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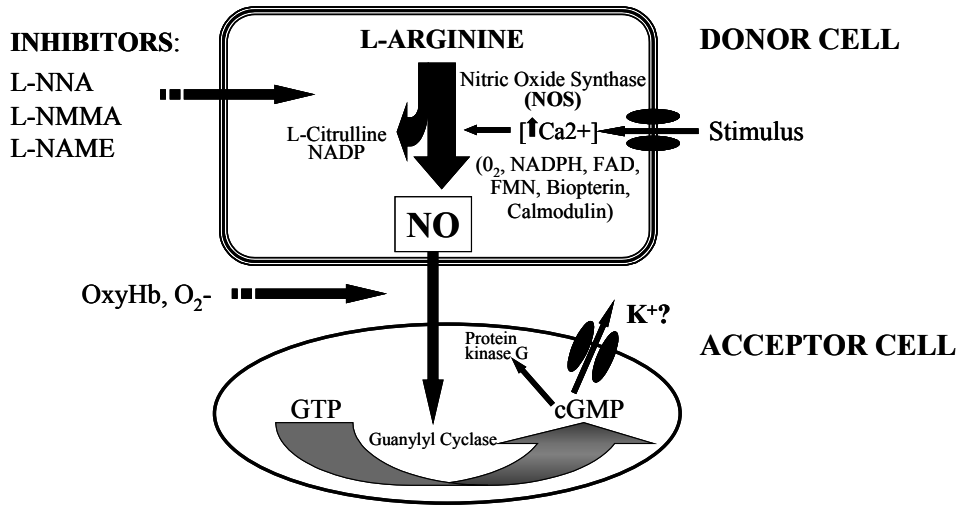

Adnan Tanović

February, 2003

Universitat Autònoma de Barcelona

Facultat de Veterinària

MECHANISMS INVOLVED IN MOTOR RESPONSES OF THE RAT SMALL INTESTINE IN HEALTHY CONDITIONS AND AFTER *TRICHINELLA SPIRALIS* INFECTION

Study presented by

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CERTIFICAN:

Que la memoria titulada “Mechanisms involved in motor responses of the rat small intestine in healthy conditions and after *TRICHINELLA SPIRALIS* infection” presentada por Adnan Tanovic para optar el grado de Doctor en Veterinaria, ha sido realizada bajo su dirección y considerándola finalizada autorizan su presentación para que sea juzgada por el correspondiente tribunal.

Y para que así conste, firman este certificado,

M. Jiménez E. Fernández

Bellaterra, Febrero de 2003.

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INDEX

ABSTRACT

The objective of this study is to analyze neural and muscular mechanisms involved in contraction and relaxation of the longitudinal muscle of the rat small intestine in healthy conditions and after *Trichinella spiralis* infection. Whole thickness preparations taken from jejunum and ileum of both healthy and *Trichinella spiralis*-infected rats were mounted in a muscle bath. In such conditions motor responses elicited by agonist addition or by electric field stimulation (EFS) induced release of endogenous neurotransmitters were studied.

1) **Healthy animals.** The mechanisms mediating the response to two main nitric oxide (NO) donors, sodium nitroprusside (SNP) and morpholinosydnonimine hydrochloride (SIN-1), have been characterized and compared with the responses to endogenous nitrergic transmitter in the longitudinal smooth muscle of the rat ileum. Moreover, we also tried to determine if prolonged incubation and an excess of NO might produce by itself changes in the contractile responses to substances that act essentially at muscular level. The calcium-handling properties of the ileal smooth muscle in these conditions were also evaluated. The response to the nitrergic transmitter released from the enteric nerves bears great similarities with that caused by SNP but not by SIN-1. Exogenously added NO does not modify the pathways involved in acetylcholine-, substance P- and KCl-induced contractions, including the calcium flows involved in these responses.

2) ***Trichinella spiralis*** **infected rats.** We studied the time course of functional and morphological changes in both inflamed worm-positive and non-inflamed worm-negative intestinal segments of T*richinella Spiralis* infected rats at different times post infection. When *T. spiralis* larvae are given orally, the parasite colonises the rat proximal small intestine - the duodenum and the jejunum but does not reach the ileum. Thus, in our study indeed both inflamed (jejunum) and non-inflamed (ileum) intestinal segments. Our results have provided evidence that changes provoked by *T. spirals* infection occur in both worm-free non-inflamed and worm-positive inflamed tissue, with the most prominent changes in contractile and relaxation responses in the uninfected ileal segments. Moreover, some of the morphological and functional changes persisted long after the complete recovery of the histopathological evidence of inflammation (until day 72 post-infection).

RESUMEN

El objetivo de este estudio es evaluar los mecanismos neurales y musculares implicados en la contracción y la relajación del músculo longitudinal del intestino delgado de rata en condiciones control y tras la infestación con *Trichinella spiralis*. Los segmentos del yeyuno y el íleon de ratas sanas e infestadas se montaron en un baño de órganos. Bajo estas condiciones se ha estudiado la contractilidad intestinal frente a la adición de agonistas y la respuesta motora a los neurotransmisores liberados por estimulación eléctrica de campo.

1) **Grupo control.** Los mecanismos implicados en la respuesta a dos principales donadores de óxido nítrico (NO), nitroprusiato sódico - SNP y morpholinosydnonimine hydrochloride - SIN-1, han sido caracterizados y comparados con las respuestas del neurotransmisor nitrérgico endógeno sobre el músculo longitudinal del íleon de rata. Además, intentamos determinar si la incubación prolongada y en exceso de NO puede, por sí misma, producir cambios en las respuestas contráctiles a sustancias que actúan esencialmente al nivel muscular. La implicación del calcio intra y extracelular bajo estas condiciones también ha sido evaluada. La respuesta que induce el neurotransmisor nitrérgico endógeno muestra grandes similitudes con la que provoca SNP pero difiere marcadamente de la respuesta provocada por SIN-1. El NO exógeno no modifica los mecanismos involucrados en las respuestas contráctiles inducidas por acetilcolina, sustancia P y KCl, incluyendo las funciones del calcio implicadas en estas respuestas.

2) **Ratas infectadas con *Trichinella spiralis*.** Estudiamos las alteraciones funcionales y morfológicas tanto en los segmentos inflamados del intestino delgado como en los no inflamados y libres de larvas de las ratas infestadas con *Trichinella spiralis* a diferentes tiempos posinfección. Cuando la infestación de la rata se lleve a cabo por administración oral de las larvas de *T. spiralis*, el parásito coloniza la parte proximal del intestino delgado -duodeno y yeyuno, pero no alcanza el íleon. De esta manera pudimos estudiar tanto los segmentos inflamados (yeyuno) como los no-inflamados y libres de larvas (íleon) del intestino delgado. Nuestros resultados evidencian que los cambios provocados por la infestación con *T. spiralis* ocurren en ambas partes del intestino. Los cambios más destacados se observaron en las respuestas motoras (contracciones y relajaciones) de los segmentos no infestados del íleon. Además, algunos de los cambios morfológicos y funcionales persistieron después de la completa desaparición de las evidencias histopatológicas de inflamación (hasta el día 72 de pos-infección).

ABSTRAKT

Cilj ove studije je bio da analizira neuralne i muskularne mehanizme koji učestvuju u kontrakcijama i relaksacijama longitudinalnog mišića tankog crijeva pacova u kontrolnom uvjetima i nakon infekcije sa larvama *Trichinelle spiralis*. Odgovori integralnih segmenata pacovskog jejunuma i ileuma od zdravih i infestiranih pacova su bili studirani u organskom kupatilu. Motorni odgovor mišića tankog crijeva induciran različitim agonistima i/ili električnom stimulacijom (EFS) pod ovim uslovima je bio tema ove studije.

1) **Kontrolna grupa.** U prvom djelu analizirali smo mehanizme koji učestvuju u odgovoru koji provociraju dva glavna donora nitričnog oksida (NO), Na-nitroprusid (SNP) i morpholinosydnonimin hydrochlorid (SIN-1), u kontaktu sa longitudinalnim mišićem pacovskog ileuma. Mahanizmi ovih odgovora su bili karakterizirani i potom komparirani sa endogenim odgovorima nitričnog neurotransmitora (provocirani sa EFS) koristeći isti preparat. Također smo pokušali da ustanovimo da li bi prolongirana inkubacija sa NO ili sam NO u suvišku mogli inducirati promjene u kontrakcijama provociranim sa supstancijama koje eksluzivno djeluju na nivou glatke muskulature. Pod tim okolnostima mobilnost kalcijuma u glatkomišićnoj ćeliji ileuma također je bila tema ove studije. Odgovori od endogenog nitričnog neurotransmitora, oslobođenog iz enteričnih nerava, pokazuju veliku sličnost sa odgovorima provociranim sa SNP ali ne i sa SIN-1. Egzogeno dodati NO nisu modificirali mehanizme implicirane u kontrakcijama provociranim sa acetilholinom, substancijom P ili KCl, uključujući tu i kalcijumovu mobilnost induciranu ovim agonistima.

2) **Pacovi zarazeni sa *Trichinellom spiralis*.** U drugom djelu izučavali smo funkcionalne i morfološke promjene u inflamatornim i upalom ne zahvaćenim segmentima tankog crijeva pacova tokom upalnog procesa i u post-upalnom periodu. Da bi smo inducirali upalu izabrali samo eksperimentalni model infestiranih pacovima sa T*richinellom spiralis*. Kada se pacovima larve *T. spiralis* daju *per oralno*, paraziti nastanjuju proksimalni dio tankog cijeva pacova - duodenum i jejunum, ali ne dosežu ileum. Na ovaj način bili smo u mogućnosti da studiramo upalom zahvaćene dijelove tankog crijeva, kao na primjer jejunum, kao i djelove bez upalnih procesa i bez prisustva parazita koristeći intestinalne segmente ileuma. Naši rezultati su dokazali da promjene inducirane sa *T. spirals* zahvataju i parazit-negativna podrucja bez indikacija upale kao i parazit-pozitivna podrucja sa izraženom upalom, kao i to da najizraženije promjene u kontraktilnim i relaksatornim odgovorima su uočene u neupaljenim segmentima ileuma. Neke od morfoloških i funkcionalnih promjena su bile prisutne dugo nakon nestanka histopatoloških znakova inflamacije i trajale su čak do 72. dana nakon infestacije pacova.

INTRODUCTION

STRUCTURE OF THE SMALL INTESTINE

The small intestine starts from the distal part of the pylorus until the ileo-cecal junction. Based on its anatomical structure it is divided into duodenum – the most cranial segment, jejunum,- and ileum - the most caudal segment of the small intestine. Regarding its histological structure, four different layers can be observed. The **mucosal** layer is in direct contact with intestinal lumen and plays an important role in nutrient absorption. Single-layer prismatic mucosal epithelial cells laying on the lamina propia build up the intestinal mucosa. In the intestinal mucosa we can observe a large number of Kerckring's valves (*plicae circularis*) with mucosal villi, as well as crypts of Lieberkühn, all of which contribute to increase mucosal area in contact with nutrients. In the **submucosal** layer a large number of blood and lymph vessels can be observed. The submucosal plexus, located between the *muscularis mucosae* and the circular muscle layer, has an important role in the control of secretory function. The **muscular** layer is divided into two sublayers; the ***circular*** and the **longitudinal** muscle layer, which is in contact with the intestinal **serosa**, which is part of the visceral peritoneum. The intestine contain also a large number of intrinsic neurons which form networks in close a position to two muscular layers.

THE ENTERIC NERVOUS SYSTEM

Recognition of the particular nature of the enteric nervous system (ENS) as a separate division of the autonomic nervous system (ANS), alongside the parasympathetic and sympathetic system, was generally stated by Langley in 1921 (Langley, 1921), who introduced the term "enteric nervous system". Throughout the last century ENS has been considered as the "third portion of the ANS" or as a displaced part of central nervous system (CNS), being also called "brain in the gut". Intrinsic neurons allow the intestine to exhibit sophisticated patterns of activity. In fact, although most neurons of the ENS do not receive a direct innervation from the brain or spinal cord, the ANS, gastrointestinal hormones and paracrine mediators can modulate them. The ENS controls motility (Costa *et* Brookes, 1994; Furness *et* Bornstein, 1955), exocrine and endocrine secretions (Cooke, 1994), microcirculation in the gastrointestinal tract (Surprenant, 1994) and is also involved in the regulation of immune and inflammatory processes in the gut (Lundgren *et al*., 1989).

Thus, the ENS contains sensory neurons, motor neurons and interneurons required to carry out its functions.

In the ENS, nerve cell bodies are grouped into small ganglia that are connected by nerve processes forming two major plexuses - the **myenteric (Auerbach's) plexus** and the **submucous (Meissner's) plexus**. The myenteric plexus lies between the longitudinal and the circular muscle layers and primarily provides motor innervation to both muscle layers and secretomotor innervation to the mucosa. The submucosal plexus, located in the submucosa between the circular muscle and the *muscularis mucosae*, plays a role as an important secretory controller. The sensitive fibres of both plexuses can transform different stimuli through inter- and motor- neurons into action potentials, provoking excitatory or inhibitory responses in the final effector systems. The majority of motor neurons projecting into the longitudinal muscle layer are excitatory, while most motor neurons projecting into the circular layer are inhibitory. This determines the so-called "inhibitory tone" that affects the circular layer. Thus, the circular muscle can contract only when inhibitory motor neurons are inactivated ("off" state), while when they are active ("on" state) the circular muscle is relaxed. In the circular muscle layer there is another aganglionic nerve plexus called "**deep muscular plexus**", formed only by fibres mainly supplying inhibitory inputs to the smooth muscle cells.

INTESTINAL MOTILITY

Intestinal motility can be defined as a series of co-ordinated contractions of the muscular layers that allow and enhance processes such as digestion and absorption of nutrients as well as progression of remaining material, most commonly, in aboral direction (Bayliss *et* Starling, 1899). Intestinal motility is controlled by the intrinsic properties of the smooth muscle cells, the ENS, electrical activity of Interstitial Cells of Cajal (ICC), and by the influence of hormones and extrinsic nerves as well. The gastrointestinal tract exhibits different patterns of motor activities that are involved in the intestinal motility including: [1] *peristalsis*, [2] *segmentation*, [3] *migrating motility complex*, [4] *retrograde motility during emesis*, [5] *control of sphincters* and [6] *physiologic ileus*. [1] The ***peristaltic reflex*** or ***peristalsis*** was the first gastrointestinal function attributed to the ENS. At the beginning of the last century Bayliss and Starling (Bayliss *et* Starling, 1899; Bayliss *et* Starling, 1900) demonstrated that distension of an intraluminal balloon in the extrinsically denervated

canine small or large intestine evoked a contraction of the circular muscle in oral direction, and a relaxation in aboral direction to the stimulus, inducing its progression down the intestine. Nowadays, we know that neuronal blockers abolish these responses and that peristalsis results from reflex activation of *polarized* intrinsic nervous. In 1969 Kottegoda found that contractions of the circular muscle appeared to alternate with those in the longitudinal muscle during peristalsis in the small intestine (Kottegoda, 1969). Kottegoda demonstrated that both muscle layers responded differentially to transmural nerve stimulation and to various drugs, concluding that both muscle layers are reciprocally innervated: when the longitudinal muscle contracts, then circular muscle relaxes and *vice versa*. The longitudinal and circular muscle layers of the intestine are innervated by different populations of motor neurons and both muscle layers can act independently of one another. Thus, the peristaltic reflex is a wavelike propagation that consists of neurally mediated contraction of the circular muscle and relaxation of the longitudinal muscle oral to the bolus; and longitudinal muscle contraction and circular muscle relaxation aboral to the bolus, which results in its progression (Figure 1). Recent studies using tension recording or intracellular microelectrodes have shown that muscular distension elicits polarized reflexes: excitatory junction potentials (EJPs) and contraction, and inhibitory junction potentials (IJPs) and relaxation, in the circular and longitudinal muscle of the small and large intestine, oral and anal to the stimulus, respectively (Hirst *et* McKirdy, 1974). Intestinal peristalsis can be activated by distension of the gut wall, which provokes activation of mechanoceptors in the muscular layers, or by direct stimulation of the intestinal mucosa. Successive activation of afferent neurons and interneurons during peristalsis differentially polarise excitatory and inhibitory motor neurons that make junctions with the smooth muscle cells in oral and anal projection to the bolus. Alternatively, Smith and Robertson (Smith *et* Robertson, 1998) have shown that peristalsis may also be generated by the synchronous activity of both muscle layers – [I] contraction of the longitudinal and circular muscle oral (ascending excitation) to a peristaltic wave and [II] synchronous aboral muscle relaxation (descending inhibition) to a peristaltic wave. The transient anal relaxation was followed by a [III] sustained contraction (descending excitation) of both muscle layers. Thus, they concluded that peristalsis consists of synchronous activation of [I] cholinergic ascending interneurons that activate cholinergic excitatory motor neurons and anally projecting descending interneurons that induce biphasic response by activating [II] inhibitory (nitrergic and purinergic) and [III] excitatory

(cholinergic) motor neurons in both muscle layers. These results have also suggested an asymmetry in neurotransmission between ascending and descending nervous pathways.

Patterns of intestinal motility in the fasted and fed states are markedly different. Ingestion provokes phasic and irregular [2] ***segmentation*** contractions of the small intestine. Segmentation mixes intestinal contents facilitating digestion and absorption. In fasted state a pattern of cyclic motility called [3] ***migrating motility complex*** (MMC) is observed. MMCs keep the intestinal motor activity and empty the small intestine by pushing its contents caudally to the colon, avoiding bacterial overgrowth (Hirst *et* McKirdy, 1974). MMCs recur in the stomach or upper small intestine at fairly regular intervals and migrate aborally along the bowel (Szurszewski, 1969). Each MMC is composed of three different phases (Grivel *et* Ruckebusch, 1972). [4] ***Retrograde motility*** in the upper gastrointestinal tract during emesis reflects an defense mechanism in response to ingested and/or emotionally perceived factors. [5] ***Control of sphincters*** is a specialised adaptation of basic peristaltic reflex in which excitatory motorneurons and myogenic tone are tonically activated. In contrast, the term [6] ***physiological ileus*** refers to a situation in which a tonic activation of inhibitory motorneurons occurs.

MECHANISMS INVOLVED IN SMOOTH MUSCLE CELL CONTRACTION AND RELAXATION

Electromechanical hyperpolarization and depolarisation as well as pharmacomechanical coupling are the mechanisms determining propulsive intestinal activity. The excitation-contraction coupling process is due to an increase in intracellular calcium levels. The main contributors for such intracellular Ca^{2+} increases are [1] entry of Ca^{2+} through voltage-dependent L-type channels, opened during the depolarisation in the action potential, and [2] Ca^{2+} release from intracellular stores - sarcoplasmatic reticulum and mitochondria (Bolton *et al*., 1999). The increase in cytosolic calcium initiates the excitation-contraction process, which results in the phosphorylation of contractile proteins. Intracellular Ca^{2+} release is mainly induced by the activation inositol-1, 4, 5-triphosphate (IP_3) and by the Ca^{2+}-induced-Ca^{2+}-release (CICR) mechanism (Putney *et al*., 1989). Increased intracellular calcium induces formation of the calcium-calmodulin complex which activates the kinase causing the calcium-calmodulin-controlled phosphorylation of myosin light chains and its binding to a single molecule of ATP. This provokes myosin-actin interaction

and consequently a contraction (Bolton *et al*., 1999).

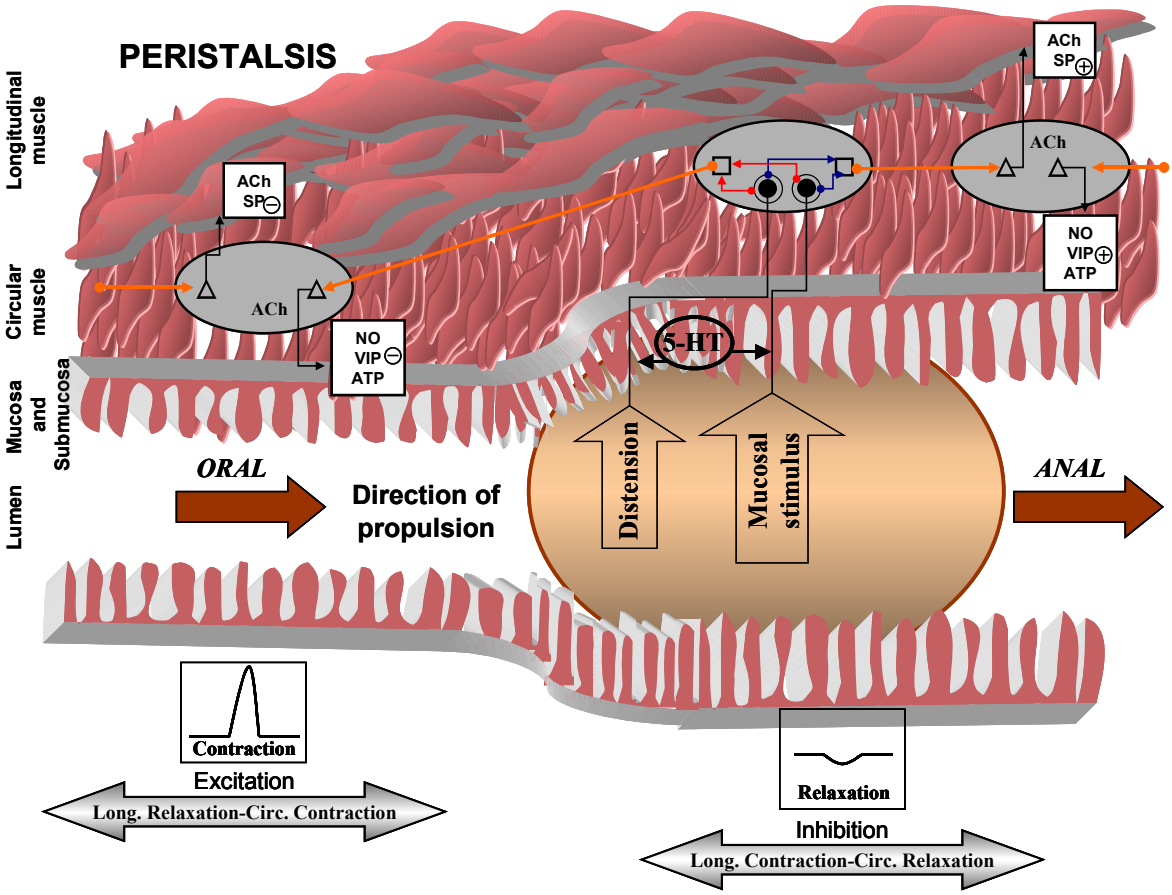

Figure 1. Peristalsis is the result of a series of local reflexes, each consisting of a contraction of intestinal muscle oral to an intraluminal stimulus and a relaxation of muscle aboral to the stimulus. The release of 5-HT by mucosal stimulation or mechanical distension of the gut lumen activates intrinsic afferent neurons. Activation of the circular muscle oral to the site of the stimulus (left), following activation of ascending cholinergic interneurons, could be due to neural inhibition of tonicaly activated inhibitory nerves. It could also be due to cholinergic excitatory inputs or a combinations of both factors. Simultaneously activation of inhibitory cholinergic interneurons in the longitudinal muscle induce its relaxation. Aboral to the stimulus site (right), descending cholinergic interneurons activate inhibitory motor neurons of the circular muscle that may contain nitric oxide (NO), vasoactive intestinal polypeptide (VIP) and ATP causing relaxation, while the activation of excitatory neurons in the longitudinal muscle result in its contraction. The resulting forces propel the bolus in anal direction. As the bolus moves, it triggers similar local peristaltic reflexes successively along the gut.

In contrast, activation of inhibitory neurotransmitter receptors induces increases of adenylyl cyclase or guanylyl cyclase in smooth muscle (Robinson *et al*., 1967; Waldman *et*

Murad, 1987). Activation of these enzymes catalyzes conversion of ATP into cyclic AMP (cAMP) or conversion of GTP into cyclic GMP (cGMP) resulting in smooth muscle cell hyperpolarization and relaxation. Thus, increased levels of cAMP or cGMP and consequent activation of the corresponding protein kinases may also be responsible for smooth muscle relaxation (Burks, 1994). Smooth muscle G proteins work as intermediary transducers between the extracellular neurotransmitter recognition site and the intracellular amplification mechanism associated with the neurotransmitter receptor. Ion channels such as small conductance calcium activated K^+ channels are also involved in muscular relaxation. Activation of these channels may also cause smooth muscle hyperpolarization and relaxation. For instance, opening of P_{2x} purinoceptors that are actually intrinsically K^+ channels able to bind ATP that may cause relaxation (Burks, 1994; Pluja *et al*., 1999).

NITRIC OXIDE

Nowadays, more then 20 substances are candidates or have been identified as neurotransmitters in enteric nerves (Gershon *et al*., 1994) and some of them co-localize in the same neuron. Thus, to the most commonly known neurotransmitters in the ENS acetylcholine, norepinephrine, histamine or serotonin we have to add many others as purines (ATP), peptides as vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) and more recently discovered gases as nitric oxide (NO) and carbon monoxide (CO).

For over 20 years it has been known that vascular endothelial cells release a highly labile factor involved in the relaxation of the underlying smooth muscle after stimulation with acetylcholine (Furchgott *et* Zawadzki, 1980; Furchgott, 1984; Palmer *et al*., 1987). The identity of this factor, originally known as Endothelium-Derived Relaxing Factor (EDRF), remained unknown until 1987, when it was demonstrated that nitric oxide (NO), produced by the enzyme nitric oxide synthase (NOS), had biological properties identical to those of EDRF (Hutchinson *et al*., 1987; Ignarro *et al.*, 1987; Khan *et* Furchgott, 1987). NO easily diffuses across the plasma membrane and triggers a number of effects in the cellular machinery. It is generally assumed that NO binds the heme group of soluble guanylate cyclase. Activation of this enzyme induces an elevation of intracellular cyclic GMP levels, which activates its final effectors (Furchgott *et* Zawadzki, 1980; Furchgott, 1984; Palmer *et*

al., 1987). The mechanism of action of cGMP to induce smooth muscle relaxation is not fully understood. Several events may account for its inhibitory effect on smooth muscle: cell membrane hyperpolarization; sequestration of cytosolic calcium; reduced sensitivity of the contractile apparatus or reduced activation of second messengers involved in excitatory pathways (Feelisch *et* Noack, 1987; Ignarro *et al*., 1987; Khan *et* Furchgott, 1987). NO is short-lived, because oxygen reacts rapidly with NO forming nitrite (NO^{2-}) and nitrate (NO^{3-}). However, the half-life of NO can be increased by superoxide dismutase, indicating the involvement of superoxide anions in its breakdown, which produces highly cytotoxic peroxynitrites (Katuski *et al*., 1977; Murad *et al*., 1978). It is well known that oxyhemoglobin, having an extremely high affinity for NO, avidly binds it and diminishes its availability (Khan *et* Furchgott, 1987; Martin *et al*., 1986; Thornbury *et al*., 1991) (Figure 2).

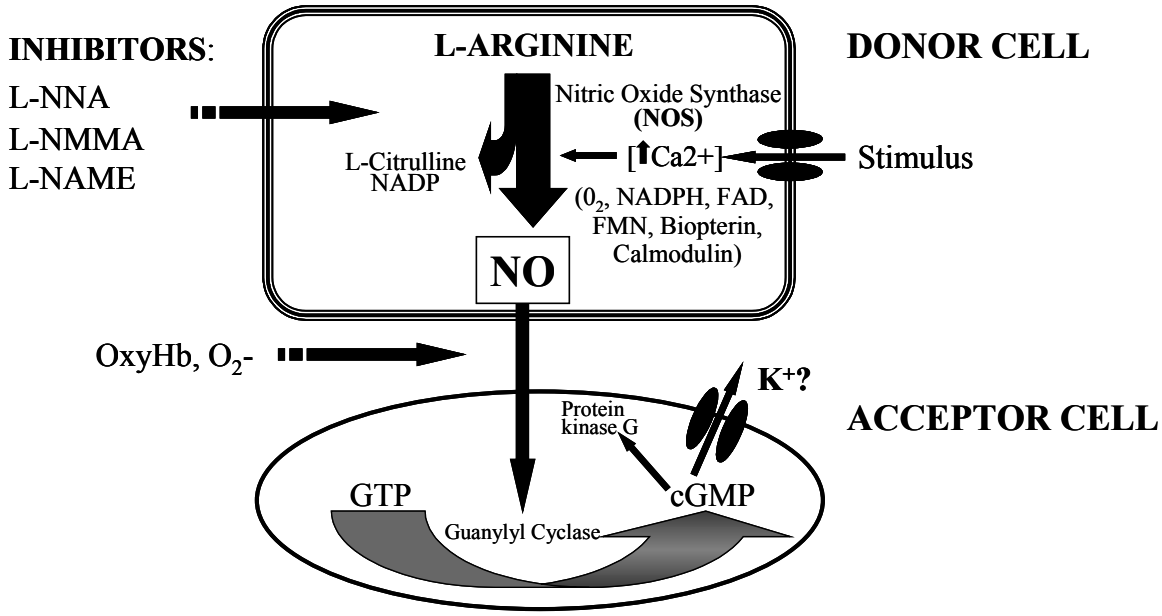

Figure 2. Pathway of nitric oxide (NO) biosynthesis from L-arginine by constitutive NO synthase (NOS). Released NO activates guanylate cyclase leading to elevation of intracellular cyclic GMP level. NOS activity can be inhibited by L-arginine analogues such as L-NNA, L-NMMA and L-NAME; superoxide anion (O^{2-}) accelerates NO breakdown; and oxyhemoglobin (OxyHb), known to bind to and scavenge NO, blocks NO-induced response.

The finding that the amino acid L-arginine is the substrate for NO synthase and the identification of the L-arginine analogues: N^G-monomethyl-L-arginine (L-NMMA) and Nω-nitro-L-arginine (L-NNA), as inhibitors of NO biosynthesis were crucial steps in understanding of this unique biochemical pathway (Katuski *et al*., 1977; Palmer *et al*.,

1988 [a]). Moreover, the specificity of effects of L-NMMA and L-NNA was demonstrated by the failure of its enantiomers, D-NMMA and D-NNA, to exert identical effects. In addition, concurrent administration of L-arginine (but not D-arginine) was able to reverse the action of L-arginine analogues used to block the enzyme (Palmer *et al*., 1988 [b]). Now it has become evident that NO extends its role far beyond the vasculature. NO is well recognised as inhibitory NANC neurotransmitter in the gastrointestinal tract. Electrical field stimulation (EFS) of isolated gastrointestinal preparations causes a neurogenic transient hyperpolarization, known as inhibitory junction potential (IJP), which results in relaxation of smooth muscle. IJPs are mediated by nonadrenergic, noncholinergic (NANC) neurotransmitters such as NO, ATP and VIP (Christinck *et al.,* 1991; Pluja *et al*., 1999). It has been evidenced that IJPs may also show two different phases, a fast and slow hyperpolarization (Pluja *et al*., 1999). This profile has been described in the rat colonic circular muscle where EFS-induced IJPs have an initial fast hyperpolarization induced by ATP, which is followed by a sustained hyperpolarization induced by NO (Pluja *et al*., 1999).

Two distinct enzymes - the constitutive and inducible form of NOS are responsible for NO synthesis (Moncada *et al*., 1991; Mourelle *et al*, 1996). The constitutive NOS (cNOS) mediates NO synthesis in physiological conditions. Two different isoforms of cNOS are described: endothelial (eNOS) and neuronal (nNOS), both of which release small amounts (picomoles) of NO and upon stimulation and during short periods. The cNOS is highly dependent on calcium, calmodulin, and NADPH for its activity (Moncada *et al*., 1991). NADPH-diaphorase activity was demonstrated to colocalize with NOS (Dawson *et al*., 1991; Hope *et al*., 1991). Therefore, NADPH-diaphorase histochemistry has provided a useful tool to explore the distribution and presence of NOS. NO synthesis has been shown to increase in response to inflammatory stimuli. Such stimuli cause expression of the inducible form of NO synthase (iNOS) (Boughton-Smith *et al*, 1993; Moncada *et al*., 1991). This highly effective calcium- and calmodulin-independent enzyme found in different inflammatory states appears to be responsible for the NO overproduction (nanomoles) and sustained release of NO. Thus, the NO produced by iNOS may be cytostatic or cytotoxic for the cells in its vicinity. In such situations, marked changes in smooth muscle contractility have been documented (Moncada *et al*., 1991). The fact that in the inflammatory state there are significant changes in functional parameters such as muscle

contractility, prompted several groups to hypothesize a causal link between NO overproduction and altered contractility, suggesting controversially both pro- and anti-inflammatory properties for NO (Hogaboam *et al*., 1995; Mourelle *et al*., 1996). Frequently such functional motor changes are unexpectedly persistent and could be involved in different motor alterations like those found in Irritable Bowel Syndrome (Kellow *et* Phillips, 1987).

INTESTINAL INFLAMMATION

It is well known that intestinal inflammation is associated with altered motility. In numerous intestinal disorders provoked by different toxins, bacteria or parasites, altered contractility has been demonstrated. However, it still remains unclear whether inflammation causes non-specific generalized damage to the neuromuscular apparatus or whether specific contractile mechanisms are affected (Depoortere *et al*., 1999; Grossi *et al*., 1993).

Irritable Bowel Syndrome (IBS) stands for a wide variety of situations in which prominent dismotility occurs. It has been difficult to find a common cause for such conditions, but in many cases it seems to be a remote sequel of an episode of intestinal inflammation. Dismotility in IBS can persist long after recovery of the normal structure of the mucosa and, in some instances, not only the inflamed area but also remote regions of the gastrointestinal tract can also be affected (Azpiroz 1999; Barbara *et al*., 1997; Goldhill *et al*., 1995; Hogaboam *et al*., 1995; Kellow *et* Phillips, 1987; Marzio *et al*., 1990).

Inflammatory Bowel Disease (IBD) is an idiopathic intestinal disease that refers to both, ulcerative colitis (UC) and Crohn's' disease (CD) (Collins, 1996). UC most often occurs in young individuals from 15 to 40 years of age and only through the inner lining of the mucosa and submucosa of the colon or rectum. CD is an inflammation and ulceration process that affects deeper layers of the lower part of the small intestine and first part of the colon (Vermillion *et al*., 1993). Both, UC and CD encompass the group of motility disorders and symptoms with unclear aetiology, which may have periods of remission and relapse. Additionally, in IBD serious complications in form of toxic megacolon, persistent haemorrhages, retrovaginal fistulas and different extra-intestinal symptoms may also occur.

There are several theories about IBS and IBD aetiology. Exacerbations are believed to occur after emotional stress and appears to be determined by behavioural, dietary or even genetic factors that could result in immune disorders (Collins *et al*., 1996). Different animal models have made possible to study functional alterations during intestinal inflammation (Grossi *et al*., 1993; Miampamba *et* Sharkey, 1999). In both, IBS and IBD increased response to physiological stimuli has been reported (Vermillion *et* Collins, 1988; Vermillion *et al*., 1993). The intestinal motor system seems to act as an extension of the immune system in host defense; the immune system recognizes foreign and potentially harmful agents in the gut lumen and recruits the motor system to assist in the eviction of these agents from the gut. Thus, this motor hyperactivity might be responsible for clinical symptoms observed in several processes of intestinal inflammation.

Many studies have focused in the study of intestinal hypermotility during inflammation. For instance, in the dog it has been documented the presence of two different patterns of "pathological" motility during inflammation: giant migrating contraction (GMC) and retrograde giant contraction (RGC), which can be associated with diarrhoea, abdominal pain and vomiting (Cowles *et* Sarna, 1990; Cowles *et* Sarna, 1991). More recently GMCs have been also observed in human IBS (Sartor, 1995).

TRICHINELLA SPIRALIS

LIFE CYCLE OF TRICHINELLA SPIRALIS

The intestinal phase of *T. spiralis* starts from ingestion of meat with encapsulated larvae and in the rat it lasts 20-23 days. The nematode *Trichinella spiralis* preferentially inhabits the proximal small intestine of rats (Dick *et* Silver, 1980). In this phase, the presence of the parasites in the mucosal and submucosal layers causes an inflammatory response and functional changes in the motility of small intestine (Blennerhassett *et al*., 1992; Castro *et al*., 1976; Palmer *et al*., 1984). In the intestinal mucosa larvae reach the adults form, copulate and yield a new generation of larvae that migrate to striated muscles. The muscular phase starts with entry of the larvae into the striated muscle fibers and larval encapsulation. This phase end with the process of larval calcification which allow long term survival of the parasite.

MOTOR CHANGES IN TRICHINELLA SPIRALIS INFECTED ANIMALS

In *T. spiralis*-infected rats, survival of the animals is warranted for long periods and the inflammation induced by the parasite is moderate in comparison to that induced by chemical compounds (Castro *et al*., 1976; Castro *et al*., 1979; Collins, 1996; Palmer *et al*., 1984; Sukhdeo *et* Croll, 1981). Though microscopic observations of *T. spiralis* infected rats confirm that the intestinal inflammatory process is overcome within 3 weeks, some neural and muscular alterations can persist for much longer periods. Indeed, mucosal inflammation is well correlated with the presence of parasites and remission of histopathological inflammatory evidence occurs soon after parasite eviction. In contrast, muscle hypertrophy and hyperplasia seems to last longer (Blennerhassett *et al*., 1992; Blennerhassett *et al*., 1999). Structural and molecular changes in intestinal smooth muscle and increase of contractile protein content have also been documented, though presence of edema, production of extracellular matrix or increases in collagen do not contribute to the apparent increase in tissue mass (Blennerhassett *et al*., 1992; Weisbrodt *et al*., 1994). For instance, agonist-related specific changes in acetylcholine (ACh)-, substance P- or KCl - induced contraction in rabbit colon has been reported (Depoortere *et al*., 1999). Previous studies have also described changes in the release of endogenous neurotransmitters in *T. spiralis* infected rats. Thus, ^{3}H-ACh and ^{3}H-norepinephrine release were significantly decreased in rat jejunal longitudinal muscle-myenteric plexus preparations (Collins *et al*., 1989; Swain *et al*., 1991), whereas another study demonstrated an increased level of SP in the rat myenteric plexus (Swain *et al*., 1992). Moreover, longitudinal jejunal muscle from mice and rats infected with *T. Spiralis* displayed a marked and long lasting increase of responsiveness to carbachol (Barbara *et al*., 1997; Sukhdeo *et* Croll, 1981) and impaired function of nitrergic inhibitory nerves (Miampamba *et* Sharkey, 1999). So, functional changes are persistent long after remission of morphological evidences of inflammation. In some cases remote areas, which are not close to the inflammatory focus, are also affected (Marzio *et al*., 1990). The rapid onset and slow recovery of structural and functional changes in *T. spiralis* infected rats is also a feature found in human gut inflammation, as persistent pathological and functional motor changes of small intestine have also been associated to Crohn's disease (Vermillion *et al*., 1993).

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