Distribution of the purinergic receptors P2X₄ and P2X₆ during rat gut development

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ABSTRACT
The purinergic receptors P2X₄ and P2X₆ are ion channels activated by ATP. These receptors are present in the gastrointestinal tract, and they are involved in synaptic transmission, taste sensation, and pain, among other functions. In this work, we studied the distribution of P2X₄ and P2X₆ receptors in proximal and distal regions of the gut newborn and adult rats. Using immunohistochemistry, purinergic receptors were found in gut epithelial cells and capillary vessels. In both proximal and distal regions of newborn rats, we observed P2X₄ signal in epithelial cells, whereas P2X₆ was present in capillary vessels in the proximal region and to a lesser extent in the distal region. In both regions of adult gut, we observed P2X₄ and P2X₆ immunostain in the capillary vessels. Semi-quantification indicated a significant difference in the amount of P2X₄ between proximal regions, whereas the P2X₆ content of both newborn regions differed from that in adult proximal gut. We conclude that P2X₄ and P2X₆ purinoreceptors are present in the gut from birth and that they are differentially distributed among regions.

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1. Introduction
Purinergic receptors were first defined in 1976 by Burnstock [4] and have been classified into two major families, P1 and P2, which show different affinities for adenosine and ATP/ADP, respectively [16,17]. The P2 receptors were further subdivided into two groups, one containing ATP activated ion channels (P2X) [7,8,24] and the second composed of G protein coupled receptors (P2Y) [20,23,25].

ATP was known initially only as an intracellular energy carrier [21], but in 1929 Drury and Szent-György observed its potent actions on the heart and blood vessels [3]. In 1970, ATP was proposed as a transmitter in the gastrointestinal tract, and later it was associated with synaptic transmission, and control of smooth muscle, pain, and inflammation [10,13,16]. ATP is now widely recognized as a fast synaptic transmitter as well as co-transmitter in the central and peripheral nervous cells (neurons and glia) [4,13,23]. On the other hand, the ATP released acts as an extracellular signaling molecule, activating G protein coupled P2Y receptors.

Seven P2X receptors, with permeability to Na+, K+, and Ca²⁺ [16,19], have been reported in distinct tissues of embryonic and adult mammals [6,15,18,22]. Purinergic receptors are composed by three subunits forming an ion pore that could form homomeric or heteromeric channels with different properties [1].

The enteric nervous system is composed of two nerve plexuses, the myenteric and submucosal plexuses formed by epithelial cells and capillary vessels. P2X₄ and P2X₆ purinergic receptor subtypes have been identified in the motor, sensory and interneurons of the myenteric and submucosal plexuses, and they are involved in synaptic neurotransmission [4,26].

The aim of our present study was to compare the expression of the P2X₄ and P2X₆ purinergic receptor ion channels in the newborn and adult rat gut, as well as in the proximal and distal regions.

2. Methods
Adult Sprague-Dawley rats were housed under a light cycle with 12 h darkness. The newborn rat pups were kept with their mothers for 8 h and then killed by decapitation; the guts were dissected and divided into proximal (1 cm after the stomach) and distal regions (8 cm after the stomach).

The adult rats were anesthetized with sodium pentobarbital (40 mg/kg, IP). As soon as they were unresponsive to nociceptive stimuli, they were killed by cervical dislocation. The small intestines were removed, divided into proximal (near from stomach) and distal regions, and then placed onto a plate with ice-cold 0.9% NaCl solution to be washed. The different regions were opened, and then fixed for 6 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7). Guts were sliced using a cryostat (Leica CM 1850) into 12 μm sections, collected on superfrost slides air dried at room temperature, and stored at −20 °C. For immunohistochemistry, the following polyclonal antisera were used: anti-P2X₄, and anti-P2X₆.
Fig. 1. Distribution of P2X_{4} receptors in the proximal and distal regions of newborn and adult rat guts. In newborn gut there was a strong immunosignal in epithelial cells (EC) of the proximal newborn gut (A) and also in the EC of newborn distal region (B). In adult gut, a weak immunosignal was observed in the proximal section (C) in capillary vessels (CV) and a moderate immunoreaction in the distal region (D). The bar 25 μm.

(Santa Cruz Technologies). Endogenous peroxidase was blocked by a 1-h pretreatment with 1% H_{2}O_{2}. Non-specific binding sites were blocked by a 1-h incubation with 3% nonfat milk. The primary antibodies were diluted 1:200 in phosphate buffer with 0.5% triton. The specimens were then incubated overnight at 4 °C. The next day, the complex was incubated with biotinylated goat anti rabbit antibody (1:750) (Santa Cruz Technologies). After washing and incubation with avidin conjugated horseradish peroxidase, the samples were washed again and color was developed with diaminobenzidine and H_{2}O_{2}. Sections were examined with an Axiosstar Zeiss microscope and scanned through an MRC Axioskam for digitization. Quantification of the intensity values was computed on digitized images of 497 × 407 pixels on gray scale with the KS300 software (Carl Zeiss). Control experiments omitting the primary antibody were

Fig. 2. Distribution of P2X_{6} receptors in the proximal and distal regions of newborn and adult rat gut. In newborn gut there was a strong immunosignal in capillary vessels (CV) of the proximal section (A), whereas a weak signal was observed in epithelial cells (EC) in the distal region (B). In adult gut there was a weak immunostain in capillary vessels of proximal region (C), and a moderate signal in the distal region (D). Bar: 25 μm.
performed to establish the specificity of the immunoreactions, and other controls were used to normalize density values at each age [12]. The data were subjected to a one-way ANOVA test, and a Tukey posttest to determine statistical differences in optical densities. \( P < 0.05 \) vs newborn proximal was considered to be statistically significant.

3. Results

The P2X\(_4\) and P2X\(_6\) purinergic receptors were present in gut epithelial cells and capillary vessels. The signal for P2X\(_4\) was strong in both newborn gut regions (Fig. 1A and B), whereas in adult proximal gut, a weak signal was observed in capillary vessels (Fig. 1C) and a moderate stain in the distal region (Fig. 1D).

The distribution of the P2X\(_6\) purinergic receptor in newborn gut was strong in the capillary vessels of the proximal region (Fig. 2A) and weak in epithelial cells of the distal region (Fig. 2B). On the other hand, adult gut capillary vessels were weakly stained in the proximal region (Fig. 2C) moderately stained in the distal region (Fig. 2D).

Semi-quantification of the immunoreactivity for P2X\(_4\) indicated a statistically significant difference between newborn and adult proximal regions (Fig. 3A). The strong immunosignal for P2X\(_6\) in newborn proximal gut was significantly greater than that in newborn distal and adult proximal regions (Fig. 3B).

In this study we made three important findings: (1) there is a strong immunosignal for P2X\(_4\) receptors in the epithelial cells of proximal and distal newborn gut. (2) There is an immunosignal for P2X\(_6\) in capillary vessels in proximal region. (3) There are significant differences in the amounts of P2X\(_4\) and p2X\(_6\) receptors between the adult proximal and distal regions.

4. Discussion

The strong immunosignals for P2X\(_4\) receptor in epithelial cells of proximal and distal regions of the newborn gut suggest a possible role for these receptors in the enteric nervous system development [4]. One possibility is that these receptors are activating calcium channels [19]. Whereas, the strong immunosignal for the P2X\(_6\) receptor in capillary vessels could be important for vascular plexus ontogeny.

The reported function of ATP as neurotransmitter, neuromodulator and co transmitter [5,9,16] was different from “Dale’s Principle”, which speculates that neuron, would store and release the same neurotransmitter. ATP has been reported to act as co transmitter with Ach and 5-HT, thereby influencing the function of their receptors during ontogeny [4].

The P2X\(_4\) immunosignal observed in adult proximal gut may indicate a lower expression of this receptor. In that case the ATP could also act as co-transmitter and modulator of transmitter release in postjunctional locations [3].

The P2X\(_6\) immunosignal observed in the villi of the distal regions of adult gut, extends the results obtained in submucosal and myenteric plexi; in those regions the P2X\(_4\) receptors were expressed widely [26]. Also extends the expression of purinergic receptors (P2X\(_1\), P2X\(_2\) and P2X\(_4\)) observed in myenteric neurons of murine small intestine [14].

It is important to consider the distribution of P2X receptors during early development, in order to explore their participation in cell growth and differentiation. In adult gut the regional distribution could be particularly relevant in the selection of drugs to treat irritable bowel syndrome [11], pain, and inflammation [2,25].

References

of stock, 1294–1302.

Developmental disease, drug

Burnstock, hippocampus, the

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