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Ontogenetic expression of the vanilloid receptors TRPV1 and TRPV2 in the rat retina

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ABSTRACT

The present study aimed to analyze the gene and protein expression and the pattern of distribution of the vanilloid receptors TRPV1 and TRPV2 in the developing rat retina. During the early phases of development, TRPV1 was found mainly in the neuroblastic layer of the retina and in the pigmented epithelium. In the adult, TRPV1 was found in microglial cells, blood vessels, astrocytes and in neuronal structures, namely synaptic boutons of both retinal plexiform layers, as well as in cell bodies of the inner nuclear layer and the ganglion cell layer. The pattern of distribution of TRPV1 was mainly punctate, and there was higher TRPV1 labeling in the peripheral retina than in central regions. TRPV2 expression was quite distinct. Its expression was virtually undetectable by immunoblotting before P1, and that receptor was found by immunohistochemistry only by postnatal day 15 (P15). RNA and protein analysis showed that the adult levels are only reached by P60, which includes small processes in the retinal plexiform layers, and labeled cellular bodies in the inner nuclear layer and the ganglion cell layer. There was no overlapping between the signal observed for both receptors. In conclusion, our results showed that the patterns of distribution of TRPV1 and TRPV2 are different during the development of the rat retina, suggesting that they have specific roles in both visual processing and in providing specific cues to neural development.

1. Introduction

The transient receptor potential channels (TRP) comprise a large family of cation channels mostly permeable to calcium. Each TRP channel is composed of six transmembrane spanning segments with intracellular amino and carboxyl terminals. Based on protein homology, the TRP family can be subdivided in seven subfamilies (Pedersen et al., 2005). The vanilloid receptors (TRPV) are one of such families, and it is composed of six members (TRPV1–6). TRPV1–4 channels are permeable to calcium and sodium, being marginally selective to calcium (reviewed by Gunthorpe et al., 2002).

TRPV1 was the first channel to be cloned in the vanilloid subfamily (Caterina et al., 1997). It is sensitive to capsaicin, the pungent compound extracted from the hot chilli pepper (reviewed by Szolcsanyi, 2004), to endocannabinoids such as anandamide and N-arachidonoyl-dopamine, and to several other exogenous lipids (Di Marzo et al., 2001, 2002). TRPV1 calcium currents can also be elicited by protons, by temperatures above 43 °C (Caterina et al., 1997) and by monoacylglycerols (Iwasaki et al., 2008). To date, TRPV2 can be endogenously activated only by temperatures higher than 52 °C (Caterina et al., 1999), whereas the exogenous compounds 2-aminoethoxydiphenyl borate (Hu et al., 2004) and probenecid (Bang et al., 2007) were only recently described as TRPV2 agonists.

Several reports have indicated that the mammalian retina presents all elements of the endocannabinoid/vanilloid machinery. The retina of different species can produce and hydrolyse anandamide, a cannabinoid CB1 and TRPV1 receptor agonist, as well as other endocannabinoids (Matsuda et al., 1997; Bisogno et al., 1999; Straiker et al., 1999a, 1999b; Yazulla et al., 1999; Straiker and Sullivan, 2003; Chen et al., 2005; Glaser et al., 2005). In the rat retina, 2-arachidonoylglycerol was found to be the main

Abbreviations: AMCA, aminomethylcoumarin; DAPI, diamidino-2-phenylindole; FAAH, fatty acid amide hydrolase; FITC, fluorescein isothiocyanate; GCL, ganglion cell layer; GFAP, glial fibrillary acidic protein; INL, inner nuclear layer; IPL, inner plexiform layer; NGF, nerve growth factor; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OX-42, type-3 complement receptor; PAN, pool of antibodies against neurofilaments of light, medium, and high molecular weights; PB, phosphate buffer; PFA, paraformaldehyde; SYP, synaptophysin; TRITC, tetramethyl rhodamine isothiocyanate; TRP, transient receptor potential channels; TRPV, transient receptor potential channels, vanilloid family.

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cannabinoid compound (Straiker et al., 1999a). TRPV1 was found in fish retinas in photoreceptor synaptic ribbons (Zimov and Yazulla, 2004) and in amacrine cells (Zimov and Yazulla, 2007). Recently, functional experiments indicated for the first time the presence of TRPV1 receptors in the rat retina (Nucci et al., 2007). Subsequently, this receptor was described in both retinal microglia and in retinal ganglion cells of rodents, where it was associated with microglial activation and with retinal ganglion cell death caused by high hydrostatic pressure (Sappington and Calkins, 2008; Sappington et al., 2009). The presence of TRPV2 receptors was previously addressed in several mammalian retinas, indicating the presence of this receptor in both plexiform layers, colocalized with somatostatin and the purinergic P2Y-1 receptor in the inner nuclear layer and in the ganglion cell layer (Yazulla and Studholme, 2004).

TRPV receptors have been implicated in various physiological processes (Leonelli et al., 2009), including neuronal transmission, sensory signaling (Di Marzo et al., 2002), synaptic plasticity (Alter and Gereau, 2008), and apoptosis (Maccarrone et al., 2000; Amantini et al., 2007). Indeed, capsaicin-treated TRPV1 transfected neuroblastoma cells are able to release neurotransmitters, in an apparently calcium-dependent way (Lam et al., 2007). It is also noteworthy that capsaicin, through TRPV1 activation, accelerated cell migration observed in hepatoblastoma cell cultures pretreated with hepatocyte growth factor (Waning et al., 2007). Moreover, TRPV1 protein interacts with tubulin dimers, and its activation improves microtubule stabilization towards diverse depolymerisation stimuli (Goswami et al., 2004). Another important aspect of the vanilloid system is that TRPV1 has been implicated in apoptosis induction. In the retina, it has been shown that capsaicin induced cell death in the inner retina of preweaning rats, but it remains uncertain whether this effect is due to TRPV1 activation or to mitochondrial impairment (Ritter and Dinh, 1992). These data indicate that TRPV receptors may have also a role in developmental processes. However, analysis of TRPV1 and TRPV2 receptors during retinal development is still lacking. Given that neurotransmitters and their receptors might play a role in the control of programmed cell death that occurs in early retinal development (Linden et al., 2005), we studied here the expressions of TRPV1 and TRPV2 in the developing rat retina, using quantitative PCR, immunoblotting and immunohistochemical techniques as a first step aimed at addressing the question of a possible role of TRPV receptors in retinal development.

2. Experimental procedures

2.1. Animal procedures

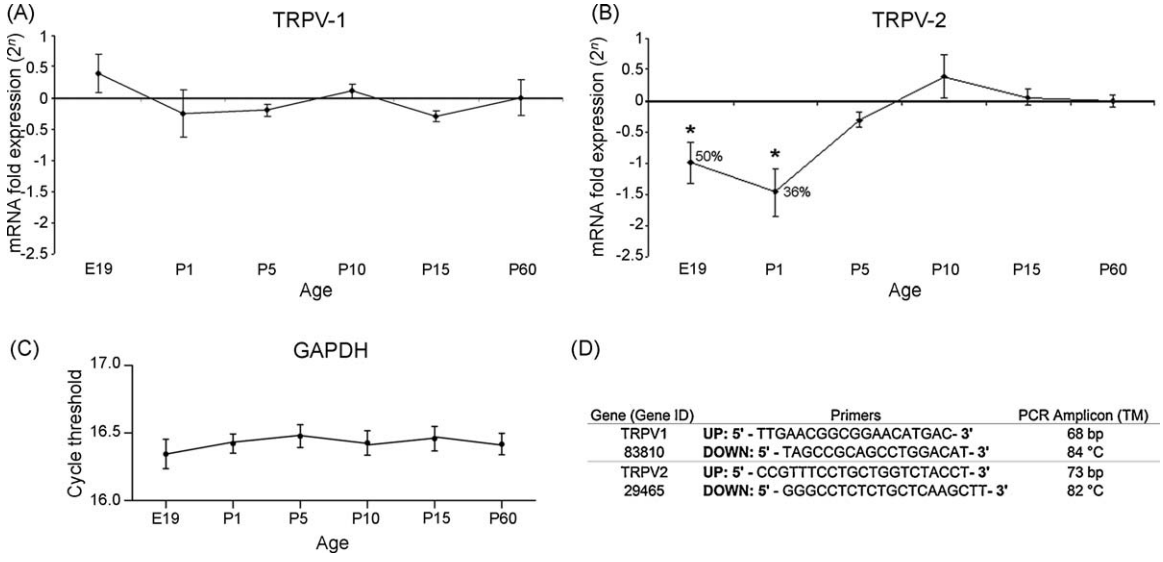
Experiments were carried out with adult, suckling and embryonic rats (*Rattus norvegicus*). The animals were kept on a 12:12 h light/dark cycle with lights on at 07:00 a.m. Postnatal day 1 (P1), P5, P15, and P60 rats ($n = 35$) were killed with an overdose of ketamine (30 mg/100 g of body weight, i.m.; Parke-Davis, Ann Arbor, MI) and xylazine (2 mg/100 g, i.m.; West Haven, CT) between 08:00 a.m. and 10:00 a.m. Pregnant rats were anesthetized with a single dosis of ketamine (5 mg/100 g, i.m.) and xylazine (1 mg/100 g, i.m.) and the embryos were removed and killed with an overdose of ketamine and xylazine as described previously. All experiments were conducted in accordance with guidelines of the NIH and the Institute of Biomedical Sciences of the University of São Paulo.

2.2. RNA isolation, cDNA synthesis, and real-time PCR

Retinas were directly homogenized in 1 mL TRIzol reagent (Invitrogen; Carlsbad, CA) and total RNA was extracted following manufacturer's suggested protocol. Following two chloroform extraction steps, RNA was precipitated with isopropanol and the pellet washed twice in 70% ethanol. After air-drying, RNA was resuspended in DEPC-treated water and the concentration of each sample obtained from A260 measurements. Residual DNA was removed using DNase I (Amersham, Piscataway, NJ) following the manufacturer's protocol. For each 20 μL reverse transcription reaction, 4 μg total RNA was mixed with 1 μL oligodT primer (0.5 μg; Invitrogen) and incubated for 10 min at 65 °C. After cooling on ice the solution was mixed with 4 μL 5× first strand buffer, 2 μL of 0.1 M DTT, 1 μL of dATP, dTTP, dCTP, and dGTP (each 10 mM), and 1 μL SuperScript III reverse transcriptase (200 U; Invitrogen) and incubated for 60 min at 50 °C. Reaction was inactivated by heating at 70 °C for 15 min. Real-time PCR was carried out as previously described (Kihara et al., 2008), using primers purified by high-performance liquid chromatography (Invitrogen). Specific primers were designed for TRPV1 and TRPV2 (Fig. 1(D)). cDNA abundance for GAPDH (forward: 5′-GATGCTGGTGCTGAGTATGTCG-3′, reverse: 5′-GTGGTGCAGGATGCATTGCTGA-3′) was determined as internal control, as previously described (Kihara et al., 2006). All PCR assays were performed as follows: after initial activation at 50 °C for 2 min and 95 °C for 10 min, cycling conditions were 95 °C, 10 s and 60 °C, 1 min. Dissociation curves of PCR products were obtained by heating samples from 60 to 95 °C, in order to evaluate primer specificity.

2.3. Data and statistical analysis

Relative quantification of target gene expression was evaluated using the comparative CT method as previously described in detail (Medhurst et al., 2000). The ΔCT value was determined by subtracting the target CT of each sample from its respective GAPDH CT value (Fig. 1(C)), used as internal control. Calculation of ΔΔCT involves using the ΔCT mean of control group (in the present study, P60) as an



Gene (Gene ID)	Primers	PCR Amplicon (TM)
TRPV1	UP: 5' - TTGAACGGCGGAACATGAC- 3'	68 bp
83810	DOWN: 5' - TAGCCGCAGCCTGGACAT- 3'	84 °C
TRPV2	UP: 5' - CCGTTTCCTGCTGGTCTACCT- 3'	73 bp
29465	DOWN: 5' - GGGCCTCTCTGCTCAAGCTT- 3'	82 °C

Fig. 1. TRPV1 and TRPV2 gene expressions during development of the rat retina. (A) TRPV1 and (B) TRPV2. Means from developmental ages (E19, P1, P5, P10, and P15) were normalized based on adult expression level (P60) and were presented as fold-expression (2^n). (C) GAPDH gene expression was used as internal standard. Whereas TRPV1 gene expression did not change during development, TRPV2 was less expressed at E19 and (50% and 36%, respectively) when compared to P60. Bars represent standard error of mean. $^*p < 0.05$ vs. P60. (D) Table describing PCR primers used in this study.