Responses of Blood Pressure and Renal Sympathetic Nerve Activity to Colorectal Distension in Anesthetized Rats

W.M. LI^{1,2}, A. SUZUKI³, and K.M. CUI¹

¹Department of Integrative Medicine, Shanghai Medical College, Fudan University, Shanghai 200032, China; ²Shanghai Research Center of Acupuncture and Meridian, Shanghai 201203, China; and ³Laboratory of Physiology, Health Science University, Yamanashi, 401-0380 Japan

Abstract: We investigated the effects of visceral stimulation by colorectal distension (CRD) on mean arterial blood pressure (MAP) and renal sympathetic nerve activity (RSNA), the latter being an index of vasoconstrictor activity, in anesthetized central nervous system (CNS)-intact and C2 spinalized rats. The CRD stimulation was induced by the distention of a balloon inserted into the colorectum. In CNS-intact rats, there were significant reductions in MAP and RSNA in response to intraballoon pressures of 60 and 80 mmHg, but not to 20 and 40 mmHg. Howev-

er, spinalized rats demonstrated significant increases in MAP in response to intraballoon pressures of 60 and 80 mmHg and increases in RSNA in response to intraballoon pressures of 40, 60, and 80 mmHg. These results suggest that noxious visceral stimulation at lower spinal levels reduces MAP by inhibiting sympathetic output in CNS-intact anesthetized rats. On the other hand, noxious visceral stimulation results in an increase in sympathetic-induced MAP in spinalized anesthetized rats.

Key words: colorectal distention, mean arterial blood pressure, renal sympathetic nerve activity, rats.

A reflex that plays an important role in maintaining homeostasis is the autonomic viscerovisceral reflex. Blood pressure (BP), an important physiological parameter of homeostasis, was reported to be regulated by visceral afferent inputs via viscerovisceral reflexes [1–3]. In animals, visceroinduced changes in BP can be elicited by electrically stimulating the vagus or splanchnic afferent nerves [4, 5] or by mechanically stimulating afferents from the mesentery or pelvic organs [6–8].

It was reported that BP was also reflexively regulated by somatic afferent inputs [9–12]. Kimura *et al.* [13] suggested that somatically induced cardiovascular reflex responses are a consequence of cardiovascular sympathetic responses and that these sympathetic responses have a strong segmental spinal reflex component and a generalized supraspinal reflex component. It would be interesting to determine whether similar systems are involved in the changes in BP that occur as a result of visceral afferent stimulation.

Colorectal distension (CRD) results in the mechanical stimulation of visceral afferents and has been widely studied in attempts to further understand the physiology and pathophysiology of viscerosensory responses [6, 7, 14, 15]. Data suggest that graded CRD stimulation produces strength-dependent responses in behavior, the rectus abdominis electromyogram (EMG), and BP in rats [6, 16].

Ness and Gebhart [6] reported that opposing BP responses occurred in animals following CRD stimulation depending on whether the animal was conscious (pressor response) or unconscious (depressor response), the latter depending on the depth of anesthesia and/or other factors. In this study, we investigated the relationship between visceral CRD stimulation and its corresponding visceral responses, specifically, mean arterial blood pressure (MAP) and renal sympathetic nerve activity (RSNA), which is an index of vasoconstrictor activity [17] in anesthetized central nervous system (CNS)-intact and spinalized rats.

MATERIALS AND METHODS

Animals. Adult male Wistar rats weighing 330–370 g ($n = 14$) were housed in a climate-controlled environment with food and water *ad libitum*. All animal experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgical preparation. The rats were anesthetized with 50 mg/kg of pentobarbital intraperitoneally for general operations, such as cannulating the trachea, artery, and vein and dissecting the renal sympathetic nerve. Because it has been reported that cardiovascular responses in anesthetized rats vary with fluctuations in the anesthetic level [6],

Received on Feb 3, 2006; accepted on Apr 5, 2006; released online on Apr 9, 2006; doi:10.2170/physiolsci.RP001106

Correspondence should be addressed to: Weimin Li, Department of Integrative Medicine, Shanghai Medical College, Fudan University, Shanghai 200032, China. Tel: +86-21-54237023, Fax: +86-21-63010641, E-mail: wml@shmu.edu.cn

a microinjection pump was used to maintain stable anesthesia during each experiment by the continuous injection of 1.0% pentobarbital saline solution at a rate of 0.5–0.8 ml/h through a cannula that was inserted into the left femoral vein. The right femoral vein was cannulated for delivery fluids. The animals were also intubated with a tracheal tube through which they were artificially ventilated (SN480-7, Shinano, Japan). End-tidal CO₂ concentrations, examined with a gas monitor (Microcap, Oridion Medical, Israel), were kept at about 3.0% by controlled respiratory rate and volume. Body temperature was kept at 37–38°C with a homeothermic blanket and an infrared lamp (ATB-1100, Nihon Kohden, Japan).

Stimulation of visceral afferents. Visceral afferents were stimulated by inserting a 2 cm air-filled balloon made from a condom into the colorectum through the anus; the tip of the balloon was placed 6 cm beyond the anal verge. The degree of distension was modulated and assessed using a 10 ml volume syringe and sphygmomanometer connected by way of a Y connector to the balloon. Pressures of 20, 40, 60, or 80 mmHg were applied for 20 s. The time interval between distentions was approximately 5 min.

MAP recordings. The right femoral artery was cannulated to measure MAP. The measurements were made with a pressure transducer (P23XL-1, Becton Dickinson and Company, USA) and were converted into digital signals by an analog-to-digital converter for analysis. The animals were kept in the supine position for 20–30 min after their operation in order to obtain a stable baseline recording. The maximum changes in MAP recorded within 30 s after the onset of CRD stimulation were compared to pre-stimulus control values.

RSNA recordings. With the rats in the prone position, a branch of the left renal sympathetic nerve was dissected retroperitoneally, and its central cut end was attached to bipolar platinum iridium wire electrodes after the nerve had been covered with paraffin oil. Muscle relaxation was induced by gallamine triethiodide (10–20 mg/kg i.v., Sigma, USA). RSNA was amplified, filtered (time constant 0.01 s, S-0476, Nihon Kohden, Tokyo), and displayed on an oscilloscope. Neuronal activity was sampled with an analog-to-digital converter (PowerLab/8, AD Instruments, Australia) at a rate of 2,000 samples/s and was rectified and integrated every 5 s. The signal recorded at the end of the experiment (after the rat died) was set as the background noise. Nerve discharge values were calculated by subtracting the background noise from the recorded RSNA values. The changes in RSNA that occurred in response to 20 s of CRD stimulation were compared with the average value in the 20 s prior to stimulation, and the responses were expressed as a percent of baseline.

Spinal transection. Full spinal cord transections were carried out at the upper C2 level in 8 rats. After surgery, their MAP was maintained around 70 mmHg by an injection

of a 4% Ficoll solution (Pharmacia Fine Chemicals AB, Sweden).

Vagotomy. To clarify the influence of vagal nerves on the MAP response to CRD stimulation, the vagus nerves were bilaterally cut at the cervical level in 4 rats.

Statistical analysis. All data are presented as the means $\pm$ S.D. A statistical analysis was performed using paired or unpaired *t*-tests (InStat for Windows, 3.05 version), and the values were considered to be significant if their *p* value was ≤ 0.05 .

RESULTS

MAP changes in response to visceral stimulation in CNS-intact rats

The changes in MAP in response to graded CRD stimulation are summarized in Fig. 1. The effects of CRD stimulation strengths of 40, 60, and 80 mmHg in one rat on MAP are shown in panels A–C. The results showed that this animal exhibited a MAP response to pressures at or above 60 mmHg. Specifically, MAP was significantly reduced by 9.5 ± 5.7 and 28.4 ± 11.3 mmHg following CRD stimulations of 60 and 80 mmHg, respectively, but CRD stimulations of 20 and 40 mmHg failed to influence MAP (Fig. 1D). The magnitude of the depressor response was dependent on the applied strength of CRD; the amplitude of the depressor response to 80 mmHg CRD stimulation was more than double that seen with 60 mmHg of CRD stimulation (Fig. 1D). These results were indepen-

Fig. 1. Changes in MAP in response to graded CRD stimulation in CNS-intact rats. Panels A–C show the original MAP responses to 40, 60, and 80 mmHg CRD stimulation in one rat. The transverse bars in the panels indicate the duration of CRD stimulation (20 s). Panel D summarizes the MAP responses to all four levels of distension ($n = 10$ trials in 6 rats). The data are expressed as the means $\pm$ SD. For statistical purposes, the maximum changes in MAP within 30 s after the onset of the CRD stimulus were compared to prestimulus values. * $p < 0.05$ and ** $p < 0.01$, using a paired *t*-test.

Fig. 2. Changes in RSNA in response to graded CRD stimulation in CNS-intact rats. Panels A–C show the original RSNA responses to 40, 60, and 80 mmHg CRD stimulation in one rat. The transverse bars in the panels indicate the duration of CRD stimulation (20 s). Panel D summarizes the RSNA responses to all four levels of distension ($n = 8$ trials in 4 rats). The data are expressed as the means $\pm$ SD. The changes in RSNA following 20 s of CRD stimulation are expressed as a percentage of the 20 s prestimulus control values. * $p < 0.05$ and ** $p < 0.01$ compared to the prestimulus control values, using a paired t -test.

dent of vagal nerve activity because in vagotomized rats the change in MAP following 60 mmHg CRD was similar to that in vagal nerve intact rats (-11.2 ± 11.9 mmHg; 8 trials in 4 rats, data not shown in the figure).

RSNA changes in response to visceral stimulation in CNS-intact rats

Figure 2 shows the effects of CRD stimulation on RSNA. Panels A–C show the original RSNA responses to 40, 60, and 80 mmHg CRD stimulation in one rat. As above, the CRD pressures of 60 and 80 mmHg significantly reduced RSNA to 79 ± 9.7 and $74 \pm 12.6\%$, respectively, of prestimulus control values; no change occurred in RSNA following 20 and 40 mmHg of CRD stimulation (Fig. 2D).

MAP changes in response to visceral stimulation in spinalized rats

Figure 3, A–C, shows the original MAP responses to 40, 60, and 80 mmHg CRD stimulation in one rat. MAP increased following 60 and 80 mmHg, but not 40 mmHg, of CRD stimulation. Figure 3D, which summarizes the MAP responses to all four levels of distension, reveals an insignificant increase in MAP in response to 40 mmHg of stimulation, and there were significant increases in MAP in response to 60 and 80 mmHg of CRD stimulation. The amplitude of the MAP response at 80 mmHg was more than double that at 60 mmHg (Fig. 3D). Although the above mean values of MAP responses were obtained from 8 trials in 4 rats, 1 of these trials showed a decrease in

Fig. 3. Changes in MAP in response to graded CRD stimulation in spinalized rats. Panels A–C show the original MAP responses to 40, 60, and 80 mmHg CRD stimulation in one rat. Panel D summarizes the MAP responses to all four levels of distension ($n = 8$ trials in 4 rats). Other details are the same as those described in Fig. 1.

Fig. 4. Changes in RSNA in response to graded CRD stimulation in spinalized rats. Panels A–C show the original RSNA responses to 40, 60, and 80 mmHg of CRD stimulation in one rat. Panel D summarizes the RSNA responses to all four levels of distension ($n = 8$ trials in 4 rats). Other details are the same as those described in Fig. 2.

MAP in response to CRD stimulation at strengths of both 60 and 80 mmHg.

RSNA changes in response to visceral stimulation in spinalized rats

Figure 4, A–C, shows the original RSNA responses to 40, 60, and 80 mmHg of CRD stimulation in one rat. Panel D summarizes the RSNA responses to all four levels of distension ($n = 8$ trials in 4 rats). The data showed that there were significant increases in RSNA to graded CRD stimulations of 40, 60, and 80 mmHg, but not to a stimulation of 20 mmHg. RSNA increased to 110.6 ± 12.4 , 119.5 ± 15.5 , and $138.8 \pm 42.4\%$ following 40, 60, and 80 mmHg of CRD stimulation, respectively.

DISCUSSION

The present study demonstrates that in anesthetized CNS-intact rats as well as in acutely spinalized rats, MAP is modulated by CRD stimulation. CRD with a 7–8 cm balloon was reported to excite visceral afferents in spinal cord segments T13–L2 and L6–S2 [18]. When such stimulation is applied to rats with a pressure of 40, 60, and 80 mmHg, it is considered noxious [16]. Our results showed that CRD stimulation of 60 and 80 mmHg significantly reduced MAP and RSNA and that these effects could be reversed by spinalization at C2. Thus there appears to be inhibitory reflex modulation of BP at supraspinal levels in CNS-intact rats.

It is well known that the vagus nerve plays an important role in the regulation of cardiovascular function. The stimulation of the vagus efferent nerve produces bradycardia, which results in a reduction in BP. Our results showed no change in MAP in response to CRD stimulation following the bilateral transection of the vagus nerves; this supported the notion that the reflex pathways resulting in the BP reduction in our experiment did not involve these nerves.

It is interesting that not all trials exhibited similar BP findings in spinalized rats. In one case, CRD stimulation resulted in a BP reduction, though the amplitude of this reduction was not as great as that seen in CNS-intact rats. Evidence showed that both sympathetic vasoconstrictor and parasympathetic vasodilator fibers innervate the genital organs [19, 20]. Although our data suggest that an increase in sympathetic vasoconstrictor activity directly contributed to the CRD-induced pressor response in spinalized rats, it is also possible that parasympathetic vasodilator fibers played a role.

In conclusion, our results suggest that the inhibition of visceral sympathetic output occurs in response to noxious visceral afferent activation at lower segmental levels in anesthetized CNS-intact rats. This inhibition was reversed by spinal transection at C2.

This work was supported by grants from the Science Foundation of the Shanghai Municipal Commission of Science and Technology (No: 04dz19836-04, 05dz19747) and the 973 State Key Basic Research Project (No: 2005CB523306).

REFERENCES

1. Chahal PS, Rendig SV, Longhurst JC. Bradykinin BK2 receptors contribute to reflex cardiovascular responses during brief abdominal ischemia. *Am J Physiol.* 1998;274:H308-13.
2. Saleh TM, Connell BJ, Allen GV. Visceral afferent activation-induced changes in sympathetic nerve activity and baroreflex sensitivity. *Am J Physiol.* 1999;276:R1780-91.
3. Saleh TM, Saleh MC, Connell BJ. Estrogen blocks the cardiovascular and autonomic changes following vagal stimulation in ovariectomized rats. *Auton Neurosci.* 2001;88:25-35.
4. Mileva S. On the mechanism of the depressor reaction upon afferent vagal stimulation in cats. II. Role of the beta-adrenergic structures for the development of the depressor reaction. *Acta Physiol Pharmacol Bulg.* 1976;2:30-8.
5. Lembeck F, Donnerer J. Reflex fall in blood pressure mediated by capsaicin-sensitive afferent fibers of the rat splanchnic nerve. *Naunyn Schmiedeberg Arch Pharmacol.* 1983;322:286-9.
6. Ness TJ, Gebhart GF. Colorectal distention as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. *Brain Res.* 1988;450:153-69.
7. Ness TJ, Follett KA, Piper J, Dirks BA. Characterization of neurons in the area of the medullary lateral reticular nucleus responsive to noxious visceral and cutaneous stimuli. *Brain Res.* 1998;802:163-74.
8. Ness TJ, Lewis-Sides A, Castroman P. Characterization of pressor and visceromotor reflex responses to bladder distention in rats: sources of variability and effect of analgesics. *J Urol.* 2001;165:968-74.
9. Sato A, Schmidt RF. The modulation of visceral functions by somatic afferent activity. *Jpn J Physiol.* 1987;37:1-17.
10. Ohsawa H, Okada K, Nishijo K, Sato Y. Neural mechanism of depressor responses of arterial pressure elicited by acupuncture-like stimulation to a hindlimb in anesthetized rats. *J Auton Nerv Syst.* 1995;51:27-35.
11. Sato A, Sato Y, Schmidt RF. The impact of somatosensory input on autonomic functions. *Rev Physiol Biochem Pharmacol.* 1997;130:1-328.
12. Shimura M, Uchida S, Suzuki A, Nakajima K, Aikawa Y. Reflex choroidal blood flow responses of the eyeball following somatic sensory stimulation in rats. *Auton Neurosci.* 2002;97:35-41.
13. Kimura A, Ohsawa H, Sato A, Sato Y. Somatocardiocardiovascular reflexes in anesthetized rats with the central nervous system intact or acutely spinalized at the cervical level. *Neurosci Res.* 1995;22:297-305.
14. Brink TS, Mason P. Role for raphe magnus neuronal responses in the behavioral reactions to colorectal distension. *J Neurophysiol.* 2004;92:2302-11.
15. Kozłowski CM, Green A, Grundy D, Boissonade FM, Bountra C. The 5-HT₃ receptor antagonist alosetron inhibits the colorectal distention induced depressor response and spinal c-fos expression in the anaesthetized rat. *Gut.* 2000;46:474-80.
16. Cui KM, Li WM, Gao X, Chung K, Chung JM, Wu GC. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. *Neurosci Lett.* 2005;376:20-3.
17. Weaver LC, Stein RD, Meckler RL. Characteristics of ongoing and reflex discharge of renal postganglionic neurons. *Miner Electrolyte Metab.* 1989;15:24-32.
18. Ness TJ, Gebhart GF. Characterization of neurons responsive to noxious colorectal distension in the T13-L2 spinal cord of the rat. *J Neurophysiol.* 1988;60:1419-38.
19. de Groat WC, Booth AM. Physiology of male sexual function. *Ann Intern Med.* 1980;92:329-31.
20. Sato Y, Hotta H, Nakayama H, Suzuki H. Sympathetic and parasympathetic regulation of the uterine blood flow and contraction in the rat. *J Auton Nerv Syst.* 1996;59:151-8.