● **EFFECT OF ANTAGONIZING 5-HT$_1$ AND 5-HT$_2$ RECEPTORS ON PERISTALSIS IN THE GUINEA-PIG ILEUM** *T. Feng, R.W. McCallum & T.K. Smith.* **Division of Gastroenterology, Departments of Internal Medicine and Physiology, University of Virginia, Charlottesville, VA 22908.**

**In the small intestine, 5-HT$_1$ and 5-HT$_2$ receptors are generally considered to be located on enteric neurons and smooth muscle cells respectively. Activation of 5-HT$_{1A}$ receptors causes membrane hyperpolarization and presynaptic inhibition of neurotransmitter release; effects that are inhibited by the 5-HT$_1$/5-HT$_2$ antagonist spiperone (spip) but not by the specific 5-HT$_2$ antagonist ketanserin (ket). Activation of 5-HT$_2$ receptors causes contraction; effects antagonized by spip and ket. A modified Trendelenburg technique was used to investigate the role of 5-HT$_1$ and 5-HT$_2$ receptors in peristalsis using the above antagonists.**

**Guinea-pigs were euthanized with pentobarbital and a 5cm segment of ileum removed and mounted in a peristaltic chamber containing Krebs' at 36°C. In addition to fluid ejection pressure (EP) and ejected fluid volume (EFV), we measured the tension of the circular muscle within 5 mm of the oral and anal ends of the segment. The lumen was perfused with Krebs' via a peristaltic pump at 0.08 ml/min to initiate regular peristaltic waves that were associated with a transient oral contraction (OC) and a transient anal dilation (AD) followed by an anal contraction (AC).**

**Both spip (0.001 to 5 μM) and ket (0.001 to 5 μM) dose dependently reduced the amplitude of EP and AC but not OC and also reduced the EFV and ket but not spip increased the frequency of peristaltic waves. Ket (1 μM) was significantly more effective than spiperone (1 μM) in altering these parameters expressed as % of control:**

| | Ketanserin (1 μM) | Spiperone (1 μM) |
|---|---|---|
| % P | 51.0 ± 4.7 ($p<0.001$)** | 73.2 ± 12.8 ($p<0.05$)* |
| % OC | 78.7 ± 15.1 ($p>0.05$) | 77.3 ± 12.8 ($p>0.05$) |
| % AC | 42.2 ± 2.8 ($p<0.001$)** | 61.1 ± 4.1 ($p<0.001$)** |
| % EFV | 63.6 ± 11.9 ($p<0.05$)* | 78.1 ± 5.5 ($p<0.05$)* |
| % Freq | 127.2 ± 9.3 ($p<0.05$)* | 108.2 ± 4.8 ($p>0.05$) |

***In conclusion, ketanserin and spiperone had similar inhibitory effects on peristalsis, although ket was more effective than spip. It is concluded that 5-HT$_2$ receptors are activated during peristalsis and are probably on enteric neurons in local excitatory or descending inhibitory pathways since neither of the antagonists significantly effected muscle tone; the role of 5-HT$_{1A}$ receptors is unknown.*** **Grants NIH DK45713 and Glaxo UK.**

● IMPORTANCE OF THE L-TYPE CALCIUM CHANNEL PATHWAY IN THE PROCESS OF INTERNAL CALCIUM STORES REFILLING IN INTESTINAL SMOOTH MUSCLE. O. Feron, C. Dessy, M.O. Christen* and T. Godfraind. Laboratoire de Pharmacologie, Université Catholoique de Louvain, UCL 5410, Avenue Hippocrate, 54, B-1200 Bruxelles, Belgium, *SOLVAY PHARMA LTM, rue Rouget-de-Lisle, 42, 92151 Suresnes, France.

**Objectives.** Calcium mobilization constitutes the determinant of actin-myosin crossbridge cycling and subsequent smooth muscle contraction. In this work, we studied the involvement of L-type voltage-dependent $Ca^{2+}$ channels (L-VDCCs) in the refilling process of emptied intracellular $Ca^{2+}$ pools in intestinal smooth muscle, and the selective blockade of these channels by pinaverium bromide (PB).
**Methods.** The isometric contraction has been measured simultaneously with the front-surface fluorometry of fura-2 loaded strips of longitudinal smooth muscle isolated from guinea-pig ileum. Radioligand experiments performed on CHO cells transfected with recombinant $Ca^{2+}$ channel α-1 subunits allowed us to analyse the isoform selectivity of PB.
**Results.** The transient calcium signal evoked by histamine in $Ca^{2+}$-free medium was used as an index of the extent of refilling of $Ca^{2+}$ stores previously emptied by repetitive agonist stimulations. Using this protocol, we showed that both histamine-evoked $Ca^{2+}$ signal and contraction were completely blocked in presence of nimodipine and stimulated by the $Ca^{2+}$ channel activator BayK8644. Our experiments also revealed that ryanodine and caffeine are both responsible for a $Ca^{2+}$ release through reticulum endoplamic calcium channels, which, in our experimental conditions, is also blocked in presence of nimodipine.
We showed that, in guinea-pig ileum smooth muscle, PB (1μM), a quaternary ammonium compound which competitively interact with the dihydropyridine binding sites, completely inhibited both the influx of extracellular $Ca^{2+}$ through L-VDCCs and the contraction. Since we demonstrated the existence of a specific splicing pattern in the L-VDCC α-1 subunit issued from intestine smooth muscle, we performed radioligand experiments on CHO cells transfected with different recombinant α-1 subunit isforms.We observed that PB, at low concentrations, exhibited a higher affinity for the isoform which is precisely more expressed in intestinal smooth muscle than in other tissues.
**Conclusions.** We showed that, whatever the $Ca^{2+}$ release process involved in intestinal smooth muscle, the refilling of internal $Ca^{2+}$ stores requires $Ca^{2+}$ influx through L-VDCCs contrary to vascular smooth muscle where this process is mainly resistant to $Ca^{2+}$ channel blockers. L-type $Ca^{2+}$ channel blockers constitute therefore a major class of drugs in the treatment of intestinal motility disorders and more particularly, we gave molecular evidences of the high selectivity of PB for the gastrointestinal tract.

● ACTION OF THE CALCIUM CHANNEL BLOCKER, PINAVERIUM BROMIDE, ON THE CHOLECYSTOKININ-MEDIATED COLONIC MOTOR RESPONSE TO A MEAL IN RATS. J. Fioramonti, M.O. Christen*, I. Dupré, L. Buéno. Department of Pharmacology, INRA, Toulouse and *Solvay Pharma/LTM, Suresnes, France.

Pinaverium bromide is a calcium-channel blocker used in the treatment of the irritable bowel syndrome in many countries. It has been recently shown that, in vitro, pinaverium bromide as well as other calcium antagonists, such as diltiazem and nicardipine, inhibit the contraction of intestinal isolated smooth muscle cells induced by CCK (Bobo et al., Life Sci., 1994; 54:1957-1954). The aim of this study was to determine whether pinaverium bromide inhibits the postprandial colonic motor response that is controlled by a mechanism involving CCK.

In two groups of 6 male Wistar rats chronically equipped with nichrome electrodes, myoelectric activity of the proximal colon was recorded, after a 15h fast, for 15 min before and 60 min after a 3 g meal (group 1) or i.v. administration of CCK at a dose of 2 μg/kg (group 2). The effects of pinaverium bromide administered orally at the doses of 2, 5, 10 and 50 mg/kg, 1h before the meal or CCK, were compared to those of the CCK-A and CCK-B antagonists, devazepide and L365260 (100 μg/kg, i.p.), and those of the calcium antagonist, diltiazem (10 mg/kg, per os). Controls consisted of vehicle administrations.

Before the meal, the frequency of colonic spike bursts was 9.4±1.5/10 min. For the 10 min after the 3 g meal and CCK administration (2 μg/kg) the spike burst frequency reached 21.5±1.8 and 15.7±2.3/10 min, respectively. The postprandial spike frequency was significantly ($P<0.05$) reduced, in a dose-related manner, by pinaverium bromide (from 26.4 to 75.2 % for doses ranging from 2 to 50 mg/kg). It was reduced by 34.7, 28.1 and 19.8 % after devazepide (100 μg/kg), L365260 (100 μg/kg) and diltiazem (10 mg/kg), respectively. The colonic response to CCK was reduced by pinaverium bromide at the dose of 2 mg/kg (81.4 %) with a maximal effect (-116.3 %) at the dose of 5 mg/kg. It was also significantly reduced (-74.4 %) by L365260, but not by devazepide and diltiazem.

Calcium channel blockers (pinaverium bromide and diltiazem) reduce the colonic motor response to eating in rats. Pinaverium bromide inhibits the CCKergic component of the response. In our experimental conditions, diltiazem does not exhibit significant effect on that response. The difference between in vivo and in vitro effects of diltiazem may be explained by its rapid absorption leading to a low concentration at the target organ, and emphasizes the interest of a gastrointestinal selective calcium antagonist such as pinaverium bromide.

ESOPHAGEAL ACIDIFICATION INCREASES LOWER ESOPHAGEAL SPHINCTER PRESSURE (LESP) IN CATS. M Firouzi, J Fields, G Urban, D Winship & A Keshavarzian. Depts Med & Pharm, Loyola Med Sch, Maywood IL; Med & Res Svces, VAH, Hines IL.

**Bkgd.** Changes in LESP are believed to be important in the development of gastroesophageal reflux disease (GERD). However, conclusions from studies on the effect of acid on LESP are at variance. Although it was originally shown by several groups studying man and/or cat that acidification of distal esophagus increases LESP, a recent study reported no change in LESP in cat following acidification. This latter study, however, included only 6 cats. Accordingly, the aim of our study was to determine the effect of acidification of the distal esophagus of cat on LESP. **Methods.** LESP was recorded using an 8-lumen esophageal catheter with a 3-cm Dent Sleeve, and a high-pressure, low compliance infusion system. Sixty cats underwent a total of 96 experiments. Light anesthesia was induced with IV ketamine. The stable resting LESP was recorded before and after wet swallows, again after infusion of acid, and then saline (3 ml at distal esophagus - 6 cm above LES). Comparisons were made using paired t-tests. **Results.** Acid-induced LESP (see Table) was higher ($p < 0.001$) than saline. Acidification also resulted in significantly higher ($p < 0.008$) contraction amplitudes (of distal esophagus) and more prolonged ($p = 0.017$) contractions. Velocity was not affected. **Conclusions.** Acidification of distal esophagus in cats results in: 1) increased LESP; 2) increased amplitude of contractions; 3) increased duration of contractions.

| | Wet Swallows | Saline | Acid |
|---|---|---|---|
| LESP-mmHg | 37 ± 4.9 | 37 ± 2.5 | 57 ± 2.9* |
| contraction amplitude, distal esophagus-mmHg | 78 ± 5.8 | 84 ± 4.9 | 92 ± 4.7* |
| contraction duration-sec | 2.9 ± 0.2 | 3.0 ± 0.1 | 3.2 ± 0.1* |