Research Article

Differential distribution of TASK-1, TASK-2 and TASK-3 immunoreactivities in the rat and human cerebellum

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Received 25 February 2004; received after revision 19 April 2004; accepted 28 April 2004

Abstract. In this work, the distributions of some acid-sensitive two-pore-domain K+ channels (TASK-1, TASK-2 and TASK-3) were investigated in the rat and human cerebellum. Astrocytes situated in rat cerebellar tissue sections were positive for TASK-2 channels. Purkinje cells were strongly stained and granule cells and astrocytes were moderately positive for TASK-3. Astrocytes isolated from the hippocampus, cerebellum and cochlear nucleus expressed TASK channels in a primary tissue culture. Our results suggest that TASK channel expression may be significant in the endoplasmic reticulum of the astrocytes. The human cerebellum showed weak TASK-2 immunolabelling. The pia mater, astrocytes, Purkinje and granule cells demonstrated strong TASK-1 and TASK-3 positivities. The TASK-3 labelling was stronger in general, but it was particularly intense in the Purkinje cells and pia mater.

Key words. TASK immunopositivity; astrocyte culture; cerebellum; endoplasmic reticulum; human brain.

Introduction

Based upon sequence similarities between the pore-forming (or α) subunits of the K+ channels, three major classes (known as superfamilies) are distinguished. Besides the voltage-gated and inward rectifier K+ channels [for reviews see 1, 2], a new superfamily with four transmembrane domains has been described more recently [3]. In these channels, the functional channel is a dimer [4], where the subunits consist of a tandem of pore-forming domains; hence these channels are often referred to as tandem-pore-domain (TPD), two-P or PP domain channels. Several classes of the TPD channels have been described in the past few years and more recently (TWIK [4–6], TREK [7–10], TRAAK [11], TASK [12–19], TALK [20, 21], THIK [22] and TRESK [23]) and the number is continuously growing. Regulation of these channels seems to be extraordinarily complex, as they may be gated by mechanical stretch of the membrane, heat, free fatty acids, volatile anaesthetics, protein kinases and various other chemical stimuli, including the alteration of the intra- or extracellular pH.

TASK (or TWIK-related acid sensitive K+) channels are particularly sensitive to changes in the extracellular pH [12, 13, 15, 19]. Certain members of the TASK family (TASK-1, TASK-3 and TASK-5) are more closely related to each other than to TASK-2 and TASK-4. In fact, the latter two channels are now classified as members of the TALK (TWIK-related alkaline-pH-activated K+ channel) group, and TASK-4 is often referred to as TALK-2 [20, 21].

TASK (and in particular TASK-1) channels are generally accepted to function as ‘background’ K+ channels [12], being responsible for the high K+ conductance of the various cells at rest, crucially determining, therefore, their resting membrane potential. The standing outward K+ current of
cerebellar granule cells \( (I_{\text{K}}; [24]) \) is also generated by the activity of TASK-1 channels [25]. Moreover, a recent study has demonstrated that TASK-3 channels play crucial roles in the K⁺-dependent apoptosis of cerebellar granule neurons in culture [26]. Considering the broad physiological functions of the TASK channels, it is not surprising that the presence of TASK-specific nucleic acid sequences has been reported in several different tissues, such as brain, lung, testis, pancreas and kidney [19, 27]. Besides physiological functions, TASK-3 channels may have oncogenic potential [28] and increased expression of the TASK-3 encoding KCNK9 gene has been reported in a significant portion of breast cancers [29].

When the distribution of TASK channels in the central nervous system was investigated, some rather interesting experimental results were obtained. In human brain, both TASK-1- and TASK-3-specific mRNA was noticed in a crude extract, in contrast to the almost complete lack of TASK-2 mRNA [27]. On the other hand, using immunohistochemical methods, both TASK-1 [30] and TASK-2 immunopositivities [31] were observed in the central nervous system of the rat. Particularly noteworthy is that while neurones, glial cells and ependymal cells all expressed TASK-1 channels, TASK-2 positivity was reported on neurones only and neither glial nor ependymal cells showed noticeable TASK-2 immunopositivity. These data suggested that there might be significant differences in the tissue distribution of the various TASK channels even within the same species. As for the human brain, the data available concern the presence (or absence) of TASK-specific mRNA, and this cannot provide information about the localisation of TASK channels at the cellular level, hence a more detailed investigation of the distribution of TASK-specific immunoreactivity seems to be important in the human central nervous system.

In the present study, we demonstrate the immunohistochemical distribution of TASK-1, TASK-2 and TASK-3 in the human cerebellum, and an interspecies comparison is performed between human and rat. Moreover, using rat astrocytes maintained in tissue culture, we provide evidence that both GluR- and GluT-type astrocytes express strong TASK-1 immunoreactivity. Somewhat weaker but nevertheless present TASK-2 and TASK-3 immunoreactions were also found in both types of astrocyte. The results obtained in astrocyte tissue culture were confirmed by Western blotting and RT-PCR methods.

Materials and methods

Enzymatic isolation and tissue culture of astrocytes

The enzymatic dissociation of the hippocampus, cerebellum and cochlear nucleus was performed using a technique similar to that described earlier [32] (the procedure was authorised by the Ethical Committee of the University of Debrecen). In short, 3- to 8-day-old Wistar rats were decapitated, their brain removed in ice-cold dissection medium (D1; in mM: NaCl, 136; KCl, 5.2; Na2HPO4; H2O, 0.64; KH2PO4, 0.22; glucose, 22; HEPES, 10; plus 0.06 U/ml penicillin and 0.06 μg/ml streptomycin). All chemicals were purchased from Sigma (St. Louis, Mo.), unless stated otherwise. Enzymatic dissociation of brain tissue was achieved by employing D1 solution containing trypsin (0.025 g/ml; 30 min, 37°C). At the end of the incubation period, the now gelatinous tissue pieces were transferred to minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) for 5 min (room temperature). Individual cells were obtained by applying very gentle agitation with fire-polished Pasteur pipettes. The cell suspension was diluted for a density of 100,000 cells/ml, and 0.5 ml of this suspension was transferred onto cover-slips situated in 12 wells of a 24-well tray (the marginal wells were not used in order to reduce the risk of infection). The cells were allowed to grow at 37°C in a 5% CO2 atmosphere. The feeding medium (MEM supplemented with 10% FCS) was changed on the following day and every other day thereafter. Under these conditions most of the neurones died, while the astrocytes kept on dividing and growing. Immunocytochemistry was usually conducted on 4- to 5-day-old cultures by which time 70–80% confluence was reached.

Fluorescent immunocytochemistry

Fluorescent immunocytochemistry was employed on astrocyte cultures. Prior to the immunocytochemistry, the cells were fixed with 4% paraformaldehyde (15 min, room temperature), then washed with phosphate-buffered saline (PBS) containing 100 mM glycine. Permeabilisation was carried out by bathing the cells in PBS containing Triton X-100 (0.1%) for 10 min followed by washing with PBS. Aspecific binding was prevented by incubating the cells in PBS containing bovine serum albumin (BSA, 1%) for 30 min. The cells were then incubated with the primary antibodies (table 1) for 60 min at room temperature or overnight at 4°C. The cells were rinsed in PBS (3 x 5 min), incubated with the appropriate secondary antibody (table 2) for 60 min at room temperature, followed by rinsing in PBS again (3 x 5 min). At the end of the procedure, the nuclei were stained with DAPI followed by cover-slip mounting.

Non-fluorescent immunostaining

When tissue samples from rat or human cerebellum were investigated, non-fluorescent immunohistochemistry was used. Formaldehyde-fixed, paraffin-embedded sections were prepared from the human tissue, while both formaldehyde-fixed (24 h) and frozen (in liquid nitrogen) sections were employed for rat cerebellum. The human cerebellar tissue blocks were obtained from the histo-