Effect of otilonium bromide on the plasma-membrane specializations of the gastrointestinal smooth muscle cells and interstitial cells of Cajal

Otilonium bromide (OB) is a quaternary ammonium derivative widely used for the treatment of hypermotility and hypersensitivity disorders of the intestine, in particular for treatment of irritable bowel syndrome (IBS; Poynard et al. 2001 for review). In humans, orally administered OB causes a dose-dependent increase of the whole gut transit time and eliminates the exaggerated colonic motor responses present in IBS patients (Narducci et al. 1985). A double-blind placebo-controlled study in a large number of IBS patients has established that OB is especially effective in relieving abdominal pain/discomfort (Battaglia et al. 1998). A recent trial has confirmed the OB efficacy in IBS patients and has shown for the first time the long-term beneficial effect of the drug during the follow up period in a group of patients previously treated with OB (Clavè et al., 2010).

Preclinical studies have shown that OB inhibits spontaneous or induced intestinal contractile activity and a mixed antimuscarinic and L-type calcium channels blocker action of OB has been proposed on the basis of experimental results (Evangelista et al. 1998). Recently OB has found to affect also T-type Ca\(^{2+}\) channels (Strege et al., 2010).

Binding studies and functional experiments in vitro have shown that OB can bind to the tachykinin NK\(_2\) receptor and inhibit its activation in guinea pig colon (Santicioli et al., 1999). Moreover, it has been recently shown that OB is able to inhibit the NK\(_2\) receptor mediated contraction of human colon (Gallego et al., 2010) as well as the internalization of this receptor in the smooth muscle cells (Cipriani et al., 2010). Therefore, these findings indicate that OB interferes on several mechanisms responsible for the gastrointestinal
contraction. Nothing is known, however, on the mechanisms or cell structures mediating the beneficial effects of the drug observed during the follow up period in the patients treated with OB. Likely, plasma membrane specialized structures present in both smooth muscle cells and interstitial cells of Cajal (the gut pacemaker cells) should be involved.

A recent interesting item is the involvement of caveolins in the smooth muscle contraction. The mammalian caveolin family proteins (caveolins-1, -2 and -3) are plasma membrane associated proteins discovered in the early 1990s, and are the major component of caveolae (Fernandez et al., 2002). Caveolae, originally described in the 1950s, are non-clatrin-coated plasma membrane microdomains rich in cholesterol and glycosphingolipids. They can invaginate to form 50-100 nm vesicles (Harder et al., 1997; van Deurs et al., 2003). They can exist as single caveolae or clusters. Caveolae are most abundant in terminally differentiated cells and high numbers of caveolae were found in endothelial and epithelial cells, adipocytes, pneumocytes, fibroblasts, cardiac myocytes, striated muscle fibres and smooth muscle cells (Feron et al., 1999; Shaul and Anderson, 1998). Interstitial cells of Cajal (ICC) characteristically possess many caveolae distributed all along their plasma membrane. Caveolin-1 is essential for caveolae formation in adipocytes, endothelial cells, pneumocytes, fibroblasts, and smooth muscles (Scherer et al., 1994; Tang et al., 1996). Since specific G protein-coupled and tyrosine kinase receptors as well as downstream signaling intermediaries (Ostrom and Insel, 2004; Shaul and Anderson, 1998) have been shown to be caveolae associated, these have been proposed to integrate important signaling pathways. Thus, the changes in caveolin concentrations may affect the formation of caveolae resulting in a disarray of the activation of caveolae-linked signal transduction pathways.

In the mouse intestine, caveolin-1 is present in smooth muscle cells and ICC and is required for the normal pacing activity (Daniel et al., 2004). Caveolin-1 knockout mice lack caveolae in endothelial, vascular and intestinal smooth muscle cells and show abnormalities in the ICC. Moreover, studies in the mouse intestine have shown that the disruption of caveolae and caveolin-1 in the smooth muscles and ICC with methyl-b-cyclodextrin reduced pacing.
frequencies and inhibited paced contractions (Daniel et al., 2004). In addition, using tissue from caveolin-1 knockout mice, Shakirova et al. (2006) observed that ileum longitudinal muscle had reduced contraction to endothelin-1 with no change in response to 5-HT or carbachol, whereas femoral arterial muscle contraction was increased in response to $\alpha_1$-adrenergic receptor stimulation. Collectively, these observations point to agonist- and tissue-specific role for caveolae and caveolin-1 in modulating receptor-mediated smooth muscle contraction.

Briefly, it could be interesting to assess the potential effect of OB on these mechanisms also on the basis of the afore-mentioned long-term beneficial effect of the drug (Clavè et al., 2010). Therefore, the aim of the present study is to investigate by immunohistochemistry and ultrastructural analyses the long-term effect of OB on smooth muscle cells and ICC plasma membrane specializations as well as on the mechanism of contraction involving caveolins. Moreover, the disruption of these structures could affect the proteic synthesis, improving or reducing the expression of some genes. Since the cells could use this mechanism to restore the normal condition it could be interesting to evaluate this aspect with different techniques such as PCR (Protein Chain Reaction) and Western Blotting.

References
- Clavè et al. (2010) Otilonium bromide improves frequency of abdominal pain, severity of distension and time to relapse in patients with IBS. Br Med J Submitted
• Strege PR. et al. (2010) T-type Ca2+ channel modulation by otilonium bromide. Am J Physiol GI 298: G706-G713.


**Time** necessary to the realization of the study: 2 years.