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Changes in the expression of GalR2 receptor in the colon of the pigs suffering from the swine dysenteria

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The expression of galanin (Gal) is raised in the porcine nervous system in many pathological processes (axotomy, neuronal degeneration, inflammation). The rise of Gal expression was found in the colon of pigs suffering from colitis caused by Brachyspira hyodysenteriae. The present study was aimed at the determination of changes in the expression of GalR2 galanin receptor in the muscular membrane, mucosa and lymphocytes isolated from mucosa in the B. hyodystenteria infection.

Twelve gilts (body weight. ca 45 kg) of Large White Polish race were divided into control (n=6) and experimental (n=6) group. Experimental animals were infected orally (via an intragastric catheter) with a B. hyodysenteriae culture obtained from a State Veterinary Institute in Pulawy, Poland. After developing a severe haemorrhagic diarrhoea the experimental animals were deeply anaesthetised with Thiopental (I.v.)and exsanguinated. The same procedure was used in control animals. After exsanguination the abdominal cavity was opened and the fragment of colonic wall from the centripetal turns was excised, washed in PBS and further processed. The fragment of the wall was separated into the muscular membrane and the mucosa and then the obtained fragments were preserved in the RNALater solution. From another part of the colonic wall sample the mucosa was scraped and used for lymphocytes isolation. Shortly, the mucosa was finely chopped, incubated in a dithiotreitol solution to get rid of the mucus and filtered through the Perlon wool. From the resulting cell suspension lymphocytes were isolated by the centrifugation in the Gradisol L (Polfa, Poland) gradient. The resulting lymphocyte pellet was also preserved in RNALater. Total RNA was isolated with a Total RNA mini kit (A&A Biotechnology, Poland) and the cDNA was prepared with a MMLV reverse transriptase (Fermentas, Lithuania). Real Time PCR analysis was carried out using SYBR Green Master Mix (Roche, USA) and primers designed for porcine GalR2 receptor sequence and porcine GAPDH was used as an internal standard. The analysis was carried out in the ABI 7500 Fast Real-Time thermal cycler (Applied Biosystems, USA). The results were normalised against the GAPDH expression and statistically analysed with a GraphPad Prism 3.0 statistical package.

The results showed clearly a dramatic decrease in the level of GalR2 receptor mRNA. In the muscular membrane it dropped 5-fold (from 0.005 to 0.001, relatively to GAPDH), in the mucosa it dropped more than 30-fold (from 0.0066 to 0.0002), while in the isolated lymphocytes it dropped more than 20-fold (from 0.0442 to 0.002). The differences were found to be statistically significant ($p<0.01$). The results point clearly to the very deep changes on GalR2 receptor expression in the colitis.