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Research article

THE INFLUENCE OF EPITHELIAL SODIUM CHANNELS IN ISOLATED GOAT ILEUM SEGMENT; INTERACTION WITH STEROIDS

Dhaya R Varkey, Sumith K Mathew and Manoj G Tyagi

Department of Pharmacology, Christian Medical College, Vellore 632002, TN
Author for correspondence: E-mail: tyagi239@yahoo.co.in

ABSTRACT: The contractile effect of Acetylcholine (Ach) in the isolated longitudinal ileal muscle of adult goats was studied over a varying concentration range. Ach produced a concentration dependent-response curve indicative of an interaction with muscarinic receptors in the ileum, with a maximum contraction seen at 12 μM. On the other hand, pretreatment with the ENaC blocker, Amiloride (100 μM) substantially reduced the Ach induced contractions by 67.11 %. However, pretreatment with Prednisolone (2mM) restored this effect and the relaxation induced was only 14.26 %. This change was found to be statistically significant. This study emphasizes the importance of ENaC channels in the goat intestinal smooth muscle.

Key words: Epithelial sodium channel, Amiloride, Prednisolone, Ileum, Acetylcholine

INTRODUCTION

The epithelial sodium channel (ENaC) is localized in the apical membrane of the aldosterone-sensitive distal nephron, distal colon, respiratory epithelia, and ducts of salivary and sweat glands. In these epithelia, ENaC is the rate-limiting transport mechanism for sodium absorption. ENaC is a member of the ENaC/degenerin family of non-voltage-gated ion channels which also includes the acid-sensing ion channel ASIC1. The available crystal structure of chicken ASIC1 (Haertis *et al* 2012) and recent atomic force microscopy data of ENaC (Stewart *et al* 2011) suggest that ENaC is a heterotrimer composed of three homologous subunits α, β, and γ (Refer Figure 1). Each subunit of ENaC contains two transmembrane domains, a large extracellular domain, and short intracellular amino and carboxyl termini. In humans, an additional δ-subunit exists which can functionally replace the α-subunit in heterologous expression systems (Stockland *et al* 2008). A unique feature of ENaC regulation is its proteolytic processing thought to be critical for channel activation under patho-physiological conditions (Tyagi and Shukla, 2013). In this study we evaluated the effect of Prednisolone on ENaC channel activity in the isolated goat ileum model.

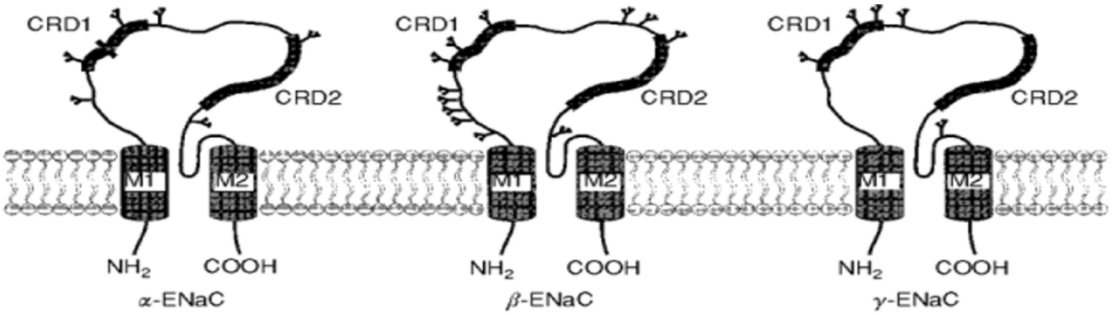

Figure 1: Subtypes of Epithelial sodium channels i.e α, β, γ (ENaC)

MATERIALS AND METHODS

The technique used was modified version of the technique used by Shamkuwar (2013). A Goat ileum was procured from local sources. A piece of goat ileum was removed, cleaned and was placed in a petri dish containing Tyrode solution. A thread was attached to the top to serve as a marker. The perfusion fluid in petri dish was aerated and debris inside the lumen was washed gently with pipette. The mesenteric membrane was trimmed for a length of ileum of approximately 2 cm. Two threads were tied to the upper and lower portion of the gut.

The thread tied to the lower portion was attached to the hook of the air-delivery tube inside the bottom of the chamber, in a water jacketed organ bath containing 20 ml Tyrode solution (composition in mM: NaCl 136.89, KCl 2.68, $MgCl_2$ 1.05. CaCl2 1.36, NaH_2PO_4 0.32, $NaHCO_3$ 11.90 and glucose 5.55) and the thread tied to the upper portion of gut was attached to the force displacement transducer. Tissues were mounted under an initial load of 1.0 g and allowed to equilibrate for 30 min. before the addition of any drug. The experiments were performed at 37 °C and bubbled with a mixture of air produced by a motorized areator. Normal rhythmic motility was recorded on a student's electric kymograph (Bio-Device, Ambala). The effect of Amiloride (100µM) with and with out pretreatment with Prednisolone (2mM) was tested on spontaneous contractions of goat ileum induced by acetylcholine (5µM). Each concentration tested was allowed a contact time of 1 min followed by washing three times with the Tyrode solution. A resting period of 15 minutes was allowed before the next addition. In a separate set of experiments, Prednisolone was added 25 minutes prior to Amiloride treatment.

RESULTS

The results obtained from the experiments are described in Table 1. These results were statistically evaluated using the students't'test. Ach produced a concentration-response curve indicative of an interaction with muscarinic receptors in the ileum, with a maximum contraction seen at 12 µM. On the other hand, pretreatment with the ENaC blocker, Amiloride (100 µM) substantially reduced the Ach induced contractions by 67.11 % ($P<0.01$). However, pretreatment with Prednisolone (2mM) restored this effect and the relaxation induced was only 14.26 % ($P<0.05$).

Table 1: Effect of Prednisolone and Amiloride on Ach induced contractions in isolated goat ileum. Statistical comparisons shown in the last column.

Pretreatment	Treatment	(Amplitude) Height in cm	Effect	% Change	'P' value
A)Nil	Acetylcholine (5µM)	3.71	Contraction		
B)Amiloride (100 µM)	Acetylcholine (5µM)	1.22	Relaxation	67.11	P<0.01 (A: B)
C)Prednisolone (2mM)	Acetylcholine (5 µM)	3.52	Relaxation	5.12	P>0.05 (A: C)
D)Prednisolone (2mM) + Amiloride	Acetylcholine (5µM)	3.18	Relaxation	14. 26	P<0.05 (A:D)

DISCUSSION

Inflammatory bowel disease (IBD) is a chronic inflammatory intestinal disorder encompassing two major entities: Crohns disease and ulcerative colitis and diarrhea is a common feature of these disorders (Cattaruzza *et al* 2011). Intestinal inflammatory processes reduce the absorption of sodium, chloride and calcium, while they increase potassium secretion. In addition, mild to severe metabolic alkalosis may occur in IBD patients, mainly depending on the severity of the disease and the part of the gastrointestinal tract being affected. Cathepsin-S has been shown to affect the ENaC in the apical membrane of the colon (Turnamian and Binder, 1989).

There is growing importance of ENaC channels in the IBD. In our study we found that Ach stimulated contractions were inhibited by Amiloride a blocker of ENaC channels. Our results also suggest that prednisolone pretreatment caused a reversal of these effects as shown in the Table 1. It is well established that the 'odd-numbered' muscarinic receptors (M_1, M_3, and M_5) typically couple *via* the α subunits of the $G_{q/11}$ family, whereas the 'even-numbered' members (M_2, M_4) couple *via* the α subunits of the G_i and G_0 and share the same proposed overall structure and a large degree of protein sequence homology (Buckley *et al* 1989). The possibility that Ach can cause contraction by inhibiting the cAMP levels has been previously reported (Tyagi *et al* 1996). On the other hand, the steroids i.e dexamethasone and aldosterone increase ENaC protein levels. These effects are similar in magnitude, additive,and presumably involve different hormone receptors. Two mechanisms have been suggested to account for induction of ENaC gene expression by adreno-corticosteroids (Masilamani *et al* 1999). The first is the increased absorption of sodium and enhanced expression of ENaC channels (Renard *et al* 1995).

Glucocorticoid receptor activation results in the stimulation of electroneutral NaCl absorption, and the glucocorticoids used to treat inflammatory bowel disease probably stimulate both electrogenic Na+ absorption and electroneutral NaCl absorption in the distal colon and rectum.
Electrogenic sodium absorption via ENaC is strongly impaired in the macroscopically but localization of ENaC is not changed. In contrast to impaired epithelial sodium transport, epithelial barrier function is not altered in non inflamed CD colon, indicating that paracellular leak flux of ions did not contribute to decreased sodium absorption. Glucocorticoids, are also supposed to play a central role in regulating ENaC expression in other smooth muscle like the airway or alveolus and in epithelia lining the male reproductive duct (Dilley and Hooper, 2004).
Thus in conclusion it can be stated that ENaC channels affect the contractile actions of Ach in the goat ileum and the steroids regulate this channel activity and this can have implications for various gastrointestinal patho-physiological conditions.

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