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Regulation and pharmacological blockade of sodium-potassium ATPase: A novel pathway to neuropathy ☆

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ABSTRACT

Inflammation causes upregulation of $Na_V1.7$ sodium channels in the associated dorsal root ganglia (DRG). The resultant increase in sodium influx must be countered to maintain osmotic homeostasis. The primary mechanism to pump sodium out of neurons is Na^+, K^+-ATPase. To test whether there is a compensatory upregulation of Na^+, K^+-ATPase after inflammation, rats received an injection of complete Freund's adjuvant (CFA) into one hindpaw and saline into the contralateral hindpaw. Three days later, L4–L6 DRGs were extracted and analyzed using gel electrophoresis and immunohistochemistry. Immunoreactivity for both the α-1 and α-3 subunits were increased in DRG associated with CFA-treatment, compared to saline-treatment. To test whether dysregulation of Na^+, K^+-ATPase may cause cell death after inflammation, we produced a pharmacological blockade with ouabain (10 mg/kg, s.c.) three days after CFA injection and paws were stimulated or not. Twenty-four hours later, DRG were removed and stained with cresyl violet. Greater cell death was seen in DRG from ouabain-treated animals on the CFA treated side than the saline-treated side. Paw stimulation doubled this difference. Control DRG showed little neuronal death. These results are evidence that regulation of Na^+, K^+-ATPase during major inflammatory disease states is critical for homeostatic protection of primary afferent neurons.

1. Introduction

To maintain an electrochemical gradient across the cell membrane or re-establish the gradient after an action potential, neurons and other excitable cells must move Na^+ ions out of the cell, so that the intracellular Na^+ concentration is less than the extracellular Na^+ concentration. This is the role of the Na^+, K^+-adenosinetriphosphatase (Na^+, K^+-ATPase); also known as the sodium pump. This enzyme is a ubiquitous integral protein of the outer plasma membrane of animal cells [1,2]. In addition to the maintenance of the resting membrane potential of most tissues, the electrochemical gradient produced by Na^+, K^+-ATPase plays a role in several cellular functions including maintenance of osmotic balance of the cell and generation of the Na^+ gradient which supplies the energy that fuels the Na^+-coupled transporter [3]. The functional macromolecule consists of two dimers composed of noncovalently-interacting α and β subunits, and a smaller γ subunit. To date, 4 α and 3 β subunit isomers have been identified [4]. Aortic smooth muscle cells, for example, express three alpha isoforms, α_1, α_2, and α_3 [5]. The α subunit is responsible for the catalytic activity whereas the β subunit appears to be involved in the insertion of the αβ complex into the membrane [6,7]. Each isoform has a unique tissue distribution and is encoded by a separate gene [8]. However, the exact functional role of each protein is unknown. A third subunit (γ) expressed in the kidney appears to modulate enzyme function, reducing the affinity of the αβ complex for Na^+ and K^+ ions and enhancing the affinity of the α-subunit for ATP [9,10]. In the DRG, approximately 80% of neurons are immunopositive for the α_1 isoform, whereas only 10–17% are immunopositive for the α_3 isoform [11].

The opening of voltage-gated sodium channels is responsible for the propagation of an action potential [12]. The $Na_V1.3$, $Na_V1.6$, $Na_V1.7$ $Na_V1.8$ and $Na_V1.9$ subtypes of voltage-gated sodium channels have

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been localized in primary afferent neurons [13,14]. Normally, neurons maintain a relatively constant overall concentration of intracellular sodium by balancing the activity of these sodium channels with the activity of Na^+, K^+-ATPase, thus contributing to the osmotic homeostasis of the cell.

We have shown that during inflammation of peripheral tissue there is an increase in $Na_v1.7$ voltage-gated sodium channels in the associated dorsal root ganglia (DRG) associated with the inflammation [15–18]. This upregulation of sodium channels has been seen in both small and large profile cells that innervate the area of inflammation [15,16,18].

Considering the profound degree of sodium channel upregulation that occurs during inflammation, it is likely that there would be an increase in intracellular sodium concentration in the cells of the DRG after neuronal firing. Without a complementary increase in a sodium pumping mechanism, an increase in sodium influx would lead to an osmotic influx of water. Consequently, the neurons would swell and, possibly, burst. However, given no abnormal circumstances other than inflammation, we have seen no swelling or cell death. Accordingly, we hypothesized that there must be an increase in one of the mechanisms that remove sodium from the neuron in order to counteract the increase in sodium influx. Because of the primacy of Na^+, K^+-ATPase in the maintenance of the voltage gradient across the membrane, we tested the hypotheses that this pump would be upregulated after inflammation, and that its pharmacological blockade would result in neuronal death.

2. Materials and methods

2.1. Subjects

All protocols were approved by the LSU Health Sciences Center Institutional Animal Care and Use Committee, and adhere to the National Research Council's guide for the care and use of laboratory animal. Male Sprague–Dawley rats, 250–350 g were housed 2 to a cage on a 12 h/12 h light/dark cycle. The colony room was temperature and humidity controlled. One hindpaw of each animal was injected with 0.1 ml of CFA (*Mycobacterium tuberculosis*; Sigma, St. Louis, MO). The hindpaw contralateral to the CFA-injected paw was injected with an equal volume of sterile normal saline to provide an internal control.

2.2. Drugs and antibodies

Ouabain was purchased from Sigma and was diluted in saline to a concentration of 10 mg/ml. Ouabain or saline was injected s.c. at a dose of 10 mg/kg three days after CFA treatment. Antibodies for the α_1 and α_3 Na^+, K^+-ATPase isozymes were purchased from Upstate (Charlottesville, VA).

2.3. Immunohistochemistry and histology

Groups of rats that had been injected with CFA were given a lethal dose of pentobarbital (200 mg/kg) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.6 at 23 °C. The tissue was allowed to fix for 1 h. The DRG from vertebral levels L4–S1 were dissected from each animal and cut into 10–30 μm-thick sections using a cryostat. All sections were collected and placed onto gelatin-coated slides. The sections were rinsed in 0.1 M PBS and then immersed in 0.1 M PBS/0.3% Triton X-10 (Sigma, St. Louis, MO)/10% normal goat serum for a minimum of 1 h. The tissue on individual slides were then incubated overnight at room temperature with 1 of 3 site-specific, anti-sodium channel antibodies for α_1, α_3, and α_2 (negative control). As a control for nonspecific fluorescence, each anti-Na^+, K^+-ATPase antibody was pre-blocked with a 500-molar excess of the peptide antigen to which it was raised. After rinsing in 0.1 M PBS, the tissue was incubated for at least 1 h at room temperature with lissamine rhodamine or AlexaFluoro-596-labeled goat anti-rabbit immunoglobulin G. The slides were then rinsed in 0.1 M PBS, dried, coverslipped, and viewed with a confocal microscope. In each of the DRG from CFA-treated animals, there was an area of neuronal profiles with Na^+, K^+-ATPase-like staining greater than the surrounding cells. This was never seen in control DRG and was presumed to be associated with the area of inflammation. Cresyl violet staining was done by the Histology Core Laboratory of the LSU School of Dentistry.

2.3.1. Western blot analysis of Na^+, K^+-ATPase α subunits

2.3.1.1. Preparation of samples for Western blot analysis. The DRG from inflamed and control sides of the same rat were collected, immediately frozen in liquid nitrogen and stored at −80 °C until the day of the experiment (1–5 days). Each DRG was homogenized in a buffer containing NaCl 140 mmol/L, Tris 10 mmol/L, $MgCl_2$ 1.5 mmol/L and protease inhibitors leupeptin, benzamidine, PMSF, pH 7.6. This crude homogenate was used for electrophoresis/Western-blot analysis. Protein was estimated by the method of Lowry et al. [19] using bovine serum albumin as a standard. The homogenates were diluted to the final concentration of approximately 2–5 mg/ml.

2.3.1.2. Gel electrophoresis and immunoblotting. 5–15 μg protein samples from each of eleven rats were loaded for alpha-1 and twelve rats for alpha-3 isoforms to the gel for electrophoresis. In preliminary experiments 12 μg of protein resulted in the best visualization of the bands. The homogenates from the control and treatment groups and prestained molecular weight standards (Bio-Rad) were subjected to polyacrylamide gel electrophoresis on 10% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate [20] and then transferred to polyvinylidene fluoride (PDVF) membrane by electroblotting [21]. After an incubation in Tris-buffered saline (in mmol/L: Tris·HCl-20, NaCl-137, pH 7.5) containing 5% (w/v) nonfat dried milk (Carnation) and 1.0% (v/v) Tween 20 for 1 h at room temperature, the blots were probed with antibodies directed against the alpha-1, and −3 subunits of Na^+, K^+-ATPase (Affinity Bioreagents, Cat#MA3-929, and MA3-915, respectively). The blots were then treated with the secondary antibody, horseradish peroxidase-labeled sheep anti-mouse Ig (Amersham). Blots were treated with Enhanced Chemilluminescence (ECL, Amersham) reagent and exposed to X-ray film for the visualization of the bands, or quantitated using a Bio-Rad gel documentation system.

2.3.1.3. Quantitation. The autoradiograms were digitized and the intensity of the bands for alpha-1 and alpha-3 were quantified as optical density units using NIH-Scion software. To normalize band density values from different blots, a known amount of internal control sample (rat kidney homogenate for α-1 and brain homogenate for α-3) were loaded to each gel and the density designated as 1 [22]. Optical density was then compared to this unit. Comparison of immunohistochemistry quantitation was compared by one-way analysis of variance. Comparisons of Western blot quantitation were compared by student's t-tests.

2.3.1.4. Thermal and mechanical stimulation. To ensure the firing of sciatic nerves in the ouabain experiment, rat hindpaws were stimulated with a focused light beam aimed at the plantar surface of the paw using a Hargreaves apparatus (IITC, Woodland Hills, CA) 1 h after drug or vehicle treatment. Each rat received four successive trials, followed by four applications of a 6.45 ga Semmes Weinstein filament. This procedure was repeated 1 and 2 h later.

3. Results

3.1. Immunohistochemistry

Compared to contralateral controls (Fig. 1A), the levels of Na^+, K^+-ATPase immunoreactivity in inflammation-associated DRG were high (Fig. 1B). Moreover, the upregulation appeared as areas of high

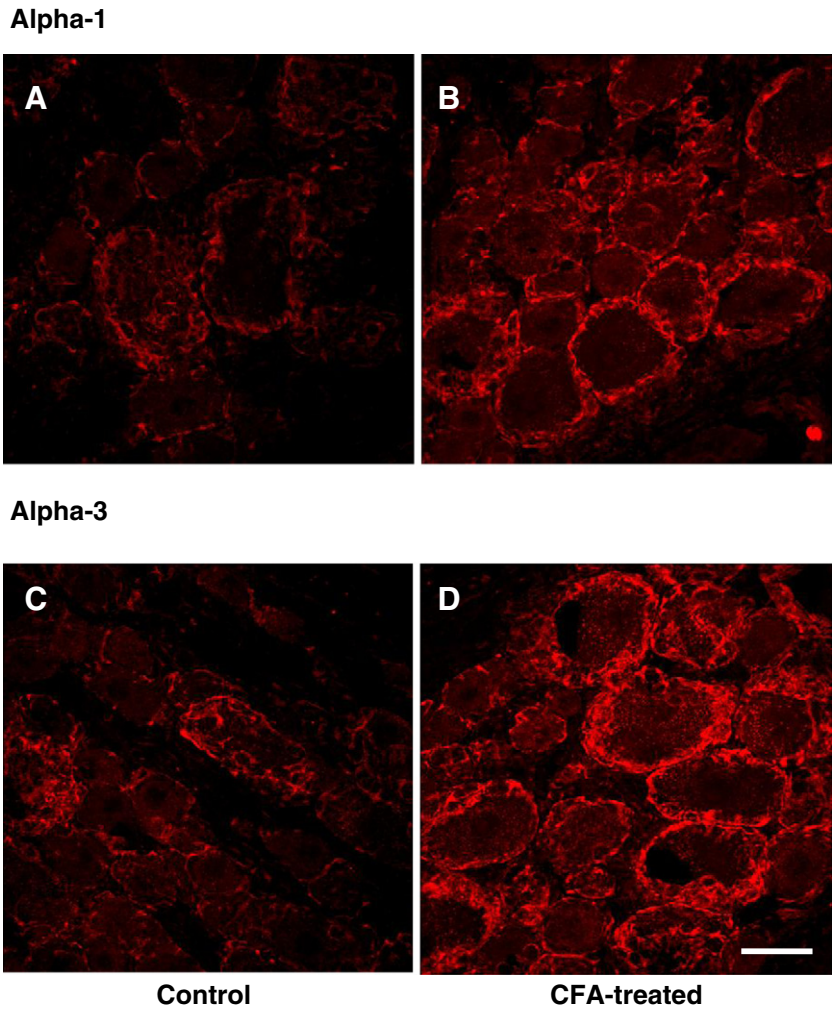

Fig. 1. Upregulation of alpha-1 and alpha-3 Na^+, K^+-ATPase subunit immunohistofluorescence following CFA injection. Groups of rats (n = 4) were injected with CFA into one hindpaw, and saline into the contralateral paw. Three days later, the rats were sacrificed, and the DRGs were removed, sectioned and labeled with antibodies for alpha-1 or alpha-3 Na^+, K^+-ATPase subunits. A: control alpha-1. B: CFA-treated alpha-1. C: control alpha-3. D: CFA-treated alpha-3. Calibration bar = 50 μm.

concentration, rather than uniformly over the somatic surface. This is reminiscent of the patches of sodium channels reported to drive somatic action potentials. Thus, there was an upregulation of Na^+, K^+-ATPase after CFA-induced inflammation.

3.2. Western blot analysis

Groups of twelve rats received an injection of CFA into the right hindpaw. Three days later, the rats were injected with a lethal dose of

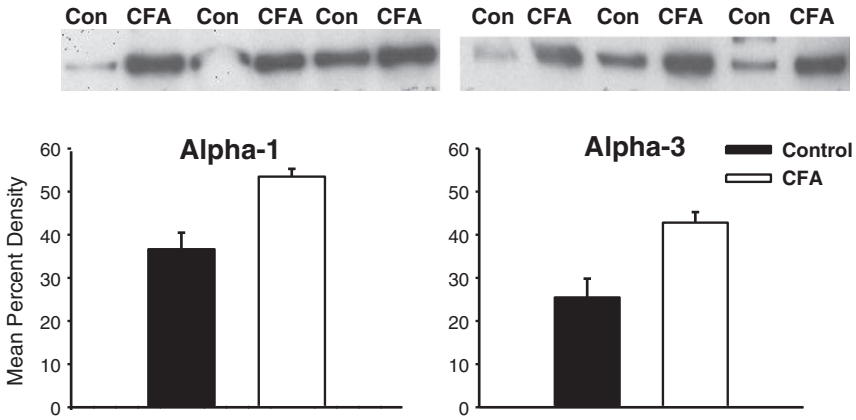

Fig. 2. Upregulation of alpha-1 and alpha-3 Na^+, K^+-ATPase subunits following CFA injection. Above the graphs, Western blots for DRG homogenates corresponding to inflamed (CFA) and control (Con) rat paws are depicted. Twelve microgram homogenates were applied to the gel. Affinity Bioreagents antibodies were used to target the alpha-1 subunit protein (left) and alpha-3 subunit protein (right). The graphs illustrate the mean percent density for samples from 11 (alpha-3) or 12 (alpha-1) animals. Differences for both comparisons: $p < .001$.

pentobarbital (200 mg/kg). The DRG from vertebral levels L4–S1 and portions of the sciatic and medial plantar nerves ipsilateral and contralateral to the CFA injection site were rapidly dissected from each animal and frozen on dry ice. To determine whether paw inflammation induces an increase in Na^+, K^+-ATPase isoform expression in DRG, we performed Western blot analysis on DRG homogenates. The DRG from the same rat corresponding to the paw that received saline injection was used as the control. After separation of proteins using standard gel chromatography, the proteins were transferred to PDVF membranes and then incubated in the primary antibody, and then in the secondary antibody linked to horseradish peroxidase. These blots were imaged on X-ray film, digitized and analyzed using NIH-Scion software. Fig. 2 depicts representative Western blots for each alpha isoform and Fig. 2 also graphically illustrates the percent increase of each of the alpha subunits. Both alpha isoforms were upregulated in DRG of CFA-treated paws, compared to control paws. Lowry analysis found no difference between protein content of samples from control and treated DRG.

3.2.1. Effect of ouabain on DRG cells after inflammation

Groups of rats (n = 3–4) received an injection of CFA into one hindpaw and saline into the other. Three days later, the rats were injected with 10 mg/kg s.c. ouabain or 1 ml/kg saline. Hindpaws were stimulated one, two and three hours later, or left in the testing apparatus without stimulation for a comparable period of time. The animals were sacrificed with pentobarbital, and perfused and the DRGs were extracted 24 h after ouabain injection. DRGs were sectioned and stained with cresyl violet. Sections from each DRG were counted for the percentage of dead cells. A cell was considered to be dead if it had no defined nucleus or nucleolus, and the Nissl substance was diffusely distributed in the cytoplasm. Rats that received saline after either CFA or saline treatment showed little or no sign of cell death (Fig. 3A–D; Table 1). This was true whether or not the paws were stimulated. Likewise, DRG from rats treated with ouabain after saline paw treatment that were not stimulated showed no sign of cell death (Fig. 3E; Table 1). However, DRG from rats treated with ouabain following CFA paw injection, and either stimulated or not, showed significant cell death (Fig. 3F and H; Table 1).

4. Discussion

We have previously demonstrated a robust upregulation of $Na_V1.7$ sodium channels in the DRG associated with CFA-induced inflammation [15–17]. In this series of experiments, the two isoforms of Na^+, K^+-

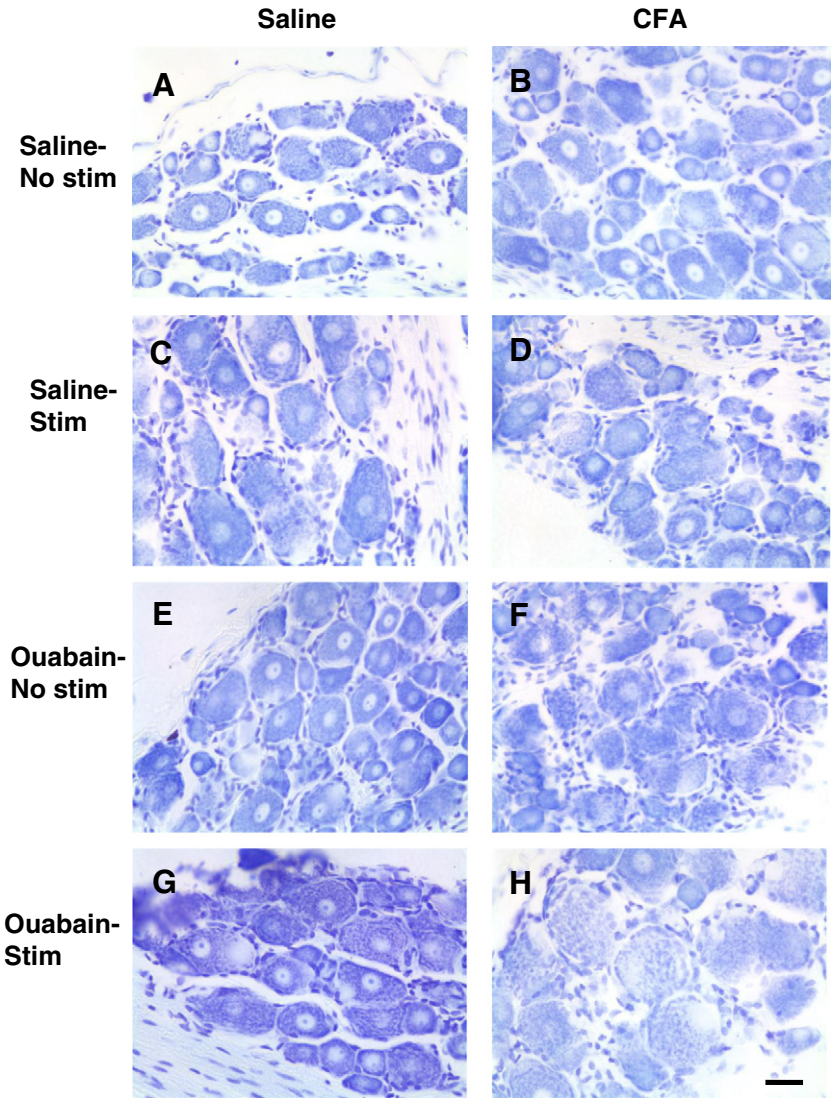

Fig. 3. Effect of ouabain on DRG cell death after CFA-induced inflammation. Hindpaws of rats (n = 3) were injected with either saline or CFA. Three days later, the rats were injected with saline or ouabain, then their paws were stimulated with the Hargreaves apparatus and the Semmes–Weinstein filament or the rat was handled with no paw stimulation. DRGs were removed 24 h later, sectioned, and stained with cresyl violet. Neuronal death was evaluated by two independent observers. Calibration bar = 50 μm.

Table 1
Cell survival after inflammation and oubain treatment. Cell survival counts represent means from at least 3 rats and 3 fields of approximately 30 cells ea.

	Percent Cells dead	
Treatment	Control	CFA-treated
Saline — no stimulation	<1	<1
Saline — stimulation	<1	<1
Ouabain — no stimulation	3 ± 1.1	42 ± 10.4*
Ouabain — stimulation	5 ± 1.2	78 ± 6.7*

* $p < .05$.

ATPase in the primary afferent neurons were upregulated following CFA-induced inflammation. It is not yet clear whether the upregulation of Na^+, K^+-ATPase is in response to the upregulation of sodium channels and consequent increase in intracellular sodium, or, alternatively, the two events are initiated by a common cellular signal. We currently favor the latter interpretation because the upregulation of both channels and pumps occur simultaneously, between 23 and 24 h after the initial inflammatory insult (data not shown).

Blockade of the sodium pumps with ouabain caused cell death in DRG associated with the CFA-induced inflammation. Ouabain treatment in rats with no inflammation had little effect on DRG cell survival. Physiological stimulation of the neurons potentiated this effect. These results are consistent with the theory that after induction of inflammation, upregulated $Na_V1.7$ channels cause greater sodium influx (both activity-dependent and leak current), and that upregulation of a sodium pumping mechanism is necessary to compensate. When sodium pumps are blocked in control DRG in the absence of inflammation, other sodium pumping mechanisms are sufficient to maintain sodium balance. However, after induction of inflammation, these alternative pumping mechanisms cannot compensate for the increased sodium influx. This should cause water to flow with the osmotic gradient, resulting in neuronal swelling and lysis.

Recent reports are indicative of the increase an intracellular sodium also affecting mitochondrial functioning [23,24] and causing the reversal in the of the Na^+/Ca^{++} exchanger [25,26]. This would result in an increase in intracellular Ca^{++} and consequent disruption of neural tubules. Disruption of mitochondrial functioning leading to impairment of Na^+, K^+-ATPase activity may contribute to neuronal injury.

These results have immediate implications for patients taking cardiac glycosides for the treatment of congestive heart failure. It would follow that these patients would be more likely to convert pain from major inflammatory diseases, e.g., rheumatoid arthritis, and inflammatory bowel disease, to neuropathic pain. In addition, disease states that interfere with sodium pump expression or function, such as diabetes [27,28], may share this risk. Sodium channel blockers, such as carbamazepine may be protective in these patients [24,29].

Conflict of Interest Statement

There are no conflicts of interest for any of the authors.

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References

[1] Lingrel JB. Na, K-ATPase: isoform structure, function, and expression. J Bioenerg Biomembr 1992;24:263–70.
[2] Reinhard L, Tidow H, Clausen MJ, Nissen P. Na+, K+-ATPase as a docking station: protein–protein complexes of the Na+, K+-ATPase. Cell Mol Life Sci 2013;70:205–22.
[3] Flemming WW. The electrogenic Na+, K+-pump in smooth muscle: physiologic and pharmacologic significance. Annu Rev Pharmacol Toxicol 1980;20:129–49.
[4] Blanco G, Mercer RW. Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity, in function. Am J Physiol 1998;275:F633–50.
[5] Sahin-Erdemli I, Rashed SM, Songu-Mize E. Rat vascular tissues express all three alpha-isoforms of Na+, K+-ATPase. Am J Physiol 1994;266:H350–3.
[6] Geering K, Theulaz I, Verrey F, Häuptle MT, Rossier BC. A role for the beta-subunit in the expression of functional Na+, K+-ATPase in *Xenopus* oocytes. Am J Physiol 1989;257:C851–8.
[7] Fink DJ, Fang DN, Li T, Mata M. Na, K-ATPase β subunit isoform experession in the peripheral nervous system of the rat. Neurosci Lett 1995;183:206–9.
[8] Van Huysse JW, Jewell EA, Lingrel JB. Site-directed mutagenesis of a predicted cation binding site of Na, K-ATPase. Biochemistry 1993;832:819–26.
[9] Arystarkhova E, Wetzel RK, Asinovski NK, Sweadner KJ. The gamma subunit modulates Na+ and K+ affinity of the renal Na, K-ATPase. J Biol Chem 1999;274:33183–5.
[10] Therien AG, Karlish SJ, Blostein R. Expression and functional role of the gamma subunit of the Na, K-ATPase in mammalian cells. J Biol Chem 1999;274:12252–6.
[11] Dobretsov M, Hastings SL, Stimers JR. Functional Na^+/K^+ pump in rat dorsal root ganglia neurons. Neuroscience 1999;93:723–9.
[12] Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 1952;117:500–44.
[13] Novakovic SD, Eglen RM, Hunter JC. Regulation of Na+ channel distribution in the nervous system. Trends Neurosci 2001;24:473–8.
[14] Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. Annu Rev Neurosci 2010;33:325–47.
[15] Gould III HJ, England JD, Liu ZP, Levinson SR. Rapid sodium channel augmentation in response to inflammation induced by complete Freund's adjuvant. Brain Res 1998;802:69–74.
[16] Gould III HJ, Gould TN, Paul D, England JD, Liu ZP, Reeb SC, et al. Development of inflammatory hyper-sensitivity and the augmentation of sodium channels in rat dorsal root ganglia. Brain Res 1999;824:296–9.
[17] Gould III HJ, England JD, Paul D. The modulation of sodium channels during inflammation. In: Krames E, Reig E, editors. The management of acute and chronic pain: the use of the "Tools of the Trade", Proceedings of the Worldwide Pain Conference, San Francisco, USA, Monduzzi Editore, Balogna, Italy; 2000. p. 27–34.
[18] Gould III HJ, Gould TN, England JD, Paul D, Liu ZP, Levinson SR. A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain. Brain Res 2000;854:19–29.
[19] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
[20] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–3.
[21] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350–4.
[22] Liu X, Songu-Mize E. Effect of Na+ on Na+, K+-ATPase alpha-subunit expression and Na+-pump activity in aortic smooth muscle cells. Eur J Pharmacol 1998;351:113–9.
[23] Persson A-K, Kim I, Zhao P, Estacion M, Black JA, Waxman SG. Sodium channels contribute to degeneration of dorsal root ganglion neuritis induced by mitochondrial dysfunction in an in vitro model of axonal injury. J Neurosci 2013;33:19250–61.
[24] Persson A-K, Liu S, Faber CG, Merkies ISJ, Black JA, Waxman SG. Neuropathy-associated $Na_V1.7$ variant I228M impairs integrity of dorsal root ganglion neuron axons. Ann Neurol 2013;73:140–5.
[25] Stys PK, Waxman SG, Ransom BR. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and $Na(+)$–Ca^{2+} exchanger. J Neurosci 1992;76:430–9.
[26] Lehning EJ, Doshi R, Isaksson N, Stys PK, LoPachin Jr RM. Mechanisms of injury-induced calcium entry into peripheral nerve myelinated axons: role of reverse sodium–calcium exchange. J Neurochem 1996;66:493–500.
[27] Casey GP, Paul D, Gould III HJ. Insulin is essential for the recovery from allodynia induced by complete Freund's adjuvant. Pain Med 2010;11:1401–10.
[28] Gould III HJ, Casey GP, Paul D. Painful diabetic neuropathy: current perspective on development and management from bench to bedside — a review. In: Gotsirize-Columbus N, editor. Analgesics. Hauppauge, NY: New Research Nova; 2012. p. 39–74.
[29] Fern R, Ransom BR, Stys PK, Waxman SG. Pharmacological protection of CNS white matter during anoxia: actions of phenytoin, carbamazepine and diazepam. J Pharmacol Exp Ther 1993;266:1549–55.