Japanese Journal of Physiology, **49**, 283–288, 1999

The Neural Mechanism of Rectal Motility Response Induced by the Epicardial Application of Lactic Acid

J. KOLEY, A. K. BASAK*, M. DAS, M. Z. HAQUE, and B. N. KOLEY†

Department of Physiology, Electrophysiology Unit, University College of Science, 92, A.P.C.Road, Calcutta 700009, India; *Department of Physiology, Nepalgunj Medical College, Chisapani, Banke, Nepal; and †Department of Physiology, College of Medical Sciences, Bharatpur, Chitwan, Nepal

Abstract: The epicardial application of lactic acid induced a biphasic rectal motility response in lightly anaesthetised, open-chested and artificially ventilated cats. This rectal biphasic response is reflexogenic in nature as epicardial lignocaine abolished such response. This rectal biphasic response is abolished by cardiac sympathectomy and reprecipitated by left inferior cardiac afferent nerve stimulation. Such response is also abolished by sacral ventral rhizotomy and reproduced by stimulation of the peripheral cut end of split sacral ventral roots. This indicates that the afferent and efferent pathways for such reflex are lying in the cardiac sympathetic and sacral pelvic nerves, respectively. The higher centers involved for such reflex are lying above the mid-collicular level of the brain as decerebration at the mid-collicular level completely abolished such type of rectal response. Furthermore, the relaxation phase and contraction phase of such rectal response are mediated through nitric oxide release and cholinergic neurones, respectively, as N^G-nitro-L-arginine and atropine abolished relaxation and contraction phase of the rectal response, respectively. [Japanese Journal of Physiology, 49, 283–288, 1999]

Key words: epicardial lactic acid, rectal biphasic response, sympathetic afferents, nitric oxide.

Cardiac disorders like myocardial infarction and ischemia are often associated with different gastrointestinal symptoms [1–3]. These responses may be elicited after the stimulation of cardiac nociceptors [4, 5] by the epicardial application of different algesic agents like bradykinin, prostaglandin, nicotine, etc., and also by experimental coronary artery occlusion [6]. It has also been demonstrated that, during cardiac ischemia or during coronary artery occlusion, there is a rise in lactic acid concentration in the cardiac muscle [7]. Recently, Koley and her group [8, 9] showed that coronary artery occlusion or epicardial nicotine application causes a change in rectal movement. But as, during coronary artery occlusion or cardiac ischemia, there is a significant rise of lactic acid concentration in the ischemic myocardium, it seems reasonable to propose that this lactic acid might have some role in initiation of the rectal response. So, in the present study, attempts have been made to investigate the role of epicardial lactic acid on rectal movement and the neural mechanism underlying it.

METHODS

Experiments were carried out on 38 cats of either sex, having a body weight of 2–3 kg, after overnight fasting with water ad libitum. The animals were anesthetised with α-chloralose (60 mg/kg, I.V.) after an initial induction with anesthetic ether. Anesthesia was maintained throughout the experiment with a maintenance dose of chloralose (10 mg/kg, I.V.) when required. The femoral artery, femoral vein and trachea were routinely cannulated. A 5% glucose solution in 0.9% physiological saline was administered by drip feeding into the femoral vein throughout the experiment to maintain body fluid and pH. Blood pressure was recorded from the femoral artery on a Beckman RM Dynograph using a Bell and Howel pressure

Received on January 7, 1999; accepted on April 15, 1999
Correspondence should be addressed to: Juthika Koley, Department of Physiology, Electrophysiology Unit, University College of Science, 92, A.P.C. Road, Calcutta 700009, India. Tel: +91-33-3506387/8386, Fax: +91-33-3519755

transducer (Type 4-327-0129). For monitoring body temperature, a thermometer was placed into the anus and body temperature was maintained at 37°C with a heating pad placed below the dissecting table. Studies were performed on animals given pancuronium (1 mg/kg, I.V.), a skeletal muscle relaxant, to eliminate the participation of external anal sphincter and other adjoining skeletal muscles in the resting intrarectal pressure [10].

Opening of the chest. Before the chest was opened, the animals were kept under artificial respiration with a Starling Ideal Respiratory Pump (INCO, India). The left thorax was opened after removing thoracic ribs 2–6. The pericardium was cut longitudinally and a pool was made with the cut ends of the pericardium. The heart was kept moistened with a cotton film soaked with warm physiological saline time to time. An operating table lamp was also used to maintain the epicardial temperature at 37°C. The left stellate ganglion and left inferior cardiac sympathetic nerve (LICN) were exposed and cleaned carefully from the surrounding connective tissues under a dissecting microscope (Vickers Instrument, UK). LICN was sectioned and the central cut end of LICN was stimulated electrically by a Grass SD 9 stimulator with square-wave pulses (40–60 Hz, 6 V, 0.6 ms for 30–60 s) when requried.

To stimulate the cardiac sensory receptors, lactic acid (800 μg/ml) was applied over the epicardial surface of the left ventricle with the help of a very light cotton applicator for 60 s, taking care to prevent any mechanical disturbance of this region. After removal of the cotton applicator, the ventricular surface was washed with warm physiological saline at least three times to remove all traces of lactic acid and allowed to rest for 30 min before repeating the procedure.

For dissecting the inferior mesenteric ganglia, the abdomen was exposed retroperitoneally by a lateral incision below the kidney level and the inferior mesenteric ganglia was isolated and cleared from the external peritoneal wall under a dissecting microscope. Before sectioning, it was immersed in an ice bath for 10 min and sectioned when required. For sectioning ventral roots of the sacral (S_2–S_4) region, the spinal cord was exposed by laminectomy at the L_1–S_4 segment following the method of Koley and Mukherjee [11]. The ventral roots were isolated under a dissecting microscope and sectioned when required. For spinal preparation, the spinal cord was opened by laminectomy at the C_1–C_2 region following the same method, and before transection, 2% lignocaine was injected into the cord to avoid spinal shock. The cord was transected when required after tying with thread. Decerebration was made in cats under ether anesthesia following the methods of Sherrington [12]. The animals were then kept undisturbed for 2–3 h, after which experimental protocol was followed.

Recording of rectal motility. A flaccid balloon (1.0–1.5 cm of a condom), distended with 8–12 ml of warm saline via a polyethylene tube, was introduced into the rectum aborally by a small incision in the descending colon and fixed at the incision point. Rectal motility was recorded on an INCO polygraph [13] through a pressure transducer (Model 301, INCO, India) in the form of intrarectal pressure (IRP).

Statistical analysis. Results were expressed as mean (±SEM). A significance test was performed using Student's *t*-test. In the case of control rectal response, significance tests were performed between the average initial IRP (mmHg) and IRP during reflex relaxation and contraction phase. The percentage change in IRP during reflex relaxation and contraction were compared between the control group and each experimental group.

Drugs used. α-Chloralose (Kochlight Lab, UK); lactic acid (Sigma, USA); pancuronium bromide ("Pavulon," Infar (India) Ltd., India); lignocaine ("xylocaine," Astra-IDL, India); atropine sulfate (Bengal Immunity, India); and N^G-nitro-L-arginine (Sigma, USA).

RESULTS

1. Role of epicardial lactic acid on rectal motility

The local application of lactic acid (800 μg/ml for 60 s) over the epicardial surface of the left ventricle resulted in a biphasic rectal response—initial relaxation followed by a sustained contraction (Fig. 1). These changes were associated with the fall of blood pressure (Fig. 1).

In 8 out of 68 observations, there was no relaxation

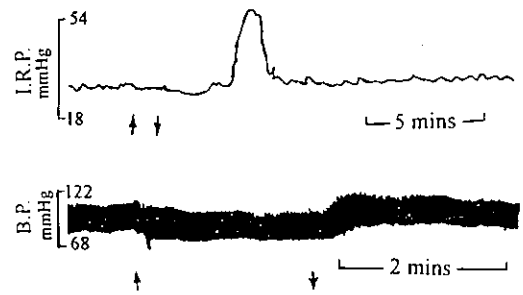

Fig. 1. Typical intrarectal pressure (IRP) changes (upper) and blood pressure (B.P.) changes (lower) in response to the epicardial application of lactic acid. The arrows (↑↓) indicate the duration of epicardial lactic acid application.

Table 1. Intrarectal pressure (IRP) during control and rectal biphasic response induced by epicardial lactic acid under different experimental conditions.

Experimental conditions	Initial Mean IRP±SEM (*n*)	Epicardial lactic acid			
		Relaxation		Contraction	
		Mean IRP ±SEM (*n*)	% fall ±SEM (*n*)	Mean IRP ±SEM (*n*)	% rise ±SEM (*n*)
Control	28.02±1.36 (18)	22.22±1.27*** (18)	20.90±1.09 (17)	41.44±1.61*** (18)	49.77±2.68 (17)
Lignocaine	26.50±1.74 (8)	25.93±1.69 (8)	2.16±1.03*** (8)	27.50±1.84 (8)	3.73±0.96*** (8)
LICN sectioning	27.25±1.88 (10)	26.5 ±1.96 (10)	3.01±1.14*** (10)	27.44±1.92 (9)	3.0 ±1.29*** (9)
Vagotomy	28.75±2.17 (8)	22.57±1.83**(8)	22.89±2.78 (8)	43.0 ±2.23*** (8)	49.71±3.13 (9)
Splanchnicotomy	34.8 ±1.8 (6)	27.76±1.53*** (6)	20.25±1.01 (6)	47.96±1.8*** (6)	39.08±1.45 (6)
Ventral rhizotomy	29.92±1.36 (10)	28.92±1.47 (10)	3.5 ±0.96*** (8)	30.92±1.32 (10)	4.29±1.08*** (10)

Number in parentheses indicates the number of observations. In all the cases, the value of mean IRP is in mmHg. ** $p<0.01$; *** $p<0.001$.

but inhibition of the spontaneous motility followed by contraction was observed. During the relaxation phase, the mean IRP was reduced to 22.22±1.27 mmHg ($p<0.001$), and during the contractile phase, the mean IRP was increased to 41.44±1.61 mmHg ($p<0.001$) from the normal intrarectal pressure (Table 1).

The desensitisation of ventricular receptors by the epicardial application of 2% lignocaine for 5–10 min completely abolished the epicardial lactic acid induced alteration of intrarectal pressure (Table 1).

2. Effect of cardiac vagotomy on lactic acid induced rectal response

In bilateral cardiac vagotomised animals, epicardial lactic acid–induced rectal biphasic response remained unaltered (Fig. 2B). There was no significant difference of IRP between the control group and vagotomised animals (Table 1).

3. Effect of cardiac sympathectomy on reflex rectal biphasic response

Sectioning of either the stellate ganglia or the LICN arising from stellate ganglion, abolished lactic acid induced biphasic rectal response completely (Fig. 2C). There was a significant ($p<0.001$) percentage alteration in IRP after LICN sectioning (Table 1).

Electrical stimulation (40–60 Hz, 6 V, 0.6 ms for 60 s) of the central cut end of LICN induced a biphasic rectal response (Fig. 2D) similar to that observed after epicardial lactic acid application to intact animals. On LICN stimulation, the mean IRP decreased from 32.3±1.37 to 25.49±1.29 mmHg and increased to 46.89±1.50 mmHg during relaxation and contraction phases, respectively. These changes are statistically significant ($p<0.001$).

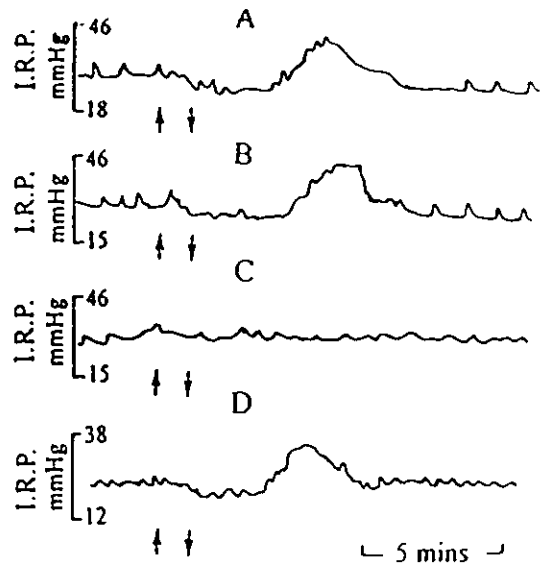

Fig. 2. Typical intrarectal pressure (IRP) changes in response to the epicardial application of lactic acid in control (A), cardiac vagotomised (B), and sympathectomised (C) animals. D shows the rectal movement in response to LICN afferent stimulation. The arrows (↑↓) indicate the duration of epicardial lactic acid application.

4. Effect of sectioning of splanchnic nerve or crushing of the inferior mesenteric ganglia (IMG) on reflex rectal biphasic response

After sectioning of the splanchnic nerve or crushing of the IMG, epicardial lactic acid induced a similar type of biphasic rectal response to that observed in intact animals. There was an insignificant percentage alteration of mean IRP in nerve-sectioned animals as compared to intact animals (Table 1).

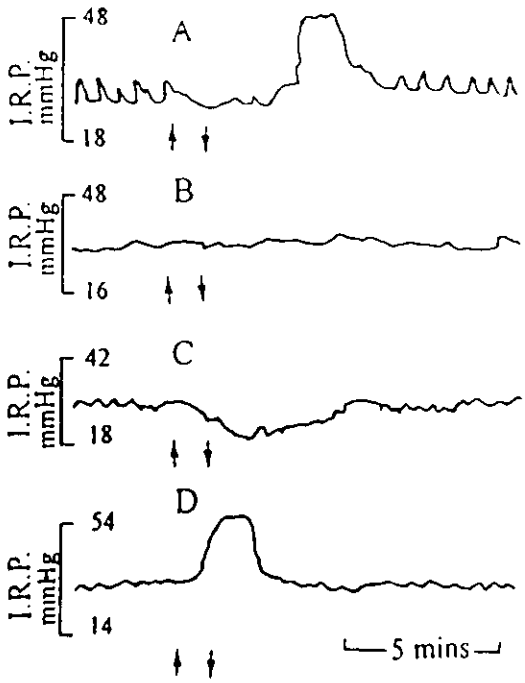

Fig. 3. Typical intrarectal pressure (IRP) changes in response to the epicardial application of lactic acid in control (A), and sacral ventral rhizotomised (B) animals. **C** and **D** show the rectal movement in response to electrical stimulation of the peripheral cut end of sacral ventral roots separately. The arrows (↑↓) indicate the duration of epicardial lactic acid application and electrical stimulation of nerves.

5. Effect of ventral rhizotomy on reflex rectal response

The rectal biphasic response induced by epicardial lactic acid was completely abolished in sacral (S_2–S_4) ventral roots–sectioned animals (Fig. 3B). There was a significant reduction in the percentage of change in mean IRP during both the relaxation and contraction phases in ventral rhizotomised animals as compared to that of control animals (Table 1).

The peripheral cut ends of the sacral ventral roots were split into a number of fine strands using a stereoscopic dissecting microscope, and each of the strands was stimulated electrically. It was observed that stimulation of the peripheral cut ends of some split strands of ventral roots resulted in relaxation only, whereas stimulation of other split strands caused contraction only (Fig. 3C, D). During the relaxation phase, the mean IRP was reduced to 24.88±2.35 mmHg ($p<0.05$), and in the case of contraction, the mean IRP was increased to 41.67±12.65 mmHg ($p<0.001$) from the initial IRP (30.0±2.47) (i.e., IRP before stimulation).

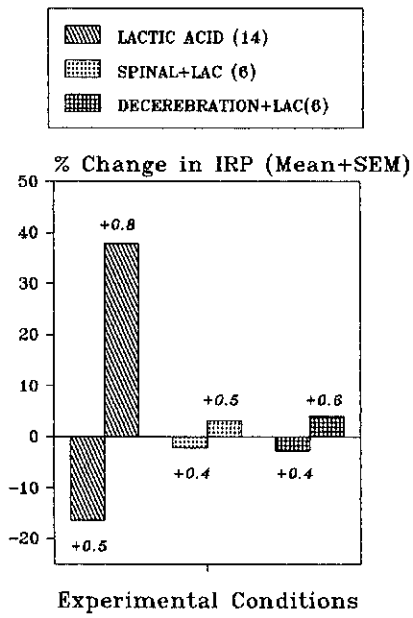

Fig. 4. The average percentage change in intrarectal pressure (IRP) in response to epicardial lactic acid in intact, spinal transected and decerebrated animals. Number within the parentheses indicates the number of observations.

6. Reflex rectal response in spinal transected or decerebrated animals

Spinal transection at the C_1–C_2 level or decerebration at the mid-collicular level abolished the epicardial lactic acid–induced biphasic rectal response. There was a significant reduction in the percentage of change in IRP in spinal transected or decerebrated animals as compared to intact animals (Fig. 4).

7. Effect of atropine and N^G-nitro-L-arginine (LNNA) on reflex rectal response

Epicardial lactic acid after 30 min of atropinisation (1 mg/kg, I.V.) failed to induce the contractile phase of rectal response, keeping the relaxation phase unchanged. During such contractile phase, the IRP was insignificantly changed from the initial IRP. The IRP during the lactic acid–induced contractile phase in control and atropinised animals varied significantly ($p<0.001$) (Fig. 5).

On the other hand, intra-arterial administration of a nitric oxide synthase inhibitor, LNNA (4 mg/kg), counteracted the relaxation phase significantly ($p<0.001$) without altering the contractile phase (Fig. 5).

DISCUSSION

It is well documented that the excitation of cardiac sensory receptors by epicardial application of chemical substances (e.g., bradykinin, nicotine, prosta-

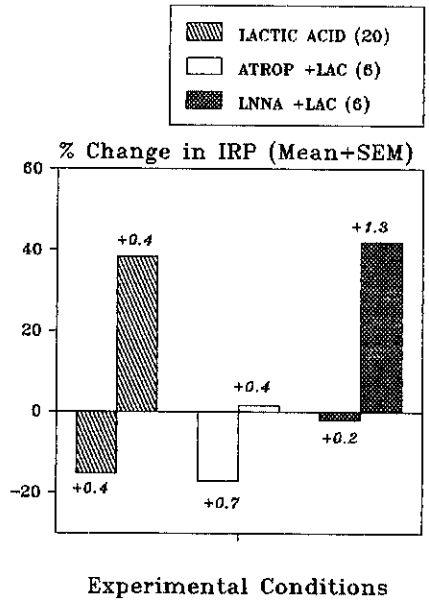

Fig. 5. The average percentage change in intrarectal pressure (IRP) in response to epicardial lactic acid in control, atropinised and LNNA-pretreated animals. Number within the parentheses indicates the number of observations.

glandins, lactic acid) may elicit bradycardia or hypotension [1, 2, 7]. Recently, Koley and her groups [8, 9, 13–15] reported that stimulation of cardiac nociceptors by left anterior descending coronary artery occlusion or by epicardial application of nicotine initiates increased forelimb movement, contraction of the nictitating membrane, increased urine flow and also alteration of the urinary bladder and rectal motility. The present study also reports that epicardial application of an algesic agent, lactic acid, excites the cardiac sensory receptors which in turn initiate biphasic rectal response. Moreover, these changes in rectal motility are reflexogenic, as they are totally absent after desensitisation of the cardiac sensory receptors by the epicardial application of lignocaine due to its endo-anesthetic and membrane stabilising properties [16]. It is now known that the sympathetic nervous system conveys afferent information from cardiovascular reflexogenic areas [17, 18]. It is apparent from the present study that cardiac receptors cause cardio-rectal reflexes when excited with the application of lactic acid directly over the epicardial surface. Such cardio-rectal reflexes can be abolished by cardiac sympathectomy. Similar biphasic cardio-rectal responses can be elicited by stimulation of the central cut end of the left inferior cardiac sympathetic nerve (LICN). This indicates that afferents for such cardio-rectal reflexes are lying in the cardiac sympathetic nerve. The efferent pathway is lying in the pelvic nerve as sectioning of the ventral roots of the sacral (S_2–S_4) spinal segment, which raises the pelvic nerve fibre to the rectum and lower abdominal structures [19, 20], completely abolished such rectal biphasic response. Electrical stimulation of some strands of these ventral roots could induce contraction and relaxation of the rectum with short latency. However, reflex responses induced by the local application of lactic acid are mostly of the long latency type as it traverses a long pathway along with several synapses, whereas in the case of electrical stimulation of the peripheral cut end of sacral ventral roots, the inhibitory or excitatory rectal response with short latency is evoked as impulse traffic has to traverse a much shorter path. Furthermore, this response is mediated through the supra spinal centres located above the mid-collicular level, as the biphasic response is absent in spinal transected and decerebrated (mid-collicular level) animals. It has been reported that parasympathetic outflow to the large intestine is composed of two sets of axons. One set synapses with intramural cholinergic neurones, which are excitatory for smooth muscle, whereas other set of axon that synapses with nonadrenergic, non-cholinergic (NANC) neurones, are inhibitory for smooth muscles [21, 22]. In the present study, it was observed that, in atropinised animals, the epicardial lactic acid–induced rectal contractile phase is absent, whereas the relaxation phase is abolished in nitric oxide blocker–pretreated animals. Therefore, the neuro-transmitter involved in the contractile phase of the lactic acid–induced biphasic response appears to be acetylcholine and that for the relaxation phase is nitric oxide. From the present observation, it may be inferred that local accumulation of lactic acid during cardiac ischemia results in evacuation of the bowel by altering rectal movement due to involvement of autonomic nerves where cardiac sympathetic nerves play as the afferent pathway and sacral parasympathetic nerve play as the efferent pathway. So it may be concluded that the defecatory response associated with severe cardiac pain is mostly of cardiac origin.

We gratefully acknowledge the financial assistance of the Indian Council of Medical Research (45/18/92-BMS, Dec. 9, 1993). We are also indebted to Dr. P. C. Das (Durham, NC, USA) for his generous gift of N^G-nitro-L-arginine.

REFERENCES

1. Abrahamsson H and Thoren P: Reflex relaxation of the stomach elicited from receptors located in the heart. An analysis of the receptors and afferents involved. Acta Physiol Scand 84: 197–207, 1972
2. Johannsen UJ, Summers R, and Marks AL: Gastric dilation during stimulation of cardiac sensory receptors.

Circulation 63: 960–964, 1987
3. Koley BN, Majumder C, and Koley J: Visceral reflexes of cardiac origin. *In*: Advances in Physiological Sciences, ed. Manchanda SK, Selvamurthy D, and Mohan Kumar V, MacMillan India Ltd., Delhi, pp 121–128, 1992
4. Wennmalm A, Chanh PH, and Junstad M: Hypoxia causes prostaglandin release from perfused rabbit hearts. Acta Physiol Scand 91: 133–134, 1974
5. Koley BN, Pal P, and Koley J: Cardiac ischemia and pain: role of sympathetic afferents. *In*: Current Trends in Pain Research and Therapy. Basic Mechanism and Clinical Applications, ed. Sharma KN and Nayar U, Indian Society for Pain Research & Therapy, New Delhi, Vol. 1, pp 85–93, 1985
6. Brown AM: Excitation of afferent cardiac sympathetic nerve fibers during myocardial ischemia. J Physiol (Lond) 190: 35–53, 1967
7. Uchida Y: Afferent aortic nerve fibres with their pathways in cardiac sympathetic nerves. Am J Physiol 228: 990–995, 1975
8. Koley J, Basak AK, Das M, Sinha S, and Koley BN: Rectal response of cardiac origin in the cat: involvement of nitric oxide and acetylcholine. Eur J Pharmacol 325: 181–187, 1997
9. Koley J, Basak AK, Das M, and Koley BN: Cardiac nociception induced rectal response: relation with haemodynamic changes. Ind J Physiol Pharmacol 42: 259–265, 1998
10. Rattan S, Sarker A, and Chakder S: Nitric oxide pathway in rectoanal inhibitory reflex of opossum internal anal sphincter. Gastro-enterology 103: 43–48, 1992
11. Koley BN and Mukherjee SR: Spinal preparations and spinal shocks. J Exp Med Sci 8: 2–3, 1964
12. Sherrington CS: Methods of decerebration. J Physiol (Lond) 22: 319–327, 1898
13. Koley J, Basak AK, Das M, Sinha S, and Koley BN: Role of cardiac nociceptors on rectal motility. Ind J Physiol Allied Sci 49: 24–33, 1995
14. Koley BN, Sinha S, and Koley J: Pulmonary J receptors, stress and cardiac affectivity. Ann Natl Acad Med Sci 31: 97–108, 1995
15. Koley J, Das M, Basak AK, Sinha S, and Koley BN: Vesicular reflexes of cardiac origin: role of cardiac nociceptors. Ind J Physiol Allied Sci 49: 107–115, 1995
16. Polwing W, McDonald FM, Brinkmann C, Hirche H, and Addicks K: Lignocaine desensitisation of epicardiac receptors. J Cardiovasc Pharmacol 9: 6–11, 1987
17. Coleridge HM and Coleridge JCG: Cardiovascular afferents involved in regulation of peripheral vessels. Annu Rev Physiol 42: 413–427, 1980
18. Pal P, Koley J, Bhattacharya S, Sengupta J, and Koley BN: Cardiac nociceptors and ischemia: role of sympathetic afferents in cat. Jpn J Physiol 39: 131–149, 1989
19. Garry RC and Gillespie JS: The responses of the musculature of the colon of the rabbit to stimulation *in vitro* of the parasympathetic and of the sympathetic outflows. J Physiol (Lond) 128: 557– 565, 1955
20. Hulten L: Extrinsic nervous control of colonic motility and blood flow. Acta Physiol Scand 335: 1–11, 1969
21. Furness JB: An electrophysiological study of the innervation of the smooth muscle of the colon. J Physiol (Lond) 205: 549–556, 1969
22. Jule Y and Gonella J: Modifications del'activite electrique du colon terminal delapin par stimulation des fibres nerveuses pelvicnes et sympatheques. J Physiol (Paris) 64: 599–607, 1976