

Inner and outer portions of colonic circular muscle: ultrastructural and immunohistochemical changes in rat chronically treated with Otilonium Bromide

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Rat colonic circular muscle layer (cml), main target of otilonium bromide (OB) spasmolytic activity [1], is subdivided in an inner (icl) and an outer (ocl) portion. The icl is particularly rich in organelles such as caveolae, smooth endoplasmic reticulum, mitochondria [2]. The expression of specific markers in comparison with the ocl was investigated. The possible changes of these organelles and related markers, and of muscarinic receptors (Mr2) were studied after OB chronic exposition (2-20mg/kg for 10 or 30 days). Colon specimens were processed for electron microscopy, immunohistochemistry and western blot. In colonic strips the contractility response to Mr2 agonist was investigated. The results show that in controls the icl displays a higher expression of Caveolin-1 and Mr2, but not of eNOS, calreticulin and calsequestrin, compared to ocl. Chronic OB treatment caused similar ultrastructural and immunohistochemical changes in both portions. Organelles, Caveolin-1 and eNOS were increased at 10 days; Mr2 expression and muscle contractility induced by metacholine were increased at 30 days. Our findings 1) provide new information on the immunohistochemical properties of the icl suggesting a distinct role for this portion in colonic motility; 2) demonstrate that chronically administered OB interferes with cell structures and molecules responsible for calcium handling and storage, and modifies cholinergic transmission.

References

- [1] Clavè et al. (2011) Sarcoglycan OBIS Study Investigators. Randomised clinical trial: otilonium bromide improves frequency of abdominal pain, severity of distension and time to relapse in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 34:432-434.
- [2] Fausssone-Pellegrini et al. (1984) Ultrastructural peculiarities of the inner portion of the circular layer of the colon. *Research in the human. Acta Anat* 120:185-189.

Keywords

Smooth muscle cells, rat colon, contractility, transmission electron microscopy, immunohistochemistry, western blot.