Chinese Journal of Physiology 45(1): 19-24, 2002

Anatomical and Functional Study of Localization of Originating Neurons of the Parasympathetic Nerve to Gallbladder in Rabbit Brain Stem

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Abstract

The present study was to investigate the localization of preganglionic parasympathetic neurons of gallbladder in brain stem by anatomical and functional approaches. Male or female rabbits (n = 11) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Cholera toxin B conjugated to horseradish peroxidase (CB-HRP) was injected into the gallbladder wall. Four days later, animals were re-anesthetized and perfused transcardially with paraformaldehyde solution in a 0.1 M phosphate buffer. The rabbit brain was then frozenly sectioned. The sections were processed for HRP label and stained with neutral red. Another group of rabbits (n = 54) were anesthetized by urethane (1 g/kg) after fasting for 18-24 hours, Gallbladder pressure (GP) was measured by inserting a frog bladder filled with normal saline into the gallbladder. Myoelectrical activity of the sphincter of Oddi (SO) was induced by a pair of copper electrodes. A glass tube (30 μm tip diameter) connected with a microsyringe was directed to the dorsal vagal complex (DVC) for microinjection. Majority of retrogradely labeled cells was found bilaterally in dorsal motor nucleus of the vagus nerve (DMV) throughout the length, except the rostral and caudal part. These cells were distributed in subnuclei parvicellularis or mediocellularis of DMV. Some labeled perikarya located in the medial subnucleus of the solitary tract (mNTS). Thyrotropin-releasing hormone (TRH, 1.3 mmol/L, 0.2 μl) microinjected into the rostral portion of the DVC (including DMV and NTS) enhanced the motility of gallbladder and SO. Microinjection of TRH at the middle part of DVC seldom induces excitatory effects on the gallbladder or SO. TRH microinjected into the caudal portion of the DVC elicited weaker response of gallbladder and SO than rostral portion. Our results indicated that DMV is one of the most important original nuclei of gallbladder's vagus nerves and mNTS may be also involved in the control of gallbladder's parasympathetic activity. Neurons that innervate the gallbladder distribute at most part of DVC, and are relatively dense at rostral and caudal position of DMV.

Key Words: dorsal motor nucleus of the vagus, nucleus tractus solitari, gallbladder, horseradish peroxidase, cholera toxin, retrograde tracing study

Introduction

The dorsal vagal complex (DVC), including the dorsal motor nucleus of vagus nerve (DMV), nucleus of the solitary tract (NTS), and nucleus ambiguous (NA), constitutes the basic neural circuit of vago-vagal reflex control of gastrointestinal motility (2). There is a topographic representation of visceral organs in DMV (6). In rat, the parasympathetic preganglinoic neurons innervating different part of the stomach are distributed at different areas in DVC (14). In Cat, the neurons that project to stomach are mainly located between 0.56 and 1.56mm rostral to obex in DMV (15). Katz also reported that individual vagal target organs had discrete and topographic representations with cytoarchitecturally distinct subnuclei of the DVC (7). Our recent research indicated that, electrical and

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Received August 28, 2001; Revised November 16, 2001; Accepted November 20, 2001.

chemical stimulation of DVC enhanced the gallbladder motility via vagus nerve, so we concluded that there is originating neurons of the parasympathetic nerves to gallbladder in DVC (10, 11, 16). But there is no anatomical data about the distribution of these neurons. Glutamate and nitric oxide could enhance the gallbladder motility if microinjected into DMV, and most effective points of these two chemicals are accumulated at rostral part of DMV (3, 16). Thyrotropin-releasing hormone (TRH) is also a transmitter in DVC (1, 2, 4, 5, 11). There are lots of TRH-immunoactive fibers in DVC (5). Microinjection of TRH into DVC enhanced the stomach and gallbladder motility (2, 4, 5, 11). But where are the TRH receptors that associated with the gallbladder motility regulation located is unclear. In this study, both of anatomical and functional methods were used to investigate (1) the distribution of originating neurons of the parasympathecic nerves to gallbladder, and (2) the location of TRH rececptors that participating in gallbladder motility regulation.

Materials and Methods

Anatomical Study

Eleven rabbits (1.5 kg -2.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv). A middle-line abdominal incision was made to expose the gallbladder. A total of 20 μl of Cholera toxin B conjugated to horseradish peroxidase (CB-HRP) was injected at 16-20 points into the wall of gallbladder via a glass pipe affixed to a 1 or 5 μl syringe. Each injecting site was sealed with vaseline ointment. Following injection, the abdominal cavity was rinsed with warm saline (37°C) and a wad of Gelform was placed around the gallbladder, isolating it from the rest of the viscera. The middle-line incision was closed, and each animal received penicillin and gentamycin after the operation to prevent infection.

After survival for 4 days, the animals were deeply anesthetized with urethane (1 g/kg), and perfused through the left ventricle with a heparinized saline (100 IU/ml, 2 ml) followed by a 3% paraformaldehyde solution in a 0.1 M phosphate buffer. Rabbit brains were then removed, fixed in the buffered 3% paraformaldehyde solution for 24 hours at 4°C and then transferred to 20% sucrose buffer until osmotic equilibrium was attained and the tissues were sank to the bottom of the container. Serial sections of 40 mm slices of the appropriate brain regions were cut on a freezing microtome, and collected in 0.1 Mol/L phosphate buffer.

Tissue sections were processed according to the tetramethylbenzene (TMB) reaction protocols for HRP histochemistry (13). Alternate sections from each case were mounted onto chrome-alum-gelatin-coated microscope slides, allowed to air dry, lightly counterstained with neutral red and rapidly dehydrated. The sections were then observed with bright illumination of a Olympus microscope, photographed, and reproduced with drawings at appropriate intervals.

Functional Study

After fasting for 18-24 hr, 54 rabbits, weighing 1.8-2.0 kg, were anaesthetized with urethane (1 g/kg), trachectomized and artificially ventilated, and the femoral artery was catheterized to monitor arterial pressure via pressure transducer. In order to monitor the GB motility, the gallbladder was exposed through a middle-line abdominal incision, and a frog bladder connected with pressure transducer was inserted into gallbladder. Myoelectrical signals of SO were induced by a pair of copper electrodes. The reasons that we used the frog bladder to measure the gallbladder pressure in this animal model is due to that [1] it is an animal organ and may not stimulate the mucus of gallbladder; and [2] its wall is very thin with high compliance. The change of gallbladder pressure (GP), blood pressure (BP) and myoelectrical signals of SO were simultaneously recorded by a polygraph (RM-6000, Nihon, Khoden, Japan). Anal temperature was kept at 37. 5°C -38. 5°C.

After operation, the animal's head was fixed in a stereotaxis frame (SN-38712, Narishige). According to Messen's topography, DMV was located at 0.5-0.7 mm lateral middle-line of brain stem, -1 -+3.5 mm rostral to obex and 0.5-1.0 mm ventral to the brain stem surface. NTS was located at 0.7-1.5 mm lateral midline of brain stem, -1 - +3.5 mm rostral to obex and 0.7-1.5 mm ventral to the brain stem surface.

The method of microinjection was described in our recent published paper (16). Simply, a micropipette (with 15 μm internal diameter and 30 μm external diameter) filled with drug solution was used. Individual drugs (TRH or normal saline) were microinjected into the nucleus. The time for one injection was 1 min. There are at least 60 min internals between the two successive microinjections.

Results

Experiment 1: Distribution of HRP Labeled Cells

Four days after injection of CB-HRP into the gallbladder wall, labeled cells were found in the dorsal motor nucleus of the vagus nerve (DMV) and medial subnucleus of the solitary tract (mNTS). Only cell bodies that the margin could be clearly identified, with evidence of a definable nucleus and containing granular reaction products in perikarya were counted as labeled cells.

115 HRP-labeled neurons were found in the DMV on both sides, throughout the length, except at its very rostral and caudal parts (Fig. 1).

There were 68 labeled that were found in the caudal portion of DMV, 56 of them were distributed in the caudalis Intermedius Paricelularis (cIP) and others in the caudalic Ventralis Mediocelkularis (cVMe). There were more labeled cells in cIP than in cVMe. The distribution of the labeled cells is from dorsomedial part to intermediate one (Fig. 1, Fig. 2A).

27 labeled cells were distributed in the middle portion of DMV, 13 of them were located within the posterior Dorsalis Hetercellularis (pDH) and others were found in the posterior Intermidius Mediocellularis (pIMe). Hence, from levels near the obex to more rostral , there also existed a distributive trend from the dorsomedial part to the intermediate part. Number of labeled cells in this portion was less than that in caudal portion (Fig. 1, Fig. 2B).

20 labeled neurons were located at the rostral portion of DMV, 14 of them were visible within the anterior Ventralis Parvicellularis (aVP), others were in the anterior Dorsalis Parvicellularis (aDP). So the labeled cells occupied the ventral and medial margin. Number of labeled cells was also less than that in caudal portion (Fig. 1, Fig. 2C).

As the whole DMV is concerned, distribution of the labeled cells is from dorsal part to the intermediate one, then to ventromedial direction (Fig. 1.). Within each subnucleus , labeled cells were middle or small, ovoid or fusiform, and with two prominences that have a similar orientation to cells bodies' longitudinal axis (Fig 2.).

Several labeled cells were observed within the medial subnuleus of NTS as well. They located in its rostral portion. Few labeled cells were found at nucleus intercalatus (Ic), nucleus praepositus hypoglossi (Prph) and subnucleus reticluaris ventralis (Rv) (Fig. 1). Their size and shape were similar to that in DMV.

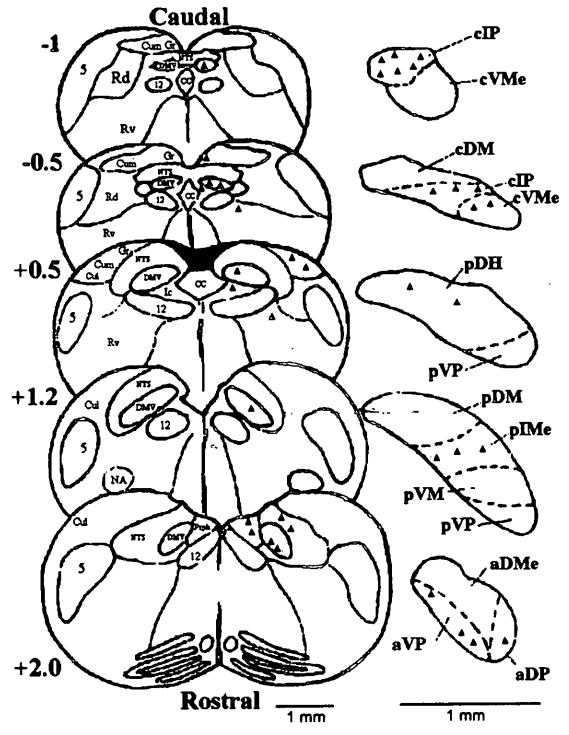

Fig.1 Schematic drawing of sections made across several different levels of brain stem showing the distribution of CB-HRP labeled cells. The triangles indicates the distribution of labeled cells. Left: Distribution of different nucleus. Right: Distribution of different subnuclei of DMV. aDP, anterior sorsalis parvicellularis; aDMe, anterior dordalis mediocellularis; aVP, anterion ventralis parvicellularis; cDM, caudalis dordalis magnocellularis; cIP, caudalis intermedius paricelularis; cVMe, caudalis ventralis mediocelkularis; Ic, nucleus intercalatus; pDH, posterior dorsalis hetercellularis; pDM, posterior dosalis magnocellularis; pIMe, posterion intermidius mediocellularis; Prph, nucleus praepositus hypoglossi; pVM, posterior ventralis magnocellularis; pVP, posterior ventricles parvicellular; Rd, subnucleus reticularis dorslalis medullae oblongatae; Rv. subnucleus reticularis ventralis medulla oblongata.

Experiment 2: Effects of TRH Microinjected into DVC (Including DMV and NTS) on Gallbladder and SO Motility

There are 112 microinjection points which were located at the rostral position of the DVC (2mm-3.2mm rostral to obex), and 65 of them in DMV, others in NTS. In the 65 points in DMV, 40 points (62%) induced excitation of gallbladder and SO motility (Fig..3 and Fig. 4). Thirteen of them (20%) excited the myoelectrical activity of SO only, and 3 of them (4. 6%) excited only gallbladder motility (Fig. 4). There are 47 points that were located in the rostral position

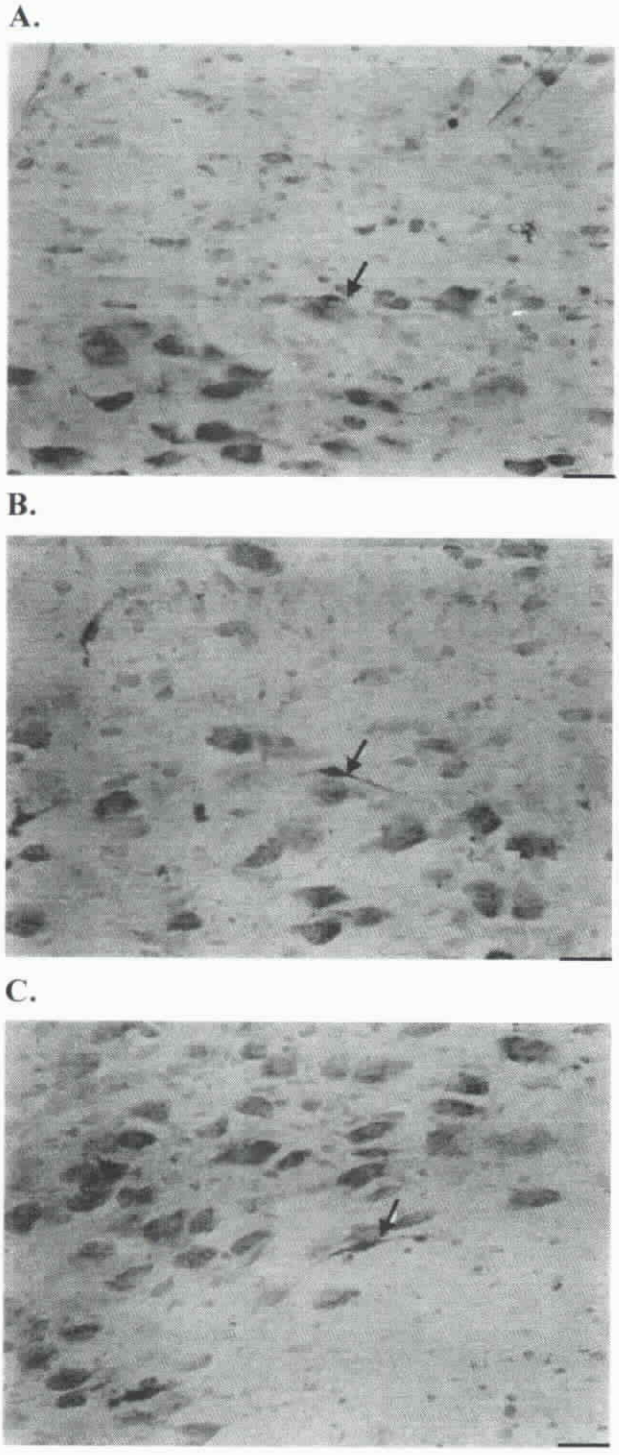

Fig.2 HRP labeled cells in the caudal (A), middle (B) and rostral (C) parts of DMV. The arrows indicates the labeled cells. The length of the bar is 50 μm.

of NTS. After microinjection of TRH (1.3 mmol/L, 0.2 ul), 6 of them (13%) excited both the gallbladder and SO, 5 points (12%) only excited the myoelectrical activity of SO, 4 (10%) points only enhanced the myoelectrical activity of SO, others (65%) had no effect

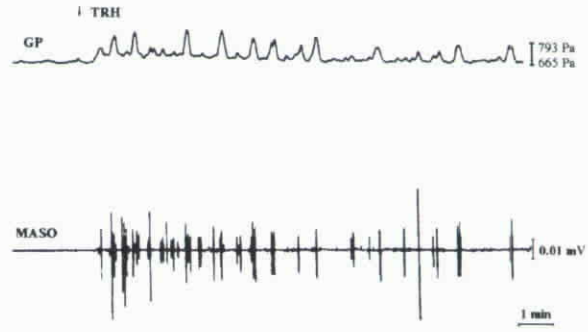

Fig.3 Thyrotropin - releasing hormone (TRH, 1.3 mmol/L, 0.2μl) microinjected into rostral part of DMV enhanced the motility of gallbladder and sphncter of oddi. GP, Mean gallbladder pressure; MASO, myoelectrical activity of sphincter of Oddi.

on the motility of gallbladder and SO. (Fig. 4)

There are 42 microinjection points that were located in middle part of DVC. Thirty of them are in DMV and others in NTS. Of the points that were in middle DMV, 4 (13%) induced the excitation of gallbladder motility, and 1 (3%) enhanced the myoelectrical activity of SO, others (83.3%) did not influence the motility of gallbladder and SO. Of the points that were in middle NTS, none of them affect the motility of gallbladder and SO. (Fig. 4)

Of the 17 points that located into caudal portion of DVC, nine were in DMV and eight were in NTS. In caudal DMV, after microinjection of TRH, 3 points (33. 3%) enhanced the motility of gallbladder and SO, others have no effect. In caudal NTS, none of the 8 points affect the motility of gallbladder and SO (Fig. 4).

Discussion

It has been well known that visceral parasympathetic ganglion locates in organs' wall or beside them, and receive synaptic contacts from the terminals of the pre-ganglion fibers. When HRP was injected into an organ's wall, it could be uptaken by these terminals and transported to their cell bodies along the axons. Therefore the visceral pre-ganglion neurons of parasympathetic nerve could be located by labeling the cells that contain HRP. Our present results indicate that, after injecting CB-HRP into gallbladder wall, labeled neurons are mainly presented in DMV. These observations suggest that DMV is the chief originating nucleus of the parasympathetic nerves that innervate gallbladder in the brain stem. The result that microinjection of TRH into DMV enhanced the motility of gallbladder further confirmed this finding.

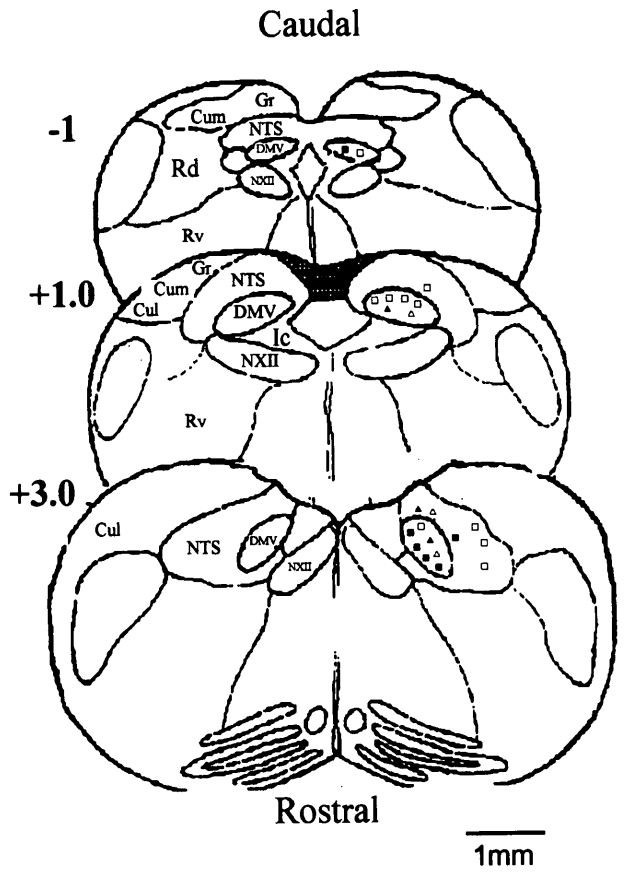

Fig. 4 Schematic drawings of sections made across several different levels, showing the positions at which thyrotropin-releasing hormone (TRH) was microinjected within dorsal motor nucles of the vagus (DMV) and nucleus of solitary tracts (NTS). ■,Effective points of gallbladder (GB) and sphincter of Oddi (SO); ▲,Effective points of GB only; △ , Effective points of SO only; □,No-effective points of both GB and SO. cUl, Nucleus Cuneatus Lateralis; Cum, Nucleus Cuneatus Laterlis; Gr,Nucleus Gracilis; Ic, Nucleus Intercalatdus; Rd, subnucleus reticularis dorslalis medullae oblongatae; Rv, Subnucleus reticularis ventralis medullae oblongatae; NXII, Nucleus Nervi Hypoglissi

In DMV, HRP labeled cells are mainly found at cIP, pIMe and aVP. So we concluded that, in DMV, originating neurons of the parasympathetic nerves to gallbladder are distributed from the caudal dorsal part to the intermedial one, then to the rostral ventromedial direction. They were site-specially organized.

Katz (7) made an elaborate research about DMV of pigeon, and segregated DMV into parvicellular, mediocellular, and magnocellular subnuclei along longitudinal axis on transverse sections. We found that the distribution of cells within DMV of the rabbit appeared particularly similar to the pattern in the pigeon. Malone (12) demonstrated the relationship between morphology and function of the neurons and suggested that small neurons innervated the smooth muscle, while large ones regulated the activity of cardiac muscle. Our data indicate that neurons projecting to the gallbladder are intermediate and small ones. These results suggest that different neurons in DMV may have different functions.

Furukawa (3) demonstrated that, after stimulation of different part of DMV in dogs, most of the parts in rostral position enhanced the motility of gallbladder, and few of the caudal points have this effect. This finding is similar to our observation in rabbits. However we did not find any points in DMV that could inhibit the motility of extrahepatic biliary system as reported by Furukawa. The discrepancy may be incurred by the different methods used. Furkawa (3) used the electrical stimulation, which could excite the nerves that passing through DMV. In the present study, microinjection was used, which only affect the excitability of neurons in nucleus.

Our anatomical and functional studies indicate that the neurons that innervate the gallbladder distributed most part of DVC, and relatively denser at rostral and caudal position of DMV. There is a little inconsistency between the results obtained from the anatomical and functional methods. By CB-HRP, more labeled neurons were found in caudal part of DMV than in rostral, while in another group, more points in rostral part of DMV excited the motility of gallbladder and SO when TRH were microinjected into them. Hornby (5) reported that dense network of TRH-immunoreactive fibers and terminals were distributed at rostral part of DMV. The result of present functional study is consistent with this finding. That is to say, in DMV, most TRH receptors participating in regulating gallbladder motility are distributed at rostral part. There are also other transmitters in DMV, such as substance P (2, 8, 9), calcitonin gene-related polypeptide (2, 9) and neutral endopeptidase (NEP) (8). Ladic reported that the majority of NEP cells was observed caudal to the obex. In this study, the labeled cells in the caudal part of DMV may regulate the gallbladder through other transmitters, such as NEP. We will test this possibility in the future study.

Labeled small neurons were also found in the mNTS (Fig. 1), and a few points enhanced the motility of gallbladder after microinjection of TRH (Fig. 6). All of these results indicate that neurons in the NTS give rise to fibers to innervate the gallbladder.

In conclusion, the result of this anatomical and functional study indicate that, in DMV, most of originating neurons of the parasympathetic nerve to gallbladder are distributed at the rostral and caudal part of DMV, and are site specially organized. THR receptors that associated with gallbladder motility are mainly located at rostral part. The labeled neurons found at caudal part may influence the gallbladder activity through other transmitters. Some neurons in NTS also are involved in the control of gallbladder's parasympathetic activity.

Acknowledgements

These experiments were supported by the Natural Scientific Foundation of Shandong Province (Y95C1032). The authors gratefully acknowledge the assistance provided by Professor Paulus S. Wang (Department of Physiology, National Yang-Ming University, Taipei, ROC) in the preparation of the manuscript and Professor Song Gang (Department of Physiology, Medical School of Shandong University, Jinan, PRC) in the performance of these experiments.

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