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Identification of acid-sensing ion channels in bone

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Abstract

Bone balances serum pH variations and both osteoclasts and osteoblasts are regulated by subtle changes in pH. The aim of the current study was to identify molecules in bone that can sense pH. Interesting candidates are the acid-sensing ion channels (ASICs). In bone, ASIC2 and ASIC3 were most abundant, while in chondrocytes it was ASIC1. Isolated human monocytes expressed ASIC1, -2, and -3, which persisted after induction to osteoclast differentiation, albeit to a lower level. In human osteoblasts ASIC1, ASIC2, and ASIC3 mRNAs were shown. Western blot and immunostaining confirmed this at protein level. ASIC4 expression was always very low abundant. For the first time, we demonstrated ASICs in human skeleton, providing a means to sense and respond to differences in extracellular pH.

Keywords: pH sensors; Bone; Osteoblasts; Oosteoclasts; Chondrocytes

A stable physiologic systemic pH is critical for the survival of mammals. Homeostasis of the intracellular hydrogen ion concentration is pivotal to the maintenance of cell function and viability, but pH fluctuations are present in both intracellular and extracellular compartments in normal physiological and pathophysiological conditions [1–3]. Bone cells, both osteoblasts and osteoclasts, respond to pH changes in the physiological range [4,5], but the sensor(s) that can mediate these pH changes are largely unknown.

Candidates are the acid-sensing ion channels (ASICs), which belong to the epithelial sodium channel (ENaC)/degenerin gene family, a new class of ion channels with a high degree of functional heterogeneity [6]. ASICs are almost ubiquitous in the mammalian nervous system and are activated in response to a drop in pH to below 7.0 [7]. To date, four genes encoding the channel subunits ASIC1, ASIC2, ASIC3, and ASIC4 have been cloned. ASICs show 45–60% amino acid sequence identity between the four genes. ASICs form homo- or heterotetrameric channels that autoassemble in the membrane, with the subunit composition determining their kinetic characteristics [6,8]. Knowing the importance of mechanical loading for bone metabolism, ASICs are the more so interesting as in various organisms members of the ENaC/DEG gene family have been linked to mechanical sensation [9,10] and degenerin can interact with the extracellular matrix [11].

The aim of our current study was to identify ASICs in bone to provide a means by which bone can sense changes in extracellular pH in order to adapt its metabolic activity.

Materials and methods

Cell culture and biochemistry. Immortalized human pre-osteoblast (SV-HFO) cultures and biochemical assays were performed as described earlier [12]. Briefly, cells were cultured in αMEM supplemented with 20 mM Hepes, pH 7.2, 7.5, and 7.7, streptomycin/penicillin, 1.8 mM $CaCl_2$, 2% charcoal-treated heat-inactivated FCS, 1 μM dexamethasone, and 10 mM

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β-glycerophosphate (Sigma) to facilitate mineralization. Calcium content was determined colorimetrically with a calcium assay kit (Sigma). Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors and isolated by density centrifugation with Lymphoprep (Axis-Shield PoC AS, Oslo, Norway). Briefly, the buffy coat preparations were diluted 1:1 with Hanks' balanced salt solution (HBSS). Twenty milliliters of diluted PBMCs was overlayered onto 15 ml of Lymphoprep and centrifuged at 1000*g* for 30 min at ambient temperature. PBMCs were recovered from the interface and washed twice with HBSS supplemented with 2% FCS, prior to separation on a Percoll gradient (Pharmacia, Uppsala, Sweden) consisting of three density layers (1.076, 1.059, and 1.045 g/ml), the middle layer of which contained predominantly monocytes, which were seeded in 96-well culture plates at a density of 10^5 cells/well and cultured for 3 weeks in DMEM supplemented with 10% FCS and 1% antibiotic–antimycotic solution containing 30 ng/ml human M-CSF (R&D systems, Minneapolis, MI, USA) and 20 ng/ml human RANKL (Peprotech, London, UK), which was replaced twice a week. After 3 weeks of culture, cells were scraped for gene expression as outlined below.

RNA isolation, cDNA synthesis, quantitative RT-PCR. RNA extraction from cells is reported elsewhere [12]. Human osteoarthritic femoral head biopsies (five donors; 51–70 years of age from whom an informed consent was obtained) were separated into bone and cartilage. For details on RNA isolation from tissues, cDNA synthesis, real-time RT-PCR, and relative quantification of gene expression according to the $2^{-\Delta C_t}$ method see Mandl et al. [13]. Data are presented as described elsewhere [14] and statistically analysed using GraphPad InStat. Primer (F, forward; R, reverse) and probe (FAM) sequences for Taqman assays with corresponding accession numbers of REFSEQ nucleotide sequences were as follows: ASIC1 (NM_020039), F_CTACGCCTACGAGGTCATTAAGC, R_CCGCACTGCTCCTTTTGG, FAM_AAGCTGTGCCGACGAGGAAAATGC; ASIC2 (NM_183377), F_GAGACAGAG-GAAACGACATTTGAA, R_TGGAGCCACCCCAAAGC, FAM_CACAGTCAGTCT-GAGCCACCTTTCATCCA; ASIC3 (NM_020322), F_GCTTCCGTGGCCAACCTT, R_CCCATCAGCGCCAGAGTTAA, FAM_ACTTCACCACGATCTTCACCCGGATG; ASIC4 (NM_182847), F_GGTACCTGGCGAGGAAGTACAA, R_CAGGGCCTCAAAGAAGACATCT, FAM_CGCAACGAGACCTACATACGGGAGAACTT; SPP1 (osteopontin, NM_000582), F_CTCAGGCCAGTTGCAGCC, R_CAAAAGCAAATCACTGCAATTCTC, FAM_AAACGCC-GACCAAGGAAAACTCACTACC.

Antibodies. Primary antibodies against ASIC subunits were purchased from ADI (Alpha Diagnostic International, San Antonio, TX 78238, USA) and used in 1:1000 (ASIC1, ASIC2, and ASIC4) and 1:1500 (ASIC3) dilutions for Western blotting, and in 1:500 (ASIC1, ASIC2, and ASIC4) and 1:700 (ASIC3) dilutions in immunolocalization experiments.

Immunohistochemistry. Cells were fixed 15 min in 4% (w/v) paraformaldehyde in phosphate-buffered saline (1× PBS), blocked in PBSB (1× PBS, pH 7,4; 10% BSA; 0.05% Tween 20) for 90 min, and incubated with rabbit anti-ASIC primary antibodies in 0.5× PBSB for 16 h at 4 °C prior to detection with goat anti-rabbit IgG (AlexaFluor488; Molecular Probes, 1:300). Negative controls included control rabbit IgG (Alpha Diagnostic International, San Antonio, USA) and omission of primary antibodies. Specimens were mounted in VectaShield (Vector Laboratories, Burlingame, USA) and examined with a Zeiss Axioplan 2.

Protein extraction and Western blotting. SV-HFO cells were harvested in 2.5 ml hypotonic PEC homogenization buffer (10 mM phosphate buffer, pH 7.0; 8 mM EDTA); one tablet per 25 ml complete protease inhibitor cocktail (Roche Diagnostics BV, Almere, The Netherlands) per 75 cm^2. All handlings were at 4 °C with cells and devices pre-chilled on ice. Cells were homogenized (RW20, IKA Werke GmbH & KG, Staufen, Germany) and cell extracts were then successively centrifuged at 4 °C: (1) 10 min at 700*g* to remove cell debris, (2) supernatant was centrifuged for 10 min at 7000*g* removing the mitochondria, and (3) 90 min at 100,000*g* giving a cytosolic (supernatant) and a 'microsome' or membrane fraction (pellet). Protein fractions were mixed 4:1 (v/v) with Roti-Load 1 (Carl Roth GmbH, Karlsruhe, Germany), denatured 5 min at 95 °C, and separated on a 12% Laemmli sodium dodecyl sulphate polyacrylamide gel [15] at a neutral pH. Total protein load per lane was 5 μg (membrane fraction) and 5 μg (cytoplasmic fraction). Separation and blotting on Immobilon-P (Millipore BV, Amsterdam, The Netherlands) was performed as described previously [16]. Membranes were blocked in PBSB and incubated with primary antibodies for 16 h at 4 °C. After washing, secondary antibody incubation was performed for 2 h at room temperature with a 1:1500-diluted goat anti-rabbit HRP-labeled antibody (P0448; DAKO, Carpinteria, CA). Antibody binding was detected using ECL Western Blotting Analysis System (RPN2109; Amersham Biosciences, UK) according to supplier's instructions.

Results and discussion

It is well known that the skeleton participates in pH homeostasis as buffering organ to ensure a stable physiologic systemic pH. The activity of both osteoclasts and osteoblasts can be regulated by changes in pH [17]. The latter is exemplified by our studies: when human osteoblasts were cultured in the presence of media with only small differences in pH (i.e., pH 7.2, 7.5, and 7.7), a pH-dependent mineralization was observed (Fig. 1A). At increasing pH, the onset of mineralization was earlier and reached higher levels. In addition, changes in extracellular pH (pH_o) also affected gene expression of human osteoblasts. A clear pH-dependent differential osteopontin transcription was

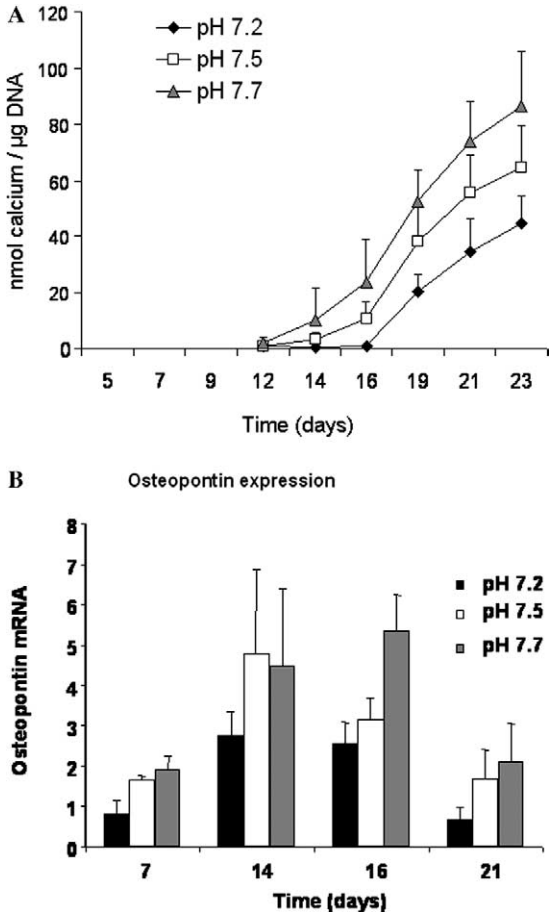

Fig. 1. pH-dependent mineralization and change in gene expression. (A) Effects of different extracellular pH-values on mineralization in cultured human pre-osteoblasts and (B) on relative expression of osteoblast marker gene osteopontin. Values are means ± SD.

observed (Fig. 1B) with a higher expression at increasing pH throughout osteoblast differentiation. Actually, knowledge on the means by which osteoblasts can sense pH is virtually absent.

In the mammalian nervous system, several pH sensors have recently been identified. In this study, we demonstrate that these sensors, the H^+-gated Na^+-channels ASICs, are also present in skeletal cells. ASIC transcripts were detected in bone and cartilage from human skeletal biopsy specimens. As demonstrated by quantitative real-time PCR, in bone expression of ASIC2 and ASIC3 was highest (Fig. 2), while in cartilage ASIC1 mRNA was most abundant (Fig. 2). In contrast to cartilage, bone is composed of various cell types and ASIC expression in bone biopsy material cannot directly be attributed to a distinct cell type. Therefore, we studied the two major cell types involved in bone metabolism separately in vitro: the osteoclast and the osteoblast. Human osteoclasts were generated from monocytes by treatment with M-CSF and RANKL. ASIC1, ASIC2, and ASIC3 were expressed in both monocytes and differentiated osteoclasts with ASIC2 being most abundant, while ASIC4 mRNA was virtually absent in both cell types (Fig. 3).

In general, expression of ASICs was higher in monocytes than in osteoclasts, about 4-fold for ASIC2 and 2-fold for ASIC1 and ASIC3. These data show that cells of the hematopoietic lineage express ASICs and may be regulated by pH. This is of interest as also macrophage activity can be changed by extracellular pH [18] and as inflammation facilitates ASICs expression [7,19]. The strong decrease in ASIC2 expression during differentiation from monocyte to osteoclast is intriguing. Functional ASICs are homo- or heterotetramers, with subunit composition determining channel kinetics [8]. Therefore, presently we can only speculate about the functional significance, but it may imply differences in pH-sensing characteristics. Environmental pH may regulate monocyte differentiation and osteoclast lifespan mediated by ASICs. Interestingly, Karsdal et al. [20] recently published a model for the role of pH_o in the coupling of bone formation and resorption

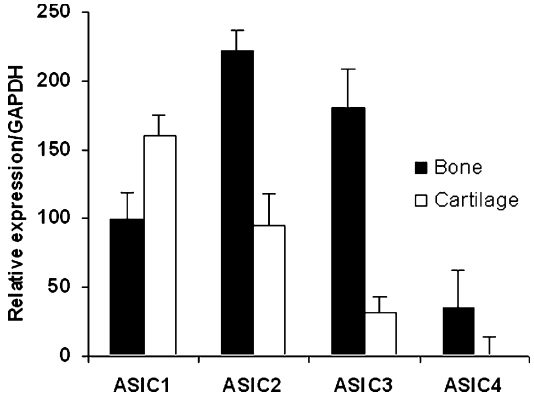

Fig. 2. Expression of ASICs in human skeletal tissues. Relative transcript abundances in human bone (black bars) and cartilage (white bars) in arbitrary units normalized to GAPDH. Values are means ± SD.

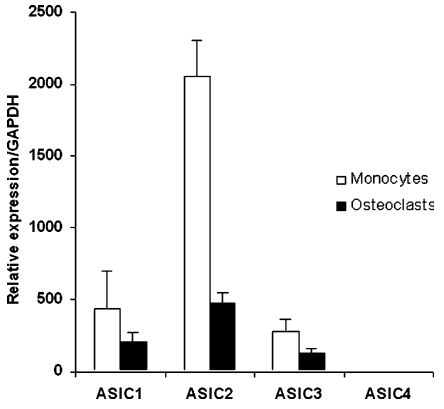

Fig. 3. Expression of ASICs in human monocytes and osteoclasts. Transcript abundances in human osteoclast precursors (monocytes) and mature osteoclasts. Arbitrary units show relative expression quantified to GAPDH. Values are means ± SD.

involving acidification attenuated osteoclastic resorption due to decreased release of proapoptotic factors.

In addition to osteoclasts, we also examined ASIC expression in human osteoblasts and also whether this relates to osteoblast differentiation. We cultured human

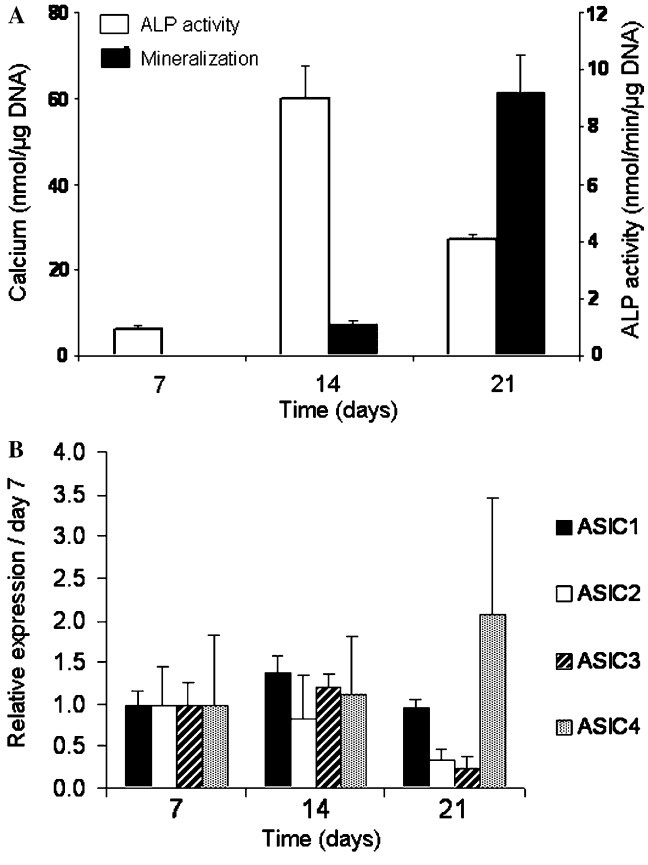

Fig. 4. ASIC expression in mineralizing pre-osteoblast culture. (A) Relative changes in alkaline phosphatase (ALP) activity during culture next to calcium incorporation in the extracellular matrix per amount of DNA. Typical time points (day 7, 14, and 21 at, pH 7.5) are depicted. (B) Fold changes in relative ASIC expression (normalized to GAPDH) in arbitrary units depicted as changes in expression relative to day 7. Means ($n = 6$) ± SD. $^*p < 0.005$; $^{\#}p < 0.0001$ versus day 7.

pre-osteoblastic cells (SV-HFO) for three weeks to induce differentiation and mineralization [12]. This resulted in a peak in alkaline phosphatase (ALP) activity at day 14 at which mineralization of the extracellular matrix started (Fig. 4A). During osteoblast differentiation ASIC1 gene expression appears to peak around day 14 while expression of ASIC 2 and 3 is significantly lower during mineralization at day 21 (Fig. 4B).

Low expression levels of membrane proteins often do not allow their detection by Western blotting. Therefore, we enhanced sensitivity by isolating cell membranes, a technique similar to that of Wemmie et al. [21]. Unfortunately, we were unable to obtain sufficient protein in the membrane fraction from osteoclasts for these experiments. In human osteoblasts we got a relatively weak signal for ASIC1 and ASIC3 while ASIC2 and ASIC4 signals were prominent (Fig. 5). ASIC1 and ASIC4 were determined to about 80 and 75 kDa, respectively. ASIC2 and ASIC3 subunits were smaller with 55 and 60 kDa, respectively. Database entries of ASIC subunit polypeptides give theoretical values from 50 to 70 kDa, depending on channel subunit and isoform. ASIC1-specific signals of 55 and 90 kDa, and 55 and 75 kDa for ASIC2, and 80 or 90 kDa for ASIC3 are known [22], while an in vitro translated, non-glycosylated human ASIC3 product of 59 kDa was also reported [23]. In human intestine, ASIC subunits have been reported with 54–56 kDa [24], whereas glycosylated ASIC subunits from rats in transfected HEK293 cells are 70–80 kDa [25]. Apparently there is quite a variation in reported sizes of ASIC subunits. Though the subunit-specificity of our antibodies has been proven [26], we cannot exclude a possible cross-reactivity with isoforms or alternative glycosylation products. ASIC1 was the most abundant transcript in osteoblast culture (Fig. 4B) but revealed only a weak signal on Western blots (Fig. 5) while the opposite holds true for ASIC4. This discrepancy may be explained by posttranslational regulation, but signal intensities may also vary due to different antibody qualities and quantitative conclusions should be drawn with precaution. Interestingly, besides in membrane fractions all ASIC subunits were also detected in the cytoplasm (Fig. 5). The immunosignal of ASIC3 is even stronger in the cytoplasmic fraction than in the membrane preparation. A prominent ASIC2, ASIC3, and ASIC4, and a weaker ASIC1 immunohistochemical staining in SV-HFO confirmed the presence of ASIC subunits in human osteoblasts. The evenly spread immunosignals in these cells (Fig. 6) may support that ASIC are also present in the cytoplasm. Remarkably, Hildebrand et al. [27] recently identified ASIC3 in the cytoplasm of mouse ganglion cells, too. It is tempting to hypothesize a yet unidentified intracellular function for this subunit.

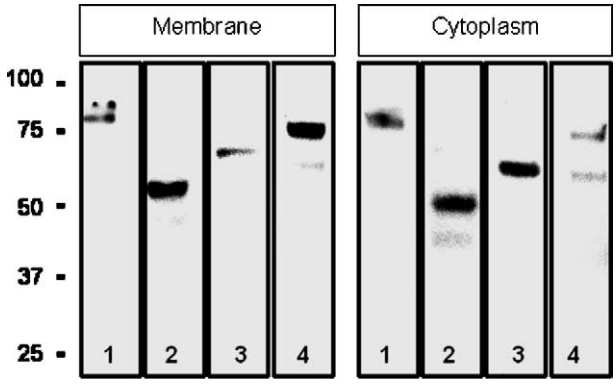

Fig. 5. Western blots showing ASIC expression in mineralizing human pre-osteoblasts at day 14. Immunosignals of ASIC1 (1), ASIC2 (2), ASIC3 (3), and ASIC4 (4) in membrane fraction and cytoplasm. Ruler indicates MW in kiloDalton.

Besides in bone cells, ASICs were also detected in cartilage from osteoarthritic patients (Fig. 2B). As we were not able to obtain undiseased cartilage we do not yet know whether ASICs are also expressed in healthy cartilage. Chondrocytes exist in an avascular, hypoxic environment, and produce large amounts of lactate [28,29], resulting in an extraordinary low pH_o (pH 6.9) even in healthy cartilage [30]. Lactate, in turn, enhances the sensitivity of ASICs to pH by lowering the extracellular concentration of bivalent ions such as Ca^{2+} [31]. Maintenance of chondrocyte pH is an important parameter controlling cartilage matrix turnover rates [30] and ASICs may thus be involved in regulation of chondrocyte function.

The physiological role of ASICs, particularly in bone, is still unclear and no inherited human diseases caused by mutations in ASICs have yet been identified to elucidate this question. Although ASIC1a, ASIC2, and ASIC3 [32]

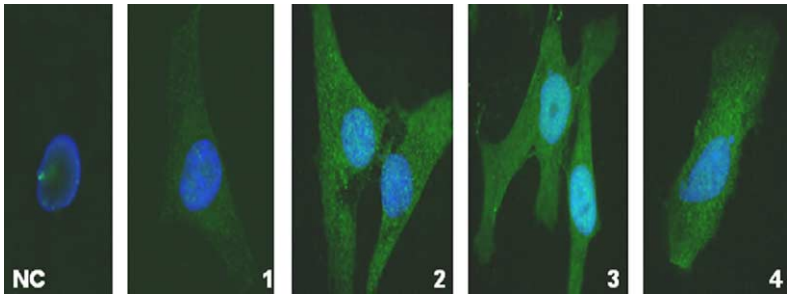

Fig. 6. Immunolocalization of ASIC expression in human pre-osteoblasts. Monolayer cultures were stained at day 14 against ASIC1 (1), ASIC2 (2), ASIC3 (3), and ASIC4 (4), and nuclei were counterstained with DAPI. On left: representative pre-immune serum negative control (NC); (650×).

as well as ASIC2/3 double knockout mice [33] have been generated, in general these mice show no obvious phenotype in unchallenged situation. Nevertheless, using ASIC1a knockout mice an important role in acid induced ischemic brain injury was recently demonstrated [34]. So far, no attention has been paid to the skeletal phenotype of these mice, especially not to ultrastructural changes at the bone level, neither in unchallenged nor challenged (e.g., ovariectomy) situations.

In conclusion, the present study demonstrates for the first time the presence a new family of pH sensors in skeletal tissues. The pH-sensing ASIC channels are present in both osteoblasts and osteoclasts, and may explain how bone cell function can be modulated by environmental pH under physiological (e.g., bone resorption) and pathological (e.g., metabolic acidosis) conditions.

Acknowledgments

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References

[1] J. Lemann Jr., D.A. Bushinsky, L.L. Hamm, Bone buffering of acid and base in humans, Am. J. Physiol. Renal. Physiol. 285 (2003) F811–F832.
[2] M.A. Bumke, D. Neri, G. Elia, Modulation of gene expression by extracellular pH variations in human fibroblasts: a transcriptomic and proteomic study, Proteomics 3 (2003) 675–688.
[3] N.S. Krieger, K.K. Frick, D.A. Bushinsky, Mechanism of acid-induced bone resorption, Curr. Opin. Nephrol. Hypertens. 13 (2004) 423–436.
[4] D.A. Bushinsky, K.K. Frick, The effects of acid on bone, Curr. Opin. Nephrol. Hypertens. 9 (2000) 369–379.
[5] T. Arnett, Regulation of bone cell function by acid-base balance, Proc. Nutr. Soc. 62 (2003) 511–520.
[6] S. Kellenberger, L. Schild, Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure, Physiol. Rev. 82 (2002) 735–767.
[7] O. Krishtal, The ASICs: signaling molecules? Modulators? Trends Neurosci. 26 (2003) 477–483.
[8] P. Escoubas, J.R. De Weille, A. Lecoq, S. Diochot, R. Waldmann, G. Champigny, D. Moinier, A. Menez, M. Lazdunski, Isolation of a tarantula toxin specific for a class of proton-gated Na+ channels, J. Biol. Chem. 275 (2000) 25116–25121.
[9] M.B. Goodman, G.G. Ernstrom, D.S. Chelur, R. O'Hagan, C.A. Yao, M. Chalfie, MEC-2 regulates C. elegans DEG/ENaC channels needed for mechanosensation, Nature 415 (2002) 1039–1042.
[10] U. Hoger, A.S. French, Extracellular acid increases the open probability of transduction channels in spider mechanoreceptors, Eur. J. Neurosci. 16 (2002) 2311–2316.
[11] J. Liu, B. Schrank, R.H. Waterston, Interaction between a putative mechanosensory membrane channel and a collagen, Science 273 (1996) 361–364.
[12] M. Eijken, M. Hewison, M.S. Cooper, F.H. de Jong, H. Chiba, P.M. Stewart, A.G. Uitterlinden, H.A. Pols, J.P. van Leeuwen, 11beta-Hydroxysteroid dehydrogenase expression and glucocorticoid synthesis are directed by a molecular switch during osteoblast differentiation, Mol. Endocrinol. 19 (2005) 621–631.
[13] E.W. Mandl, H. Jahr, J.L. Koevoet, J.P. van Leeuwen, H. Weinans, J.A. Verhaar, G.J. van Osch, Fibroblast growth factor-2 in serum-free medium is a potent mitogen and reduces dedifferentiation of human ear chondrocytes in monolayer culture, Matrix Biol. 23 (2004) 231–241.
[14] P.J. Koshy, C.J. Lundy, A.D. Rowan, S. Porter, D.R. Edwards, A. Hogan, I.M. Clark, T.E. Cawston, The modulation of matrix metalloproteinase and ADAM gene expression in human chondrocytes by interleukin-1 and oncostatin M: a time-course study using real-time quantitative reverse transcription-polymerase chain reaction, Arthritis Rheum. 46 (2002) 961–967.
[15] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[16] Y. Yiangou, R. Birch, L. Sangameswaran, R. Eglen, P. Anand, SNS/PN3 and SNS2/NaN sodium channel-like immunoreactivity in human adult and neonate injured sensory nerves, FEBS Lett. 467 (2000) 249–252.
[17] N.S. Krieger, N.E. Sessler, D.A. Bushinsky, Acidosis inhibits osteoblastic and stimulates osteoclastic activity in vitro, Am. J. Physiol. 262 (1992) F442–F448.
[18] T.A. Heming, D.M. Tuazon, S.K. Dave, A.K. Chopra, J.W. Peterson, A. Bidani, Post-transcriptional effects of extracellular pH on tumour necrosis factor-alpha production in RAW 246.7 and J774 A.1 cells, Clin. Sci. (Lond) 100 (2001) 259–266.
[19] N. Voilley, Acid-sensing ion channels (ASICs): new targets for the analgesic effects of non-steroid anti-inflammatory drugs (NSAIDs), Curr. Drug Targets Inflamm. Allergy 3 (2004) 71–79.
[20] M.A. Karsdal, K. Henriksen, M.G. Sorensen, J. Gram, S. Schaller, M.H. Dziegiel, A.M. Heegaard, P. Christophersen, T.J. Martin, C. Christiansen, J. Bollerslev, Acidification of the osteoclastic resorption compartment provides insight into the coupling of bone formation to bone resorption, Am. J. Pathol. 166 (2005) 467–476.
[21] J.A. Wemmie, M.W. Coryell, C.C. Askwith, E. Lamani, A.S. Leonard, C.D. Sigmund, M.J. Welsh, Overexpression of acid-sensing ion channel 1a in transgenic mice increases acquired fear-related behavior, Proc. Natl. Acad. Sci. USA 101 (2004) 3621–3626.
[22] A.S. Leonard, O. Yermolaieva, A. Hruska-Hageman, C.C. Askwith, M.P. Price, J.A. Wemmie, M.J. Welsh, cAMP-dependent protein kinase phosphorylation of the acid-sensing ion channel-1 regulates its binding to the protein interacting with C-kinase-1, Proc. Natl. Acad. Sci. USA 100 (2003) 2029–2034.
[23] F. Bassilana, G. Champigny, R. Waldmann, J.R. de Weille, C. Heurteaux, M. Lazdunski, The acid-sensitive ionic channel subunit ASIC and the mammalian degenerin MDEG form a heteromultimeric H+-gated Na+ channel with novel properties, J. Biol. Chem. 272 (1997) 28819–28822.
[24] Y. Yiangou, P. Facer, J.A. Smith, L. Sangameswaran, R. Eglen, R. Birch, C. Knowles, N. Williams, P. Anand, Increased acid-sensing ion channel ASIC-3 in inflamed human intestine, Eur. J. Gastroenterol. Hepatol. 13 (2001) 891–896.
[25] D. Alvarez de la Rosa, P. Zhang, D. Shao, F. White, C.M. Canessa, Functional implications of the localization and activity of acid-sensitive channels in rat peripheral nervous system, Proc. Natl. Acad. Sci. USA 99 (2002) 2326–2331.
[26] D. Alvarez de la Rosa, S.R. Krueger, A. Kolar, D. Shao, R.M. Fitzsimonds, C.M. Canessa, Distribution, subcellular localization and ontogeny of ASIC1 in the mammalian central nervous system, J. Physiol. 546 (2003) 77–87.
[27] M.S. Hildebrand, M.G. de Silva, T. Klockars, E. Rose, M. Price, R.J. Smith, W.T. McGuirt, H. Christopoulos, C. Petit, H.H. Dahl, Characterisation of DRASIC in the mouse inner ear, Hear Res. 190 (2004) 149–160.
[28] P. Otte, Basic cell metabolism of articular cartilage. Manometric studies, Z. Rheumatol. 50 (1991) 304–312.
[29] R.E. Marcus, The effect of low oxygen concentration on growth, glycolysis, and sulfate incorporation by articular chondrocytes in monolayer culture, Arthritis Rheum. 16 (1973) 646–656.
[30] J.A. Browning, R.J. Wilkins, Mechanisms contributing to intracellular pH homeostasis in an immortalised human chondrocyte cell line,

Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 137 (2004) 409–418.

[31] D.C. Immke, E.W. McCleskey, Lactate enhances the acid-sensing Na+ channel on ischemia-sensing neurons, Nat. Neurosci. 4 (2001) 869–870.

[32] M.P. Price, G.R. Lewin, S.L. McIlwrath, C. Cheng, J. Xie, P.A. Heppenstall, C.L. Stucky, A.G. Mannsfeldt, T.J. Brennan, H.A. Drummond, J. Qiao, C.J. Benson, D.E. Tarr, R.F. Hrstka, B. Yang, R.A. Williamson, M.J. Welsh, The mammalian sodium channel BNC1 is required for normal touch sensation, Nature 407 (2000) 1007–1011.

[33] L.J. Drew, D.K. Rohrer, M.P. Price, K.E. Blaver, D.A. Cockayne, P. Cesare, J.N. Wood, Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones, J. Physiol. 556 (2004) 691–710.

[34] Z.G. Xiong, X.M. Zhu, X.P. Chu, M. Minami, J. Hey, W.L. Wei, J.F. MacDonald, J.A. Wemmie, M.P. Price, M.J. Welsh, R.P. Simon, Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels, Cell 118 (2004) 687–698.