4405

PINAVERIUM BROMIDE PREVENTS SPINAL FOS EXPRESSION IN ACETIC ACID-INDUCED VISCERAL PAIN IN RATS.

Valerie Sinniger, Christophe Lamy, Jacques Fournet, Marie-Odile Christen, Bruno L. Bonaz, Dept of Gastroenterology, Grenoble, France; Solvay Pharma Inc, Suresnes, France.

Pinaverium bromide (PB) is an antagonist of L-type voltage-dependent calcium channels which are known to play a role in the spinal processing of nociception (1). Patients with irritable bowel syndrome (IBS) exhibit hypermotility and chronic visceral hyperalgesia evidenced by hypersensitivity to balloon distension (2). PB is commonly used to treat IBS patients (3). Intraperitoneal injection of acetic acid (AA) in rats is used as a model of visceral pain. Spinal neurons activated by nociceptive stimuli can be localized by dectecting the protein product of the proto-oncogene c-fos (4). Aim: to study the preventive effect of PB on AA-induced spinal Fos expression in rats. Methods: AA was injected (0.6%, 10ml/kg ip) in conscious male fasted rats pretreated either with PB (100mg in 0.9%NaCl/kg/day intragastrically for 3 consecutive days; n=5) or with vehicle (n=7). Sixty minutes after AA ip, rats were transcardially perfused with 4% paraformaldehyde. Frozen sections of the spinal cord were cut in the thoracolumbar segment, where neuronal activation is maximum (4), and processed for Fos immunohistochemistry. Results: in *vehicle pretreated rats,* AA induced Fos-IR (mean number of Fos positive cells on hemisection) mainly in superficial layers (lamina I; 10.2±0.3), in deeper layers (laminae V, 3.5±0.5 and VII, 5.3±1.3) of the dorsal horn and in area X surrounding the central canal (2.9±0.5). These neurons were predominantly concentrated on the site ipsilateral to the injection side. In *PB pretreated rats*, a significant decrease (30 % decrease; P=0.001) of Fos count was observed only in the superficial dorsal horn (lamina I; 7.1±0.5) while no significant modification was observed in laminae V,VII and area X. Conclusion: ip AA increases Fos expression in the thoracolumbar segment, mainly in laminae I,V,VII of the dorsal horn and in area X, which are all known to receive visceral afferent fibers. PB, at a dose of 100mg/kg/day for 3 days significantly decreases Fos expression only in lamina I. PB seems to selectively impair the visceral nociceptive stimulus processing in lamina I which contains neuromediators, like substance P, known to be important for nociceptive information. These anatomical data confirm that PB, a gastrointestinal selective calcium channel antagonist, acts on visceral hypersensitivity, in addition to its well-established effect on motility of the gut. *1) J Neurophysiol 76: 3740-48,1996. 2) Gut 45: 17-24,1999. 3) Today's Ther Trends 13: 47-62,1995. 4) Neurogastroenterol Motil april,2000,in press.*

4406

MEASUREMENT OF ACETYLCHOLINE RELEASE DURING SUBSTANCE P-MEDIATED CONTRACTIONS IN THE DOG COLON USING IN VIVO MICRODIALYSIS.

Mikio Tsukamoto, Shunsuke Kawakami, Noriaki Makimoto, Akihito Enjoji, Junichiro Furui, Takashi Kanematsu, Kotaro Taniyama, Nagasaki Univ Sch of Medicine, Nagasaki, Japan.

The involvement of the substance P in stimulating the phasic contractions and giant migrating contractions in the dog colon has been reported. We hypothesized that the substance P-mediated phasic contractions in the dog colon were regulated by the acetylcholine release from the enteric neurons and that substance P-mediated giant migrating contractions were not regulated by the acetylcholine release from the enteric neurons but by the direct action of substance P on the smooth muscle cells. This study was designed to prove these objectives using in vivo microdialysis in dog colon. Methods: Three healthy dogs were used. Under general anesthesia, the intraarterial silastic catheter to infuse distal colon were implanted. In the infused segment, microdialysis probe was implanted in the muscle layer to measure the acetylcholine release from the enteric neurons. The strain gauge force transducer was implanted to record the circular muscle contractions of the infused segment. The dialysate was collected and measured the acetylcholine concentration using high-performance liquid chromatography. To stimulate phasic contractions, 0.1nmol/ml of substance P was infused intraarterially. The 2.0 nmol/ml of substance P was used to stimulate the giant migrating contraction. Results: Spontaneous acetylcholine release from the enteric neuron in the colon fluctuated cyclically from 0.07±0.01 pmol to 0.64±0.02 pmol, synchronizedwith the spontaneous phasic contractions. Close intraarterial infusion of 0.1nmol/ml of substance P stimulated the phasic contractions and the actylcholine release was significantly increased to 559.4±27.7% compare to basic acetylcholine release($p<0.05$, n=3). The dose of 2.0 nmol/ml substance P stimulated giant migrating contraction in the colon and acetylcholine release did not exhibit any significant increases. Conclusions: 1) Spontaneous phasic contractions are regulated by the acetylcholine, which is released from the enteric neurons. 2) The low dose of substance P increases the acetylcholine release from the enteric neurons and stimulates the phasic contractions. 3) The giant migrating contractions of the colon are not regulated by the acetylcholine release from the enteric neurons. 4)Microdialysis is the useful method to measure the in vivo acetylcholine release in the colon.

4407

THE INFLUENCE OF BISACODYL ON HUMAN COLON MOTILITY IN VITRO.

Winfried A. Voderholzer, Maria-Antonieta Morena, Norbert E. Schindlbeck, Charite, Humboldt-University, Berlin, Germany; Clin Innenstadt, Univ of Munich, Munich, Germany.

Background: Bisacodyl has a direct stimulatory effect on colon motility. The mechanism of action is unknown. Therefore, the effect of the active metabolite of bisacodyl, bishydroxyphenyl-pyridyl-methan (=BHPM) on human colon motility was investigated. *Methods:* Isolated smooth muscle strips of human colon were mounted in a standardized organ bath system (37°C, basal resting tension 20 mN). First, the effect of BHPM was characterised by using tetrodotoxin (= TTX, 10^{-6} M), atropine (10^{-6} M), nifedipine (3 x 10^{-5} M) and L-NNA (3 x 10^{-4} M). After that, the effect of stimulation with carbachol (10^{-8} - 10^{-5} M) and substance P (10^{-8} - 10^{-5} M)was tested. To investigate the influence of the mucosa, all experiments were performed by using strips with and without mucosa. *Results:* BHPM dose-dependently induced tonic contractions that were not inhibited by atropine,TTX or L-NNA. If the mucosa was left in place, segments tended to have higher contraction amplitudes than muscle strips without mucosa. Nifedipine blocked contractions by 47±54% (n=10, p=0.05) in longitudinal, and 100±35% (n=9, $p<0.01$) in circular muscle strips. In the presence of BHPM dose response curves of carbachol and substance P were shifted to the right. Moreover, BHPM (150 μg/ml) decreased maximum contraction amplitudes of carbachol by 56±19% of longitudinal and by 96±4% of circular strips. Similarly, maximum contraction amplitudes of substance P were inhibited by 55±5% (longitudinal, $p>0.05$) and by 81±3% (circular, $p>0.05$). *Summary and conclusions*: Bisacodyl has a stimulatory and an inhibitory effect on human colon in vitro. The stimulatory effect can be inhibited by calcium channel blockers and may need intact mucosa. The inhibitory effect happens in the presence of high concentrations of BHPM. In our system the effect of BHPM seems not to be mediated by nitric oxide. Further investigations are needed to investigate the therapeutic relevance of these results. Supported by Friedrich Baur Stiftung 3/96.

4408

EFFECTS OF SHORT CHAIN FATTY ACIDS ON CANINE COLONIC SMOOTH MUSCLE.

Cate M. McManus, Kathy Michel, Daniel M. Simon, Robert J. Washabau, Univ of Pennsylvania, Philadelphia, PA.

Introduction: Short chain fatty acids (SCFA) are the end products of anaerobic microbial fermentation in the mammalian gastrointestinal tract. These metabolites contribute to the maintenance of the intestinal mucosa, intestinal fluid and electrolyte balance, carbohydrate and lipid metabolism, and the energy needs of the animal. SCFAs have been shown to stimulate canine ileal motility, but these effects have not been studied in the colon, the major site of fiber fermentation in this species. Objective: To determine the effects of SCFAs (acetate, butyrate, propionate) on longitudinal colonic smooth muscle contraction in vitro. Methods: Proximal and distal longitudinal colonic smooth muscle strips were suspended in physiologic buffer solution, attached to isometric force transducers, and set to optimal muscle length (Lo) with acetylcholine (ACh; 10^{-4} M) or potassium chloride (K^+, 80 mM). Muscles were then incubated with short chain fatty acids, i.e., sodium acetate, butyrate, or propionate (1-100 mM). The mechanism of the SCFA response was investigated by incubating with nifedipine (1 μM), tetrodotoxin (1 μM), or atropine (1 μM) prior to the addition of acetate, butyrate, or propionate. In a separate set of experiments, muscle strips were treated with ACh (10^{-9} to 10^{-4} M) after incubation with 10 mM propionate or butyrate. Results: Individual short chain fatty acids elicited isometric stress responses (0.25-2.15 x 10^4 N/m^2) in longitudinal smooth muscle from proximal and distal colon. Maximal responses were attained at 50 mM butyrate and propionate concentrations. Maximal butyrate and propionate responses were 35 and 25% of the maximal ACh response (10^{-4} M) in proximal or distal smooth muscle. Sodium acetate was least effective in stimulating contractile responses. Tetrodotoxin (1 μM) and atropine (1 μM) were without effect on SCFA contractile responses, but nifedipine (1 μM) virtually abolished the acetate, butyrate, and propionate responses. Butyrate (10 mM) and propionate (10 mM) also increased the sensitivity of ACh contractions in cumulative dose-response experiments. Conclusions: SCFAs enhance basal and ACh-stimulated proximal and distal longitudinal smooth muscle contractions. The mechanism of the SCFA response appears to involve influx of extracellular calcium.