Nucleus Raphe Obscurus Participates in Regulation of Gallbladder Motility through Vagus and Sympathetic Nerves in Rabbits

***Yu-Feng Xie, Chuan-Yong Liu and Jing-Zhang Liu**

Institute of Physiology
Medical School of Shandong University
Jinan, Shandong 250012, People's Republic of China

Abstract

The aim of the present study is to investigate if the nucleus raphe obscurus (NRO) participate in regulating the gallbladder motility in rabbits. Rabbits were fasted for about 20-24 hours. After anesthetization with urethane, an incision was made at the middle of the abdomen and the gallbladder was exposed. A frog bladder connected with force transducer was inserted into the gallbladder through a small incision at the funds to record gallbladder motility (tonic contraction and phasic contraction). Glutamate and other chemicals were microinjected into NRO through a vitreous tube attached to a microsyringe. We found both the tonic contraction and phasic contraction of the gallbladder were enhanced after the glutamate was injected into NRO. GABA inhibited gallbladder motility if administrated in the same way. Microinjection of ketamine, NMDA (N-methyl-D-aspartate) receptor antagonist, into NRO inhibited the phasic contraction of gallbladder. Administration of CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione), a non-NMDA receptor antagonist, enhanced the gallbladder tonic contraction. Pretreatment of ketamine into NRO attenuated the effect of glutamate, while pretreatment of CQNX had no effect on it. Intravenous injection of atropine or vagotomy completely abolished the effect of glutamate on gallbladder phasic contraction, while intravenous injection of phentolamine or transecting the spinal cord at T3~4 inhibited that on tonic contraction. Intravenous injection of propranolol did not influence the glutamate effect. These results suggested that glutamate in NRO participates in regulating the motility of the gallbladder through NMDA receptor. When excited, the NMDA receptors in NRO enhance the phasic contraction of the gallbladder through vagus nerve and peripheral M-receptors, and enhance the tonic contraction of the gallbladder through sympathetic nerve and peripheral α-receptors. GABA in NRO is also involved in the regulation of gallbladder motility.

Key Words: nucleus raphe obscurus, gallbladder, NMDA receptor, non-NMDA receptor, GABA, motility, vagus nerve, sympathetic nerve

Introduction

Many studies have found that Nucleus Raphe Obscurus (NRO) had nerve connections with sympathetic and vagus nerves. Descending fibers from NRO projected to the ventral and lateral funiculi of the spinal cord, with arborization in the thoracic intermediolateral cell column; in laminae VII, IX, and X of the lumbosacral cord; and in the sacral parasympathetic nucleus (SPN) (2, 3). NRO also has reciprocal fiber connections with the dorsal vagal complex (DVC), and our recent study indicated that DVC modulated gallbladder motility through vagus nerves (9, 11, 12, 13, 14, 18). Scholars reported that NRO was involved in central autonomic regulation, especially the regulation of gastrointestinal functions such as gastric motility and secretion through sympathetic and vagus nerves (4, 5, 6, 15, 19). In our

Corresponding author: Dr. Liu Chuanyong, Institute of Physiology, Medical School of Shandong University, Jinan, Shandong 250012, People's Republic of China. Tel: 0086-531-8382037, Fax: 0086-531-2942485, E-mail: liucy@sdu.edu.cn

* Y.-F. Xie is studying as Ph.D student at Department of Physiology, School of Medicine, Xi'an Jiao Tong University, Xi'an, Shaanxi 710061, P. R. China.

Received: May 1, 2002; Revised: July 9, 2002; Accepted: July 23, 2002

previous study, we also found that NRO could regulate the motility of sphincter of Oddi (SO) through vagal nerve and peripheral M cholinergic receptor (20).

There are a variety of neurons in NRO that respond to different neurotransmitters. Microinjection of thyrotropin-releasing hormone (TRH) or pituitary adenylate cyclase-activating polypeptide (PACAP38) into NRO resulted in gastric excitation, whereas substance P (SP) and vasoactive intestinal polypeptide (VIP) evoked gastric relaxation through vagal mechanism (8). Glutamate microinjected into NRO also enhanced gastric motility in rat, and these responses could be abolished by peripheral muscarinic blockade (15). Glutamate is an endogenous neurotransmitter in NRO. There are several subtypes of glutamate receptors. Ionotropic glutamate receptor activated by agonist such as glutamate or NMDA caused a concentration-dependent increase of arterial blood pressure, while metabotropic glutamate receptor activated by (+/-)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-APCD) caused a concentration-dependent decrease in blood pressure. Ionotropic and metatropic glutamate receptor antagonists could block the responses activated by corresponding agonist respectively (1). These results suggest that different glutamate subtype receptors in NRO have different functions in autonomic regulation.

The motility of gallbladder in interdigestive period could be divided into two types, one is transient or phasic contraction and the other prolonged or tonic contraction. Different motility formations have different mechanisms and physiological importances (11, 12, 13, 16, 18). In rabbits, vagus nerve regulate the gallbladder phasic contraction while the sympathetic nerve modulate the tonic contraction (10, 14).

All these results suggest that NRO may also play a vital role in the regulation of gallbladder motility through autonomic nerves. The aim of this study is to investigate the role of NRO in the modulation of gallbladder motility during the interdigestive period in rabbits.

Materials and Methods

Animals

The healthy adult rabbits, weighting 1.8-2.0 kg, were purchased from Shandong University Animal Center. All animals were deprived of food but allowed access to water ad libitum for 18-24 hours before the experiments. The Shandong University's criteria for care and use of laboratory animals in research which was in compliance with Chinese regulation on the protection of laboratory animals was followed throughout the study.

Chemicals and Reagents

Urethane was made by Shanghai Chemical and Reagent factory. Glutamate and r-amino-n-butyric acid (GABA) are product of Shanghai Kangda Amino Acid Company. Propranolol, atropine and phentolamine were made by Shanghai Medicine Factory. Ketamine and 6-cyano-7-nitroquinoxaline-2,3-dine (CNQX) were the products of Sigma Chemical Co (St. Louis, Mo, USA).

Frog Urinary Bladder Preparation

Before every experiment, a frog was killed and a silicone canula was inserted into its urinary bladder through a small incision in cervix, then the urinary bladder was ligated tightly onto the silicone canula under the incision and disassociated upper the ligation. The urinary bladder had a volume about 2 ml and was emerged in saline for usage.

Surgical Procedures

Animals were anaesthetized with urethane (1 g/kg, iv), trachectomized and artificially ventilated, and the right femoral artery was catheterized to monitor arterial pressure via pressure transducer. In order to monitor the gallbladder motility, the gallbladder was exposed through a midline abdominal incision, and the frog urinary bladder connected with pressure transducer was inserted into it. The reasons why we used the frog bladder to measure the gallbladder pressure in this animal model is that: [1] it is a biological organ and does not stimulate the mucus of gallbladder; [2] and its wall is thin with high compliance. The changes of gallbladder pressure (GP) and blood pressure (BP) were simultaneously recorded by a polygraph (RM-6000, NIHON KHODEN, JAPAN). The animal's head was fixed in a stereotaxis frame (NARISHIGE, JAPAN). According to Messen's topography, NRO was located from midline to 0.5 mm off the midline of brain stem, 1.5-2.5 mm rostral to the obex and 1.0-2.5 mm ventral to the brain stem surface. A guide cannula was stereotaxically placed on the brain stem surface. Bilateral vagal nerves of some animals were cut at cervix level and the spinal cords of some animals were transected at T3-4. Rectal temperature and BP of all animals were monitored and the rectal temperature was kept at 37.5-38.5 °C by a thermostatically controlled heated operating table (MEDAX, Germany).

Microinjection

A vitreous tube attached to a 0.5 µl microsyringe (Shanghai Galss Apparatus Factory) with inside

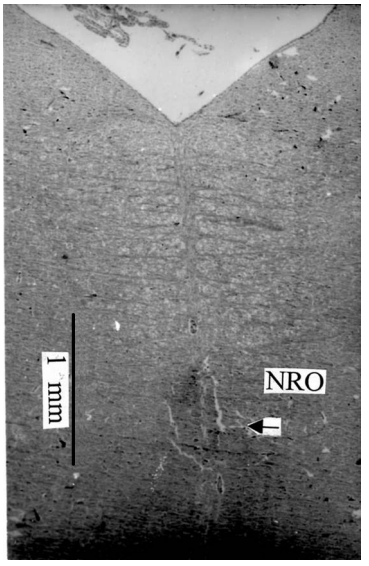

Fig. 1. The coronal section of brain stem at the level of 2.0 mm rostral to obex. The arrow indicate the ruptured tissue and swelling cells in nucleus raphe obscurus (NRO) caused by glutamate of high concentration and large dose (2 M/l, 1 μl)

diameter of 15 μm and outside diameter of 30 μm, was inserted into the nucleus via the guide cannula and 0.2 μl chemicals or reagents were injected slowly (last for one minute) through the microsyringe.

Tissue Location

After every experiment, glutamate of high concentration and large dose (2M, 1 μl) was injected into NRO within 3 seconds to destroy the nervous tissue. Two hours later the brain of animal was removed and fixed in 10% formaldehyde solution. Sections of brain (40-50 μm) were made and examined with microscope. Under the microscope we observed that, at the position where the vitreous tube reached, the brain tissue ruptured and the cells swelled (Fig. 1). Only data from those rabbits that the injection sites were correct were included for final evaluation.

Protocol

103 animals were used in this study. On every animal, the microinjection was repeated for 3-4 times. The intermission between the two successive microinjections is 90 to 120 minutes. n is the repeated times of one experiment. Control experiments were conducted to test the reliability of this method.

The animals were randomly divided as follows:

Control group: including microinjection of NS into NRO (n=17) and microinjection of Glutamate outside NRO (1.0 mm off the midline) (170 mmol/L, n=21).

Experiment group 1: microinjection of glutamate (170mmol/L) , a natural and non selective glutamate receptors agonist, into NRO (n=23).

Experiment group 2: microinjection of GABA (1 mol/L), an inhibitory neuro-transmitter, into NRO (n=23).

Experiment group 3: microinjection of CNQX (2 mmol/L), a non-NMDA glu-tamate receptor antagonist, into NRO (n=22).

Experiment group 4: microinjection of glutamate into NRO after microinjection of CNQX into NRO (n=19).

Experiment group 5: microinjection of ketamine (180 mmol/L), a NMDA glu-tamate receptor antagonist, into NRO (n=23).

Experiment group 6: microinjection of glutamate (170 mmol/L) into NRO after microinjection of Ketamine (n=23).

Experiment group 7: microinjection of glutamate (170 mmol/L) into NRO after intravenous injection of atropine (0.2 mg/kg) (n=19).

Experiment group 8: microinjection of glutamate (170 mmol/L) into NRO after bilateral vagotomy (n=18).

Experiment group 9: microinjection of glutamate (170 mmol/L) into NRO after intravenous injection of phen-tolamine (1.5 mg/kg) (n=21);

Experiment group 10: microinjection of glutamate (170 mmol/L) into NRO after transecting the spinal cord through T3-4 (n=21);

Experiment group 11: microinjection of glutamate (170 mmol/L) into NRO after intravenous injection of pro-pranolol (1.5 mg/kg) (n=20).

Experiment group 12: microinjection of glutamate (170 mmol/L) into NRO after combination of atropine and phentolamine intravenous injection (n=20).

Experiment group 13: microinjection of glutamate (170 mmol/L) into NRO after combination of vagotomy and transecting the spimal cord (n=20).

Statistics

The GP before the experiments was regarded as 0, and it was positive (+) if raised, negative (-) if

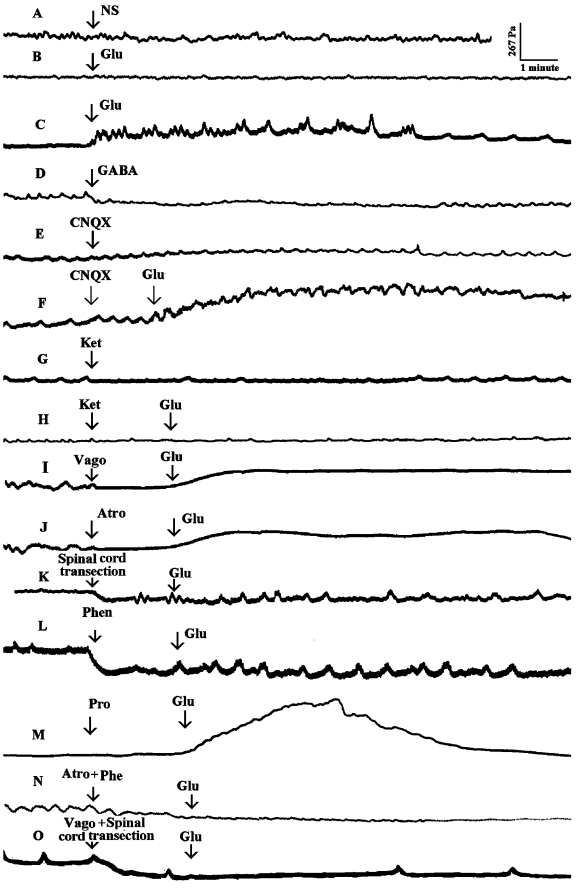

Fig. 2. The effects of several reagents microinjected into NRO on gallbladder motility.
A, Normal Saline (NS);
B, Glutamate (Glu) outside the NRO;
C, Glutamate into NRO;
D, r-Amino-n-Butyric Acid (GABA);
E, 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX);
F, Glutamate after CNQX;
G, Ketamine (Ket);
H, Glutamate after ketamine;
I, Glutamate after Vagotomy (Vago).
J, Glutamate after intravenous injection of atropine (Atro);
K, Glutamate after transecting the spinal cord;
L, Glulatame after intravenous injection of phentolamine (Phen);
M, Glutamate after intravenous injection of propranolol (Pro).
N, Glutamate after intravenous injection of atropine and phentolamine;
O, Glutamate after vagotomy and transecting the spinal cord.
The arrows indicate the treatments.

decreased during the experiments. If the GP rose above 60 pa but returned within 20 seconds, the motion was defined as phasic contraction. Experimental data including GP and frequency of phasic contraction of GB (PCGB) (/min) were expressed as means±S.D.. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between means was tested using Duncan's multiple

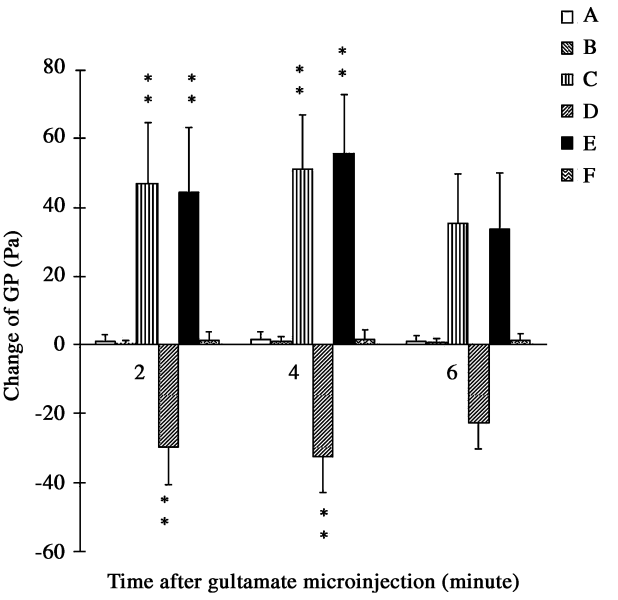

Fig. 3. The effects of microinjecting normal saline into NRO(A), glutamate outside NRO(B), glutamate (C), r-Amino-n-Butyric Acid (GABA) (D), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) (E) and ketamine (F) into NRO on gallbladder pressure (GP) .*P<0.05 *vs*. (A), **P<0.01 *vs*. (A)

range tests. The difference between two means was considered to be statistically significant when P was less than 0.05.

Results

Normal Gallbladder Motion

In the interdigestive period in rabbits, two kinds of motion of gallbladder were recorded, i.e. the tonic and phasic contractions. Tonic contraction, which means continuous and weak contraction, could maintain GP at a stable level (2670±190Pa, n=8). Phasic contraction, with a duration of 5~20 s and the frequency of 1~3 times per minute, raised GP by 60-200Pa rhythmically. When NS was microinjected into NRO or glutamate microinjected outside NRO, GP and PCGB showed no change in 20 minutes [Fig. 2(A), 2(B); Fig 3(A) and 3(B); Fig 4(A) and 4(B)].

Regulation of NRO on Tonic Contraction of Gallbladder

When glutamate was microinjected into NRO, the tonic contraction of gallbladder was enforced, the GP increased by 46±18 Pa, 51±16 Pa, 35±15 Pa at the 2nd minute, 4th minute, and 6th minute respectively after glutamate microinjection[Fig. 2(C), Fig. 3(C)]. While after microinjection of GABA, the tonic contraction of gallbladder was weaken, the GP decreased to 29.93±11.09 Pa, 29.93±10.46 Pa,

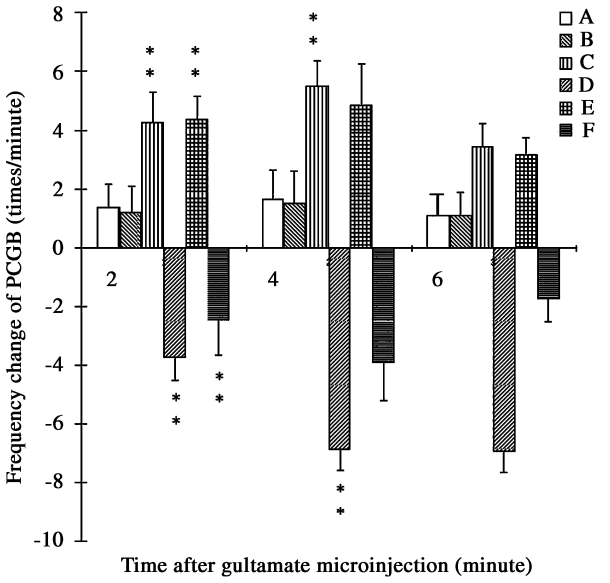

Fig 4. The effects of microinjecting normal saline into NRO(A), glutamate outside NRO(B), glutamate (C), r-Amino-n-Butyric Acid (GABA) (D), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) (E) and ketamine (F) into NRO on phasic contraction of gallbladder (PCGB). ***P*<0.01 *vs*. (A)

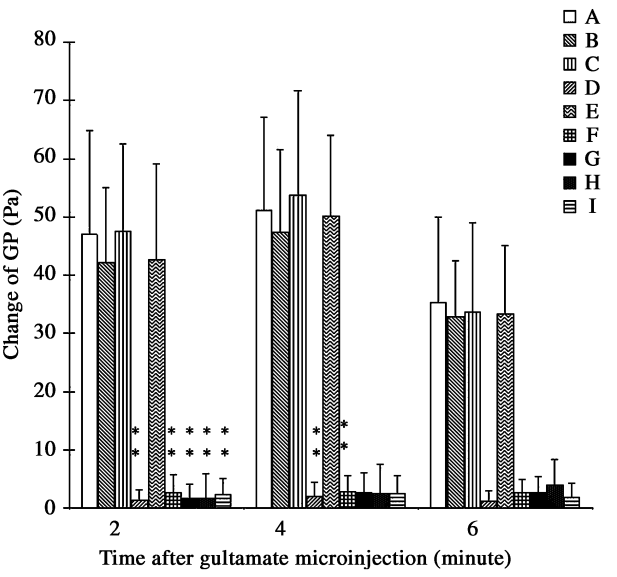

Fig. 5. The effects of glutamate administrated into NRO after microinjection of CNQX into NRO (A); that after intravenous injection of atropine (B); that after vagotomy (C); that after administration of ketamine into NRO (D); that after intravenous administration of propranolol (E); that after intravenous injection of phentolamine (F); that after transecting the spinal cord (G); that after intravenous injection of atropine and phentolamine (H); and that after vagotomy and transecting the spinal cord (I) on gallbladder pressure. ***P*<0.01 *vs* Fig. 3(C).

19.29±8.04 Pa at the 2nd, 4th and 6th minute after GABA microinjection[Fig. 2(D), Fig.3(D)].

Microinjection of CNQX enforced the tonic contractions of the gallbladder [Fig. 2(E), Fig. 3(E)] but did not influence the effects of glutamate microinjection on the tonic contraction of gallbladder [Fig. 2(F), Fig.5(A)]. Microinjection of ketamine into NRO did not affect the tonic contractions of the gallbladder [Fig. 2(G), Fig. 3(F)]. Pretreatment of ketamine into NRO completely abolished the effect of glutamate on tonic contractions of gallbladder [Fig. 2(H), Fig. 5(D)]. Intravenous injection of atropine or bilateral vagotomy did not influence the effect of glutamate microinjection on tonic contraction [Fig. 2(I, J), Fig. 5(B, C)]. Intravenous injection of phentolamine or transection of the spinal cord at T3-4 completely abolished the effect of glutamate on tonic contraction [Fig. 2(K, L), Fig. 5(F, G)]. Intravenous injection of propranolol did not influence the effects of glutamate on tonic contraction of gallbladder [Fig. 2(M), Fig. 4(E)]. After intravenous injection of atropine and phentolamine or vagotomy and transecting the spinal cord, the administration of glutamate into NRO did not affect the tonic contractions of gallbladder [Fig. 2 (N, O), Fig. 5(H, I)].

Regulation of NRO on Phasic Contraction of Gallbladder

After microinjection of glutamate into NRO, the phasic contraction of the gallbladder was enhanced. The frequency of PCGB increased by 4.5±1.1, 5.5± 1.0 and 3.0±0.8 at the 2nd minute, 4th minute, and 6th minute respectively after glutamate administration [Fig. 2(C) and Fig. 4(C)]. The strength of the phasic contraction return to normal level within twenty minutes. Microinjection of GABA into NRO inhibited the phasic contraction. At the 2nd, 4th and 6th minute after GABA administration, the frequency of phasic contraction decreased by 3.7±1, 5.5±1.1 and 3.6±1.0 [Fig. 2(D) and Fig. 4(D)].

Microinjection of CNQX enforced the phasic contraction of the gallbladder [Fig. 2(E) and Fig. 4(E)] but did not influence the effect of glutamate microinjection on the phasic contraction of the gallbladder [Fig. 2(F) and Fig. 6(A)]. Microinjection of ketamine into NRO decreased phasic contraction of gallbladder [Fig. 2(G) and Fig. 4(F)]. Premicroinjection of ketamine into NRO completely abolished the effect of glutamate on the phasic contraction of gallbladder [Fig. 2(H) and Fig. 6(B)].

Intravenous injection of atropine or bilateral vagotomy abolished the effect of glutamate microinjection on phasic contraction [Fig. 2(I, J) and Fig. 6(C, G)]. Intravenous injection of phentolamine or transection of the spinal cord at T3-4 did not influence the effect of microinjection of glutamate

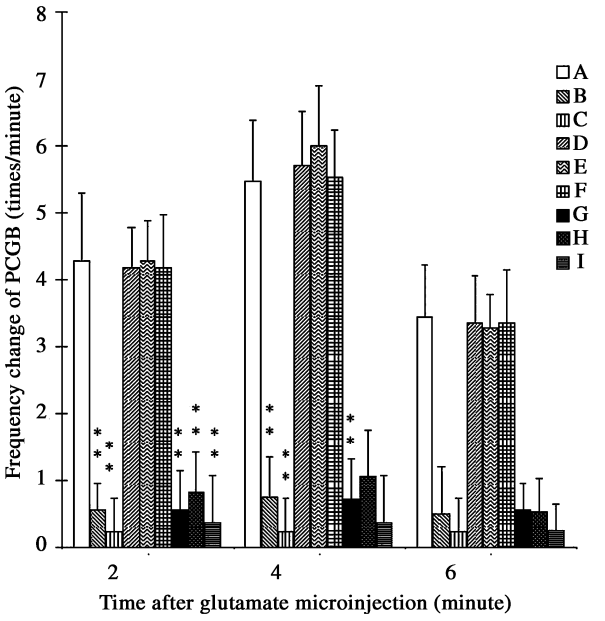

Fig. 6. The effects of glutamate administrated into NRO after microinjection of CNQX into NRO (A), that after administration of ketamine into NRO (B); that after intravenous injection of atropine (C); that after transecting the spinal cord (D); that after intravenous injection of phentolamine (E); that after intravenous administration of propranolol (F); that after vagotomy (G); that after intravenous injection of atropine and phentolamine (H); and that after vagotomy and transecting the spinal cord (I) on phasic contraction of gallbladder (PCGB). ***P*<0.01 *vs* Fig. 4(C).

into NRO on the frequency of phasic contraction [Fig. 2(K, L) and Fig. 6(D, E)]. Intravenous injection of propranolol did not influence the effect of glutamate on the phasic contraction of gallbladder [Fig. 2(M) and Fig. 6(F)]. After intravenous injection of atropine and phentolamine or vagotomy and transecting the spinal cord, microinjection of glutamate into NRO did not affect the phasic contraction of gallbladder [Fig. 2(N,O) and Fig. 6(H,I)].

Discussion

Neurons in NRO receive nerve projections from many sites such as cerebrum, brain stem, and spinal cord and also project to sites mentioned above (1, 8). There are many neurotramsmitters in the neurons of NRO, such as glutamate, serotonin, substance P, TRH and nitric oxide (5, 6, 7, 17) et al. The comprehensive fiber connection and chemical neuroanatomy of NRO may be the basis for its participating in regulation of many visceral functions. NRO has been regarded as an important centrum in regulating the visceral functions, especially the motility of digestive system.

We have reported that NRO regulated the myoelectronic activity of SO (22). In the present study, we found that microinjection of glutamate, an excitatory neurotransmitter, into NRO enforced the motility of the gallbladder in the interdigestive period in rabbits while microinjection of inhibitory neurotransmitter, GABA, could inhibit the motility of gallbladder. These results indicate that NRO may physiologically regulate the gallbladder motility. Our findings confirm that NRO is an important centrum regulating the motility of extrahepatic biliary system. The glutamate receptors are divided into 3 subtypes, ionotropic, metabotropic and L-AP4 receptor. The ionotropic glutamate receptor is further divided into NMDA and non-NMDA receptors. These kinds of glutamate receptors have different functions. D'Amico M et al found that different subtypes of glutamate receptor have different functions on blood pressure (1). In our recent study, we found that glutamate could regulate the electronic activity of SO mainly through NMDA receptor. In this study, NMDA receptor antagonist, ketamine, could decrease the phasic contraction of gallbladder, while non-NMDA receptor antagonist, CNQX, could enforce the gallbladder motility, these results indicate that glutamate in NRO regulate gallbladder motility through different receptors. Through NMDA receptor, glutamate in NRO enforce the motility while through non-NMDA receptor, glutamate inhibit the motility of gallbladder.

In this study, intravenous injection of atropine or vagotomy abolished the effect of glutamate on the phasic contraction of gallbladder, intravenous injection of phentolamine or transecting the spinal cord attenuates that on tonic contraction. Therefore we deduce that, during the interdigestive period in rabbits, endogenous glutamate in NRO regulates the gallbladder phasic contraction through vagus nerve and peripheral cholinergic receptor while regulates the tonic contraction through sympathetic nerve and peripheral α-receptor. In our previous study, we also found that DVC regulate the motility of gallbladder through the similar peripheral pathway (9, 10, 11, 12, 13, 18). Since NRO has reciprocal connection with DVC, we think that NRO may regulate the motility through DVC.

Both of the tonic and phasic contractions of gallbladder are of great importance. The former, mainly controlled by sympathetic nerve, maintains the GP at constant level. The later, controlled by vagus nerve, is responsible for stirring the bile in gallbladder and prevent the formation of gallstones (10, 13, 14). NRO regulate both of the two kinds of gallbladder motions through sympathetic and vagus nerves respectively. This result clearly indicates that in the interdigestive period in rabbits, both of the sympathetic and parasympathetic efferent activities are regulated by NRO.

In conclusion, this study indicates that NRO regulate tonic gallbladder contraction through sympathetic nerve and peripheral α receptors, while modulate gallbladder phasic contraction via vagus nerve and peripheral M receptors.

Acknowledgements

This study is supported by the Natural Scientific Foundation of Shandong Province, People's Republic of China (Q98C06129). The authors gratefully acknowledge the assistance provided by Professor Paulus S. Wang (Department of Physiology, National Yang-Ming University, Taipei, Taiwan, R. O. C) in the preparation of the maunscript and Dr. Zhang Min (Department of Biology, McMaster University, Country) for his generous donation of CNQX.

References

1. D'Amico, M., Berrino, L., Pizzirusso, A., de Novellis, V., and Rossi, F. Opposing effects on blood pressure following the activatvation of metabotropic and ionontropic glutamate receptors in raphe obscurus in the anaesthetized rat. Naunyn Schmiedebergs Arch. *Pharmacol*. 353: 302-305, 1996.
2. Hermann, G.E., Bresnahan, J.C., Holmes, G.M., Rogers, R.C., and Beattie, M.S. Descending projection from nucleus raphe obscurus to pudendal motoneurons in male rat. *J. Comp. Neurol*. 397: 458-74, 1998.
3. Holmes, G.M., Martau, J.M., Hermann, G.E., Rogers, R.C., Bresnahan, J.C., and Beattie M.S. Nucleus raphe obscurus regulation of anorectal motility in rats. *Brain Res.* 759: 197-204, 1997.
4. Hornby, P.J., Rossiter, C.D., White, R.L., Norman, W.P., Kuhn, D. H., and Gillis, R.A. Medullary raphe: a new site for vagally mediated stimulation of gastric motility in cats. *Am. J. Physiol*. 258: G637-G647, 1990.
5. Krowicki, Z.K. and Hornby, P.J. Opposing gastric motor responses to TRH and substance P on their microinjection into nucleus raphe obscurus of rats. *Am. J. Physiol*. 265: G819-G830, 1993.
6. Krowicki, Z.K. and Hornby, P.J. Serotonin microinjected into the nucleus raphe obscurus increases intragastric pressure in the rat via a vagally mediated pathway. *J. Pharmacol. Exp. Ther.* 265: 468-476, 1993.
7. Krowicki, Z.K. and Hornby, P.J. The inhibitory effect of substance P on gastric motor function in the nucleus raphe obscurus is mediated via nitric oxide in the dorsal vagal complex. *J. Auton. Nerv. Syst.* 58:177-180, 1996.
8. Krowicki, Z.K. Role of selected peptides in the vagal regulation of gastric motor and endocrine pancreatic function. *J. Physiol. Pharmacol*. 47: 399-409, 1996.
9. Li, A.j., Liu, J.Z. and Liu, C.Y. Anatomical and functional study of localization of originating neurons of the parasympathetic nerve to gallbladder in rabbit brain stem. *Chinese J. Physiol*. 45: 19-24, 2002.
10. Liu, C.Y., Liu, J.Z., Li, Z.Y., and Liu, K.J. The effects of vagus and sympathetic nerves on gallbladder pressure in the interdigestive period in rabbits. *Acta Academiae Med. Shandong*. 38: 32-35, 2000.
11. Liu, C.Y., Liu, J.Z., Zhou, J.H., Wang, H.R. and Li, Z.Y. The effects of electrical and chemical stimulation of DVC on the motility of biliary system. *Acta Acdemiae Med. Shangdong*. 35: 207-210, 1997.
12. Liu, C.Y., Liu, J.Z., Zhou, J.H., Wang, H.R., Li, Z.Y., Li, A.J. and Liu, K.J. The effects of TRH microinjected into DVC on the myoelectricity of sphincter of Oddi in rabbits and analysis of the route. *Chin. J. Appl. Physiol*. 15: 43-46, 1999.
13. Liu, C.Y., Liu, J.Z., Zhou, J.H., Wang, H.R., Li, Z.Y. and Liu, K.J. TRH microinjection into DVC enhances motility of rabbit's gallbadder via vagus nerve. *World J Gastroenterol*. 4: 162-164, 1998.
14. Liu, C.Y., Liu, J.Z., Li, A.J., Zhou, J.H., Wang, H.R., Li, Z.Y. and Liu, K.J. The influence of vagal and sympathetic nerves on the activities of PCGB in fasted rabbits. *Chin. J. Neurosci*. 14: 174-177, 1998.
15. McCann, M.J., Hermann, G.E. and Rogers, R.C. Nucleus raphe obscurus (nRO) influences vagal control of gastric motility in rats. *Brain Res.* 486: 181-184, 1989.
16. Mizumoto, A., Fujimura, M., Ohtawa, M., Ueki, S., Hayashi, N., Itoh, Z., Fujino, M. and Arimura, A. Pituitary adenylate cyclase activating polypeptide stimulates gallbladder motility in conscious dogs. *Regul. Pept.* 42:39-50, 1992.
17. Okumura, T., Uehara, A., Taniguchi, Y., Watanabe, Y., Tsuji, K., Kitamori, S. and Namiki, M. Kainic acid injection into medullary raphe produces gastric lesions through the vagal system in rats. *Am. J. Physiol*. 264: G655-G658, 1993.
18. Wang, J.Y., Liu, J.Z., and Liu, C.Y. Effects of nitric oxide in the dorsal motor nucleus of the vagus on the extrahepatic biliary system in rabbits. *Auton. Neurosci.: Basic & Clinical*. 87: 46-51, 2001.
19. White, R.L Jr, Rossiter, C.D., Hornby, P.J., Harmon, J.W., Kasbekar, D.K., and Gillis R.A. Excitation of neurons in the medullary raphe increases gastric acid and pepsin production in cats. *Am. J. Physiol.* 260: G91-96, 1991.
20. Xie, Y.F., Liu, J.Z. and Liu, C.Y. Effect of nucleus raphe obscurus on myoelectric activity of sphincter of Oddi in rabbits. *Acta Physiol. Sinica* 53: 215-218, 2001.