Gene Expression Patterns 10 (2010) 65–74

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Dynamic expression of the TRPM subgroup of ion channels in developing mouse sensory neurons

Susanne Staaf [a,b], Marina C.M. Franck [b], Frédéric Marmigère [b], Jan P. Mattsson [c], Patrik Ernfors [b,*]

[a] Department of Bioscience, AstraZeneca R&D Mölndal, 431 83 Mölndal, Sweden
[b] Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden
[c] Albireo Pharmaceuticals, 430 33 Göteborg, Sweden

ARTICLE INFO

Article history:
Received 2 February 2009
Received in revised form 10 September 2009
Accepted 12 October 2009
Available online 20 October 2009

Keywords:
Nociceptive
Non-nociceptive
Peptidergic
TRP channels
Expression
mRNA
Neuron
In situ hybridization
Dorsal root ganglion
Nodose ganglion
Sensory nervous system
TRPM8
Immunohistochemistry
PCR

ABSTRACT

Despite the significance of transient receptor potential (TRP) channels in sensory physiology, little is known of the expression and developmental regulation of the TRPM (melastatin) subgroup in sensory neurons. In order to find out if the eight TRPM subgroup members (TRPM1–TRPM8) have a possible role in the sensory nervous system, we characterized the developmental regulation of their expression in mouse dorsal root ganglion (DRG) from embryonic (E) day 12 to adulthood. Transcripts for all channels except for TRPM1 were detected in lumbar and thoracic DRG and in nodose ganglion (NG) with distinguishable expression patterns from E12 until adult. For most channels, the expression increased from E14 to adult with the exception of TRPM5, which displayed transient high levels during embryonic and early postnatal stages. Cellular localization of TRPM8 mRNA was found only in a limited subset of very small diameter neurons distinct in size from other populations. These neurons did not bind isolectin B4 (IB4) and expressed neither the neuropeptide calcitonin gene-related peptide (CGRP) nor neurofilament (NF)200. This suggests that TRPM8^{+} thermoreceptive sensory neurons fall into a separate group of very small sized neurons distinct from peptidergic and IB4^{+} subtypes of sensory neurons. Our results, showing the expression and dynamic regulation of TRPM channels during development, indicate that many TRPM subfamily members could participate during nervous system development and in the adult by determining distinct physiological properties of sensory neurons.

© 2009 Elsevier B.V. All rights reserved.

1. Results and discussion

There are several types of sensory neurons in the DRG with responsiveness to different kinds of external and internal stimuli. These stimuli, i.e. nociceptive, thermal or mechanical, activate different receptors and ion channels that are present in the nerve terminals at the sensory receptive fields and their expression in selective subsets of DRG neurons determines the response profile of individual neurons to a given stimuli. TRP channels are a group of non-selective cation channels that play important functions in sensory neurons. These channels are divided into six subgroups called TRPM (melastatin), TRPC (classical), TRPV (vanilloid), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin). Despite the significance of TRP channels for sensory functions little is known about their expression in the DRG and in particular, about the expression and regulation of the TRPM subfamily. TRPM1–M8 is functionally a very diverse group of ion channels with diverse functions and expression patterns (Fleig and Penner, 2004; Fonfria et al., 2006). TRPM8 is the only TRPM channel with a clearly assigned function in DRG neurons. It is activated by innocuous cool stimuli and responds to menthol and icilin with intracellular Ca^{2+} elevations (McKemy et al., 2002; Peier et al., 2002). Placing TRPM8 in any particular category of small size sensory neurons has been enigmatic and controversial. Originally, a lack of colocalization with known neuronal markers such as NF200, CGRP, IB4 and TRPV1 was reported (Peier et al., 2002). These findings were challenged by other groups showing coexistence of TRPM8 with TRPV1 (Okazawa et al., 2004) and NF200 (Kobayashi et al., 2005). In addition, two studies using tracers for TRPM8 in transgenic mice presented conflicting results where one showed presence of both CGRP and NF200 in a subpopulation of TRPM8^{+} neurons (Takashima et al., 2007) while the other reported lack of colocalization for TRPM8 with NF150, IB4 and CGRP (Dhaka et al., 2008).

To give some insight on which of the TRPM subfamily channels may have importance in the sensory nervous system we analyzed

* Corresponding author. Address: Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Scheelsv. 1, 171 77 Stockholm, Sweden. Tel.: +46 8 5248 7659; fax: +46 8 341960.
E-mail address: patrik.ernfors@ki.se (P. Ernfors).

1567-133X/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2009.10.003

the expression of the TRPM channels through embryonic and postnatal development in mouse thoracic and lumbar dorsal root ganglia as well as in the nodose ganglion. Except for TRPM1, we found transcripts of all channels with different expression patterns between E12 and adulthood. TRPM8 expression was investigated in more detail using *in situ* hybridization in combination with immunohistochemistry. We found by quantitative analysis that only a limited subset of very small diameter neurons, which did not label for IB4, CGRP or NF200, contained TRPM8. Consistently, size frequency histogram analysis placed the very small caliber TRPM8+ neurons in a separate category of neurons distinct from IB4+ and NF200+ neurons.

1.1. Developmental expression of TRPM channels in lumbar dorsal root ganglia

Real-time PCR analysis using cDNA from lumbar DRG at E12, E14, E18, P0, P4, P12 and adult ages was performed to study the expression pattern of TRPM1–TRPM8 throughout development. By adding an equal amount of template in each assay, using an internal reference gene (mouse ribosomal protein 36B4) and normalizing against a common calibrator gene (adult lumbar TRPV1) that was set to 1, the relative expression levels could be compared between the different channels. All reactions produced one peak in the melting point curves.

In adult tissue the mRNA expression levels were highest for TRPM3, ∼20% of TRPV1 mRNA levels, followed by TRPM2, TRPM4 and TRPM7 that expressed ∼10% of TRPV1 mRNA levels (Fig. 1). TRPM8 was expressed in lower amounts (∼3% of TRPV1 mRNA levels) and TRPM5 and TRPM6 levels were very low (<1% of TRPV1 mRNA levels). TRPM1 was not detected at all, neither in adult nor at any developmental stage analyzed. At embryonic stages, all channels showed increased expression from E12 until soon before or around birth. From that time point to P4 the expression decreased for all channels except for TRPM6, for which the mRNA levels remained unchanged. At the next stage analyzed, P12, the expression increased for all channels except for TRPM5, for which mRNA levels continued to decrease until adulthood at which time very little TRPM5 was detected. For TRPM2, M3, M4 and M6 the expression levels continued to increase between P12 and adulthood while TRPM7 and TRPM8 showed a small reduction in levels. This pattern suggests two expression waves. The second wave occurring after birth implies an importance of these channels in adult life either direct in sensory mechanisms or in more general ion homeostasis of the sensory neurons.

1.2. TRPM8 is expressed in neither peptidergic nor IB4+ small size neurons in mouse dorsal root ganglia

To define the population of neurons in mouse lumbar DRG expressing TRPM8, colocalization studies were performed with common markers for neuronal subtypes. *In situ* hybridization with a digoxigenin labeled TRPM8 RNA probe showed very strong expression of the channel mRNA in a small subset of the neurons. Total number of neurons was determined by the nuclear neuronal marker Islet 1. Labeling was seen in 6.0% of all adult DRG neurons (Fig. 2), a finding that corresponds well with other studies reporting positive labeling in 5–10% of the neurons (Dhaka et al., 2008; Peier et al., 2002). To investigate if the decreased expression seen at P4 by quantitative PCR was caused by a loss of cells expressing TRPM8 or by lower expression levels in each cell, *in situ* hybridization was conducted on E18, P4 and P12 DRG (Figs. 3 and 4). The TRPM8 probe labeled 2.4%, 3.8%, 5.0% and 6.0% of the neurons in E18, P4, P12 and adult DRG, respectively (Table 2). Statistical analysis showed significantly less cells labeled in E18 than in P4 and in P4 than in P12 or adult, whereas there was no significant difference between P12 and adult values. Hence, overall, the number of TRPM8+ neurons was found to steadily increase from E18 to adult stages. These findings suggest that increased expression levels in the DRG postnatally reflects an increase in the number of TRPM8 expressing neurons rather than marked alteration in expression levels within neurons already expressing TRPM8. However, our data suggest that the decline in mRNA levels detected by real-time PCR between E18 and P4 reflect reduced transcript levels in individual cells.

To further investigate which population the TRPM8 labeled subset of cells belong to, immunohistochemical labelings with IB4, binding to small non-peptidergic neurons, NF200, which labels large proprioceptive and mechanoreceptive neurons, and CGRP, expressed by peptidergic nociceptive neurons, were performed on the same sections (Fig. 2). The neuronal nuclear marker Islet 1 was used to count the total number of neurons. IB4 stained 39%, NF200 was expressed by 38% and CGRP occurred in 40% of the total population of neurons in adult DRG. The same markers were also employed on P4 and P12 tissue (Table 2, Figs. 3 and 4). At these earlier stages, labeling frequencies for IB4 were only 16% at P4 and 33% at P12. Neuronal numbers expressing CGRP were also found to increase from 24% at P4 to 32% at P12, whereas similar numbers of NF200 expressing cells were counted at postnatal stages (27% at P4 and 29% at P12). TRPM8+ cells did not stain for IB4, NF200 or CGRP at any stage examined. The lack of coexpression in adults with either marker was consistent with the findings of Peier et al. (2002), where *in situ* hybridization combined with immunohistochemistry indicated no overlap between TRPM8 and NF200, TRPV1, CGRP or IB4 staining. Another study showed coexistence of TRPM8 with TRPV1 in the same cells in DRG sections, i.e. 29% of TRPM8+ cells also expressed TRPV1 (Okazawa et al., 2004). Yet another report presented a 23% TRPM8 labeling frequency and that 50% of the TRPM8+ cells colocalized with NF200 in rat DRG neurons (Kobayashi et al., 2005). Interestingly, contradicting the results of Okazawa et al. (2004), but confirming the findings of Peier et al. (2002), this study showed rare colocalization between TRPV1 and TRPM8. It might be of importance that both Kobayashi et al. (2005) and Okazawa et al. (2004) were using rat tissue which might stain differently for neuronal markers and TRP channels than mice do. Using a transgenic mouse expressing EGFP in the TRPM8 locus, TRPM8 expression was found in 7.8% of DRG neurons and also showed some colocalization with TRPV1 (Dhaka et al., 2008). Nerve endings from the TRPM8 population were localized in the outermost layers of epidermis close to, but not overlapping with CGRP+ fibers. These terminals correspond to the non-peptidergic/IB4-negative sensory free nerve endings previously identified in the epidermis in the intervibrissal fur on the mystacial pad of the rat (Fundin et al., 1997). Another study with a similar approach, using GFP as a tracer for TRPM8 neurons, showed a quite extensive overlap between GFP and NF200, CGRP and TRPV1 (Takashima et al., 2007). The lack of co-labeling of TRPM8 with IB4, NF200 or CGRP at any analyzed stage in the present study indicates that new TRPM8 neurons might not be recruited from any of these subtypes, in which case a transient co-expression at early developmental stages might be expected followed by a subsequent loss of the IB4, CGRP and NF200 identity.

Differentiation of sensory neurons into specific subtypes is controlled by a dynamic interaction between extracellular signaling and control of gene expression exerted by neurotrophic growth factors and transcription factors (Marmigere and Ernfors, 2007). During embryonic development around 80% of all neurons express TrkA. The non-peptidergic population of neurons is generated from roughly half of the TrkA+ population that under influence of the runt homology domain transcription factor Runx1 starts to express Ret and extinguishes TrkA postnatally. These neurons colabel to 90% with IB4 and are dependent on GDNF for survival (Molliver

Download English Version:

https://daneshyari.com/en/article/2182120

Download Persian Version:

https://daneshyari.com/article/2182120

Daneshyari.com