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Basal internal anal sphincter tone, inhibitory neurotransmission, and other factors contributing to the maintenance of high pressures in the anal canal

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Abstract

Maintenance of the basal tone in the internal anal sphincter (IAS) is critical for rectoanal continence. Effective evacuation requires a fully functional rectoanal inhibitory reflex (RAIR)-mediated relaxation of the IAS via inhibitory neurotransmission (INT). Systematic studies examining the nature of the INT in different species have identified nitric oxide (NO) as the major inhibitory neurotransmitter. However, other mediators such as vasoactive intestinal polypeptide (VIP), ATP, and carbon monoxide (CO) may also play species-specific role under certain experimental conditions. Measurements of the intraluminal pressures in the IAS along with the force of the isolated IAS tissues are the mainstay in the basic studies for the molecular mechanisms underlying the basal tone and in the nature of the INT. The identification of NO as the inhibitory neurotransmitter has led to major advances in the diagnosis and treatment of a number of rectoanal motility disorders associated with the IAS dysfunction. Besides the IAS, the high pressures in the anal canal are affected by the external anal sphincter (EAS) function, and its malfunction may also lead to rectoanal incontinence. Different approaches including biofeedback have been attempted to improve the EAS function, with variable outcomes. There is a dire need for the innovative ways to improve the week high pressures zone in the anal canal. This viewpoint focuses on two studies that extend the above concept of multiplicity of inhibitory neurotransmitters (Neurogastroenterol Motil 2011 23 e11–25), and that high pressures in the anal canal can be improved by the EAS plication (Neurogastroenterol Motil 2011 23 70–5).

Keywords co-neurotransmission, EAS plication, Inhibitory neurotransmission, nitric oxide synthase, rectoanal continence.

BASAL IAS TONE AND RAIR-MEDIATED IAS RELAXATION

High pressures in the anal canal are the result of the sum total effect of the involuntary (autonomic) internal anal sphincter (IAS) smooth muscle, voluntary (somatic) external anal sphincter (EAS), levator ani, and puborectalis skeletal muscles.1,2 The myogenic IAS tone provides a major bulk of the contribution towards the high pressures in the anal canal, and is considered to be a primary factor in the rectoanal continence.1,3–6 Relaxation of the IAS mediated via the inhibitory neurotransmission (INT) during the rectoanal inhibitory reflex (RAIR) is equally important for the successful elimination of the digestive wastes.

NATURE OF INHIBITORY NEUROTRANSMISSION

The smooth muscle sphincters prototyped by the IAS were among the first organs to determine the nature of the inhibitory neurotransmitter/s in the gastrointestinal (GI) tract. The sphincteric smooth muscle offers distinct advantages in this regard. The true basal tone (without the exogenous administration of any contractile agonist) allows the free expression of inhibition (relaxation) of the smooth muscle in response to the RAIR. Additionally, the RAIR can be mimicked in the organ bath using isolated smooth muscle strips, via appropriate parameters of electrical field stimulation (EFS). The knowledge of INT has enormous implications in the pathophysiology and therapy of a number of gastrointestinal motility disorders.

Among different candidate inhibitory neurotransmitters examined so far, NO has fulfilled different criteria for the inhibitory neurotransmitter in the IAS. These criteria include direct relaxation of the smooth muscle that is inhibited selectively by the selective inhibitor/s, presence of its biosynthetic/biodegrading/ recycling machineries within the IAS myenteric inhibitory neurons, the actual release of the substance, and its interaction with the receptor site or enzymatic protein on the membrane or within the IAS smooth muscle cells (SMC) leading to the signal transduction cascade for the smooth muscle relaxation.1 Normal cascade of events that follow may be increase in cAMP or cGMP, followed by decrease in intracellular Ca2+, K+ efflux, and inhibition of the basic molecular machinery that supports the basal tone e.g. RhoA/ROCK and associated pathway.7,8 A systematic strategy for the identification of the inhibitory neurotransmitter carried out extensively in our laboratory and reviewed recently1 has translated into diagnosis and therapy of a number of debilitating rectoanal and other GI motility disorders.3,9,10

In addition, a direct association of the loss of the INT with the pathophysiology of the human disease characterized by the absence of IAS relaxation, e.g. the loss of neuronal nitric oxide synthase (nNOS)-containing or nitrergic nerves has been demonstrated in the

Hirschsprung's disease.11,12 There are several other such examples throughout the GI tract starting from the esophagus.10

Besides NO, other inhibitory neurotransmitter substances such as VIP, ATP and CO may also be involved in the IAS.

Using different animal species including humans in in vivo and in vitro experiments, it has been shown that NO is the major inhibitory neurotransmitter in the IAS.1,13–16 Such studies have used important functional parameters such as recording of the intraluminal pressures and the force. However, electrophysiological parameters (monitoring of the basal resting membrane potentials (RMP), and inhibitory junction potentials (IJP) have not been readily monitored. The IJPs may serve as precursors to the oncoming relaxation in the IAS. In the recent paper in the Journal, Opazo et al. in addition to force measurements, monitored electrophysiological parameters to further investigate the nature of INT in the rat IAS.17

OTHER FACTORS AFFECTING THE HIGH PRESSURE ZONE IN THE ANAL CANAL

While the IAS is the major determinant of high pressure in the anal canal, the surrounding skeletal muscles such as the EAS, levator ani, and puborectalis may also contribute towards these pressures.1,2 Although, the myogenic activity in the IAS is the primary regulator of the basal tone in the IAS, other neurohumoral factors along with the parasympathetic and sympathetic inputs may also modulate the IAS tone.1,18,19 The neurohumoral factors may either be circulating in the region or generated within the IAS SMC.1,20–22

In contrast to the major role of the basal IAS tone in the in the rectoanal continence, hypertensive IAS has been associated with the debilitating conditions such as Hirschsprung's disease, recurrent anal fissures, and hemorrhoids.1,9,23,24 A lack of understanding in the molecular mechanism is the major underlying reason for the absence of selective and side-effects free therapy in managing such rectoanal motility disorders. Recent basic studies from our laboratory have identified Rho Kinase and associated proteins (RhoA/ROCK) to be a major molecular mechanism for the basal tone in the IAS.25,26 Such data in humans may provide new avenues in the pathophysiology and management of such motility disorders.

Because of the important contribution in the rectoanal continence, the EAS malfunction may also lead to rectoanal incontinence. Such malfunction at times may be correctable using biofeedback.5 Recent studies presented in the Journal by Rajasekaran et al.27 explores new approaches to the improvement in the EAS function.

COMMENTARY

Regarding the INT, Opazo et al.17 using rat IAS show that in the basal state, it is primarily nitrergic in nature. This conclusion was derived from the data showing

complete blockade of the EFS-induced relaxation of the rat IAS by the NOS inhibitor.17 ATP (as shown by the lack of effect of P2Y1 receptors antagonism alone, using a new and selective antagonist MRS2500) had no significant effect on this relaxation. Similar data were obtained following the stimulation of the myenteric neurons by nicotine. However, data shown in the subsequent figures is somewhat contradictory. The authors show _50% attenuation of EFSinduced IAS relaxation following the NOS inhibition. In support of this limited attenuation, the authors quote other studies.15,16 A closer examination of those studies however, reveals that the NOS inhibition caused near obliteration of the human IAS relaxations as compared with the _50% inhibition in the adjoining non sphincteric region. It is noteworthy that the above rat IAS studies (in contrast with those in humans and other species) were performed in the presence of PGF2a to sustain the tone rather than in the pure basal state. Whether such exogenous contractile agonists may disturb the natural state of INT is not known.

The other important contribution made by paper is the demonstration of spontaneous nitrergic INT in the basal state of the IAS. This was shown by a significant increase in the basal IAS tone by NOS inhibition (not by ATP inhibition). This concept was also demonstrated by the increase in the RMP of the IAS SMC. Additionally, the authors examined the role of NO and ATP in the spontaneous IJPs in the IAS SMC. In that regard, data show that ATP mediates the initial-fast component of the IJPs while NO mediates the latter slow component. Similar data were obtained following 5 Hz for long train, except for some quantitative difference after L-NNA pretreatment. The authors showed further that combined antagonism of NO and ATP causes complete obliteration of the neurally mediated hyperpolarization of the IAS SMC. These data are basically similar to one murine IAS study (also in PGF2a-stimulated state) 28 but different from the other (in unstimulated state) where the authors examined the role of NO in the RAIR.29

The authors examined the role of VIP in the hyperpolarization and mechanical relaxation of the IAS by the EFS. Data show that VIP antagonism does not modify the IAS hyperpolarization and the relaxation in response to the neural stimulation. Data with the hyperpolarization is clear, but less so with the actual relaxation responses because the effect of VIP antagonist alone was not examined. Systematic studies on the field performed before have shown that VIP may partially mediate the neurally-induced IAS relaxation which in part is dependent on the release of NO from either the nerve terminals (resistant to tetrodotoxin) or the IAS SMC.30,31 Data suggest that NO is the major inhibitory neurotransmitter in the IAS, other inhibitory substances such as VIP, ATP and CO may also be involved in certain species under certain experimental conditions. This multiplicity of inhibitory neurotransmitters likely represents a back up mechanisms for such an important gastrointestinal reflex as the RAIR.1,31

In summary, above study17 shows a functional co-transmission for NO and ATP or ATP-like substance with complementary roles in the tonic and phasic effects of inhibitory motor pathways in the rat IAS. Resting tone and RMP are mainly controlled by NO whereas purines via the activation of P2Y1 receptors mediate the initial fast IJP following

the stimulation of inhibitory myenteric neurons in the rat IAS. Additionally, the study suggests a relationship between these two independent pathways: ATP acting via P2Y1 receptors may be the neurotransmitter responsible for phasic relaxations and NO for the sustained tonic IAS relaxation. From the data the authors suggest that the contribution of NO is predominant in IAS relaxation and that both pathways represent a 'reserve' physiological mechanism as abolition of one of these pathways (specially the purinergic one) does not prevent a strong IAS relaxation following stimulation of inhibitory myenteric neurons. In principle, the data are in agreement with the previous data that the mechanical relaxation of the IAS in different species including humans is mediated via the release of NO as the primary inhibitory neurotransmitter. Additionally, there appears to be a relationship between the hyperpolarization and the IAS relaxation. However, exact significance of the role of ATP in the IJP and hyperpolarization in view of the observed dissociation between the mechanical and electrical events, in the rectoanal pathophysiology remains to be determined. Concerning the role of the EAS in the high pressuresin the anal canal, the current issue of the Journal has another important paper by Rajasekaran et al.27 This study utilized a novel approach in an attempt to improve the EAS function. In accordance with the Frank Starling principle, a given muscle generates optimal or maximal force/pressure at the optimal muscle/sarcomere length.32,33 Interestingly, in the body, not all muscles are placed at the optimal length. For example, cardiac muscles operate at the suboptimal length, which allows it to increase the force of contraction (increase cardiac output) when left ventricle is distended (up to a certain limit).

This study revealed that similar to cardiac muscle, the EAS muscle also operates in the body at the suboptimal muscle or sarcomere length (approximately 20% shorter). The authors used EAS plication technique to alter its sarcomere length surgically.27 Sarcomere length increases in direct proportion to the length of EAS muscle plicated. A plication length that involved 20% length (circumference) of the EAS muscle resulted in optimal sarcomere length and maximal increase in anal canal pressure with EAS muscle stimulation. Clinical relevance of above finding is that one can manipulate EAS muscle length surgically to increase the anal canal pressure and thus anal continence related to a weak EAS muscle, regardless whether the weakness is related to partial muscle or nerve injury.

SUMMARY AND CONCLUSIONS

The two studies in the current issue of the Journal have added to our understanding of the regulation of anal canal pressure. The basal tone in the IAS and its relaxation during the RAIR are the two major functions of the IAS smooth muscle. The primary inhibitory neurotransmitter for the RAIR is NO, however, its co-neurotransmission with other inhibitory mediator such as VIP and ATP or related purine (via the activation of P2Y1 receptors in the IAS SMC) may also be involved under some conditions in a species-specific manner. In addition, besides the well known importance of the IAS in the rectoanal continence, the EAS also plays an important role. The function of the EAS may be improved experimentally by the manipulation of the sarcomere length via EAS plication. Such findings may have clinical implications towards improvement in the anal

canal pressures and thus the rectoanal continence associated with the weak EAS, regardless the origin of the weakness within the muscle or caused by the partial nerve damage.

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