and some connections of cholecystokinin neurons in the rat brain. Ann. NY Acad. Sci. **448:** 121-132.

Fukushima K, CRS Kaneko, AF Fuchs. 1992. The neuronal substrate of integration in the oculomotor system. Prog. Neurobiol. **39:** 609-639.

Godaux E, P Mettens, G Cheron. 1993. Differential effect of injections of kainic acid into the prepositus and the vestibular nuclei of the cat. J. Physiol. **472:** 459-482.

Helmchen C, H Rambold, L Fuhry, U Büttner. 1998. Deficits in vertical and torsional eye movements after uni- and bilateral muscimol inactivation of the interstitial nucleus of Cajal of the alert monkey. Exp. Brain Res. **119:** 436-452.

Innis RB, FMA Corrêa, GR Uhl, B Schneider, SH Snyder. 1979. Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. Proc. Natl. Acad. Sci. USA **76:** 521-525.

King JS, GA Bishop. 1990. Distribution and brainstem origin of cholecystokinin-like immunoreactivity in the opossum cerebellum. J. Comp. Neurol. **298:** 373-384.

King JS, RH Ho, GA Bishop. 1986. Cholecystokinin-like immunoreactivity in the cerebellum of the opossum. Neurosci. Abstr. **12:** 461.

Kiyama H, S Shiosaka, Y Kubota, HJ Cho, H Takagi, K Tateishi, et al. 1983. Ontogeny of cholecystokinin-8 containing neuron system of the rat: an immunohistochemical analysis-II. Lower brain stem. Neuroscience **10:** 1341-1359.

Kubota Y, S Inagaki, S Shiosaka, HJ Cho, K Tateishi, E Hashimura, et al. 1983. The distribution of cholecystokinin octapeptide-like structures in the lower brain stem of the rat: an immunohistochemical analysis. Neuroscience **9:** 587-604.

Lan CT, CY Wen, CK Tan, EA Ling, JY Shieh. 1995. Multiple origins of cerebellar cholinergic afferents from the lower brainstem in the gerbil. J. Anat. **186:** 549-561.

Lorén I, J Alumats, R Håkanson, F Sundler. 1979. Distribution of gastrin and CCK-like peptides in rat brain. An immunocytochemical study. Histochemistry **59:** 249-257.

Loskota WJ, P Lomax, MA Verity. 1974. A stereotaxic atlas of the Mongolian gerbil brain (*Meriones unguiculatus)*. Michigan: Ann Arbor Science.

Mantyh PW, SP Hunt. 1984. Evidence for cholecystokinin-like immunoreactive neurons in the rat medulla oblongata which project to the spinal cord. Brain Res. **291:** 49-54.

Nisimaru N, S Katayama. 1995. Projection of cardiovascular afferents to the lateral nodulus-uvula of the cerebellum in rabbits. Neurosci. Res. **21:** 343-350.

Ohtsuki H, A Tokunaga, K Ono, S Hasebe, Y Tadokoro. 1992.

Distribution of efferent neurons projecting to the tectum and cerebellum in the rat prepositus hypoglossi nucleus. Invest. Ophthalmol. Vis. Sci. **33:** 2567-2574.

Päällysaho J, S Sugita, H Noda. 1991. Brainstem mossy fiber projections to lobules VIa, VIb,c, VII and VIII of the cerebellar vermis in the rat. Neurosci. Res. **12:** 217-231.

Rehfeld JF, NW Mogensen, L Bardram, L Hilsted, HJ Monstein. 1992. Expression, but failing maturation of procholecystokinin in cerebellum. Brain Res. **576:** 111-119.

Schiffmann SN, JJ Vanderhaeghen. 1991. Distribution of cells containing mRNA encoding cholecystokinin in the rat central nervous system. J. Comp. Neurol. **304:** 219-233.

Shiraishi Y, S Nakao. 1995. Differential locations in the midbrain of distinct groups of vertical eye movement-related neurones in cat: their projections and direct connections with oculomotor neurones. Acta Physiol. Scand. **154:** 151-163.

Spencer RF, SF Wang. 1996. Immunohistochemical localization of neurotransmitters utilized by neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) that project to the oculomotor and trochlear nuclei in the cat. J. Comp. Neurol. **366:** 134-148.

Torvik A, A Brodal. 1954. The cerebellar projection of the perihypoglossal nuclei (nucleus intercalatus, nucleus praepositus hypoglossi and nucleus of Roller) in the cat. J. Neuropath. Exp. Neur. **13:** 515-527.

Vanderhaeghen JJ. 1975. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature **257:** 604-605.

Vanderhaeghen JJ, F Lotstra, J De Mey, C Gilles. 1980. Immunohistochemical localization of cholecystokinin- and gastrin-like peptides in the brain and hypophysis of the rat. Proc. Natl. Acad. Sci. USA **77:** 1190-1194.

Vanderhaeghen JJ, SN Schiffmann. 1992. Distribution of brain neuronal CCK: an in situ hybridization study. *In* CT Dourish, SJ Cooper, SD Iversen, LL Iversen, eds. Multiple cholecystokinin receptors in the CNS. New York: Oxford Univ. Press, pp. 38-56.

Yamashita A, M Hayashi, K Shimizu, K Oshima. 1990. Neuropeptide-immunoreactive cells and fibers in the developing primate cerebellum. Dev. Brain Res. **51:** 19-25.

沙鼠舌下神經周核內投射至小腦之膽囊激素性的神經元分布

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本研究係探討沙鼠舌下神經周核之小腦蚓部投射膽囊激素神經元的分布情形。利用免疫組織化學法可知膽囊激素性神經元主要分布於舌下神經周核的羅勒神經核及舌下神經前置核，而閏核則非常少；且這些膽囊激素性神經元主要為小型神經細胞及部份的中型細胞。進一步利用山葵過氧化氫酶作為神經追蹤劑，多點注射小腦蚓部的皮質，則發現在羅勒核、閏核和舌下神經前置核內有許多膽囊激素性神經元受山葵過氧化氫酶雙重標誌。進一步分析得知在舌下神經周核約有百分之九十的膽囊激素性神經元同時受此追蹤劑所標示。因舌下神經周核內含大量膽囊激素性神經元，且羅勒核和舌下神經前置核可能發出膽囊激素性苔狀神經纖維至小腦蚓部，所以我們推測膽囊激素可作為此投射中神經訊息的重要傳遞或調節物質。

關鍵詞：膽囊激素，雙重標示，舌下神經周核投射神經元，神經追蹤劑，嚙齒類。

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