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Single-Channel Properties and Pharmacological Characteristics of K_{ATP} Channels in Primary Afferent Neurons

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1. Introduction

ATP-sensitive potassium (K_{ATP}) channels first discovered in cardiac myocytes in 1983 (Noma, 1983), and then found in many other metabolically active tissues, including central nervous systems (Babenko et al., 1998). K_{ATP} channels are inhibited by physiological concentration of intracellular ATP, and are activated when the intracellular ADP/ATP ratio increases secondary to hypoxia, ischemia, or metabolic stress (Babenko et al., 1998; Miki & Seino, 2005; Nichols, 2006). The activation of K_{ATP} channels results in an enhanced outward repolarizing flow of K^+ and cell membrane hyperpolarization, and thus they regulate cell excitability and mediate cellular responses that determine cell survival during metabolic stress (Miki & Seino, 2005).

Recent electrophysiological and molecular genetic studies of K_{ATP} channels have provided insights into their physiological and pathophysiological roles, such as insulin secretion (Seghers et al., 2000; Miki et al., 1999), cardioprotection (Saito et al., 2005; Suzuki et al., 2001), vasodilatation (Chutkow et al., 2002; Miki et al., 2002), and neuroprotection (Sun et al., 2006; Yamada et al., 2001). Indeed, K_{ATP} channels are the target for a number of pharmacological agents, including inhibitors such as the sulfonylureas and a structurally unrelated group of K^+ channel openers (Babenko et al., 2000; Nichols, 2006).

In central nervous systems, K_{ATP} channels are predominantly expressed in basal ganglia, thalamus, hippocampus, and cerebral cortex (Mourre et al., 1989). Although detailed functional roles of K_{ATP} channel in central nervous systems still remain to be clarified, their activation results in K^+ efflux, leading to membrane hyperpolarization, decreased excitability, attenuation of transmitter release, and protection from cell death (Yamada et al., 2001).

In addition to central nervous systems, we have recently reported that functional K_{ATP} channels are expressed in rat sensory neurons (Kawano et al., 2009a, 2009b, 2009c). The primary sensory neuron is known to be an important site of pathogenesis for neuropathic pain, which is a common clinical condition that is difficult to treat by current methods (Amir et al., 2005; Gold, 2000; Zimmermann, 2001). Since our observations also reveal that currents through K_{ATP} channels are significantly decreased by painful nerve injury (Sarantopoulos et al., 2003; Kawano et al., 2009a, 2009b, 2009c, Zoga et al., 2010), identification of their roles in

sensory neurons, particularly regarding excitability, may reveal a contribution to the genesis of neuropathic pain. These researches may lead to development of novel therapies for neuropathic pain. Thus, the overall objective of this chapter is to discuss the characteristics and role of K_{ATP} channels in rat sensory neurons.

2. Tissue-specific molecular structure of K_{ATP} channel

K_{ATP} channels are a widely distributed family of potassium-selective ion channels, whose structure consists of an inwardly rectifying, pore-forming, K^+ channel (Kir6.x) subunit, each coupled to a regulatory sulfonylurea receptor (SUR) subunit (Babenko et al., 1998; Miki et al., 1999; Yokoshiki et al., 1998). The Kir 6.x subunits belong to the Kir family, and determine the inward rectification, ATP-sensitivity, and unitary single-channel conductance of K_{ATP} channel (Babenko et al., 1998). On the other hand, SUR subunits belong to the ATP-binding cassette superfamily, and confer responsiveness to K_{ATP} channel openers and sulfonylureas (Miki et al., 1999). The functional K_{ATP} channel is assembled as an octamer with a 4:4 stoichiometry of Kir6.x and SUR subunit (Fig. 1 and 2).

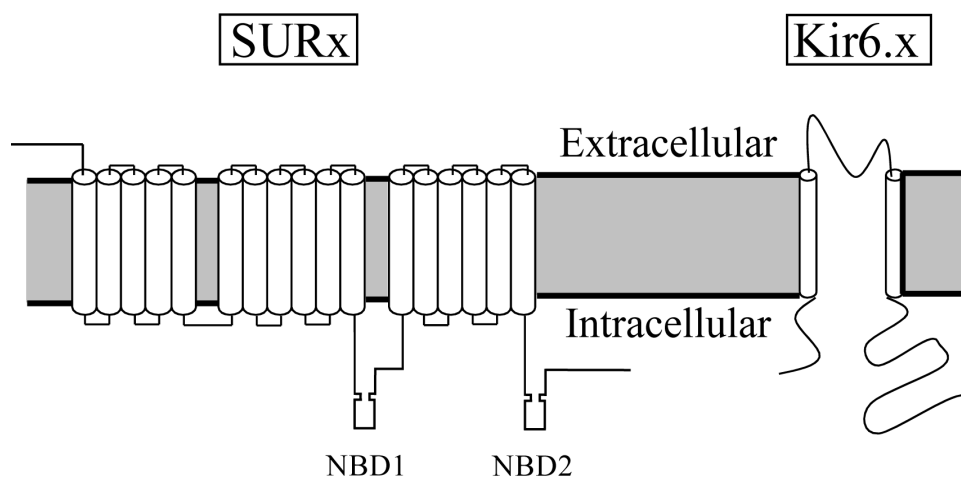


Fig. 1. Molecular structure of the K_{ATP} channel. Schematic representation of the transmembrane topology of a single sulfonylurea receptor (SUR) or inwardly rectifying K^+ channel (Kir6.x) subunit. SUR is an ABC protein bearing transmembrane domains and two nucleotide-binding domains (NBD1 and NBD2). The Kir6.x subunits presumably form the pore of the channel and determine the sensitivity of the channel to inhibition by ATP (Nichols, 2006).

Native K_{ATP} channels in different tissues show distinct single channel properties, modulating potency of nucleotides, and varying sensitivity to drugs that act as channel openers (such as diazoxide, pinacidil, nicorandil, etc) or blockers (sulfonylureas, like glibenclamide, tolbutamide, etc). These tissue-specific biophysical and pharmacological properties of K_{ATP} channels (summarized in Table 1) are thought to be endowed by their different molecular composition of Kir6.0 and SUR subunits (Yokoshiki et al., 1998). For instance, affinity for sulfonylureas is high for SUR1 but low for SUR2A and SUR2B subunits. Similarly, there are differences in response to openers, with the SUR1 and SUR2B channels responding more potently to diazoxide in contrast to the response of SUR2A channels.

Hetero-octamer comprising two subunits (4:4 SURx/Kir6.x)

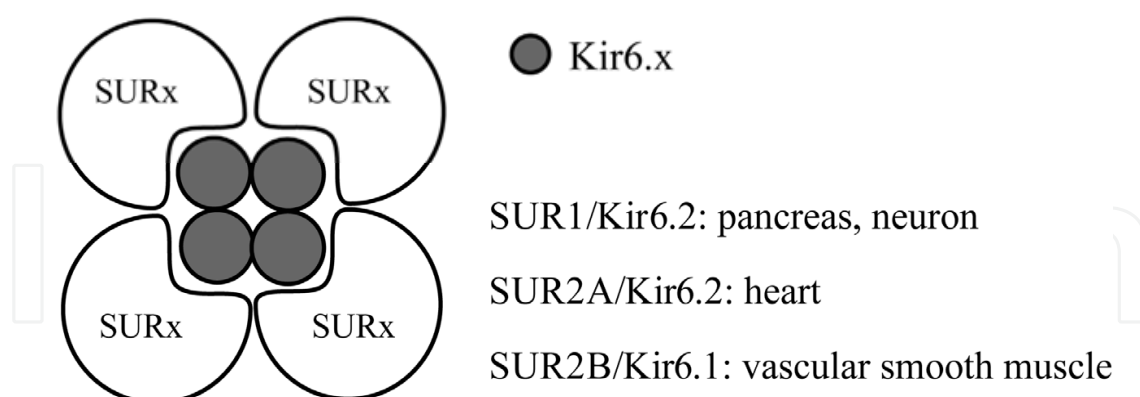


Fig. 2. Schematic representation of the octameric K_{ATP} channel complex viewed in cross section. Four Kir6.x subunits come together to form the K^+ channel pore, and each is associated with a regulatory SURx subunit. Several subtypes have been identified based on subunit combinations; co-expressing SUR1 and Kir6.2 forms the pancreatic β -cell and neuronal type K_{ATP} channel, SUR2A and Kir6.2 form the cardiac type K_{ATP} channel, and SUR2B and Kir6.1 form the vascular smooth muscle type K_{ATP} channel (Babenko et al., 1998; Miki et al., 1999; Yokoshiki et al., 1998).

	Pancreas (SUR1/Kir6.2)	Heart (SUR2A/Kir6.2)	VSM (SUR2B/Kir6.2)	Neuron (SUR1-2/Kir6.2)
Metabolic activity				
[ATP] _i	(---)	(---)	(- ~ 0)	(---)
[ADP] _i	(+)	(+)	(+)	(+)
[Acidosis] _i	(++)	(++)	(+++)	(++)
Channel openers				
Diazoxide	(+++)	(0)	(+++)	(+++)
Pinacidil	(+)	(+++)	(+++)	(+)
Nicorandil	(0)	(+)	(+++)	(+)
Sulfonylurea				
Glibenclamide	(---)	(--)	(--)	(---)
Tolbutamide	(---)	(- ~ 0)	(- ~ 0)	(---)

VSM: vascular smooth muscle.

[ATP]_i: Intracellular ATP, [ADP]_i: Intracellular ADP, [Acidosis]_i: Intracellular acidosis

(+): activation, (0): no effect, (-): inhibition.

Table 1. Tissue-specific properties of K_{ATP} channels.

3. Biophysical properties of K_{ATP} channel in primary afferent neurons

3.1 Distribution of the K_{ATP} channel subunits in primary afferent neurons

Western blot analysis and immunostaining show that peripheral sensory neurons express SUR1, SUR2 and Kir6.2 protein, but not Kir6.1 protein (Kawano et al., 2009b, 2009c). Co-

localization of Kir6.2 with SUR1 subunits is also demonstrated by staining with antibody against Kir6.2 and BODIPY-Glibenclamide, which specifically binds to SUR1 with high affinity (Zoga et al., 2010). These findings indicate that K_{ATP} channels in peripheral sensory neuron are composed of SUR1/Kir6.2 (pancreas/neuronal type) or SUR2/Kir6.2 (cardiac type) subunits.

In addition to neuronal somata, image analysis results of immunostaining show that K_{ATP} channels are present in glial satellite and Schwann cells (Zoga et al., 2010), which are known to express K^+ currents (Chiu et al., 1984). K_{ATP} channels in these sites are thought to convey glial cell-mediated clearance of extracellular K^+ , often termed " K^+ spatial buffering" (Kofuji et al., 2002).

3.2 Single-channel characteristics of K_{ATP} channel in primary afferent neurons

3.2.1 Cell isolation and plating

The L5 and L4 dorsal root ganglia (DRG) was harvested after normal adult Sprague-Dawley rats were decapitated under isoflurane anesthesia. DRG neurons were enzymatically dissociated in a solution containing 0.25 ml Liberase Blendzyme 2 (0.05%) and 0.25 ml Dulbecco's modified Eagle's medium (DMEM) for 30 min at 37 °C.

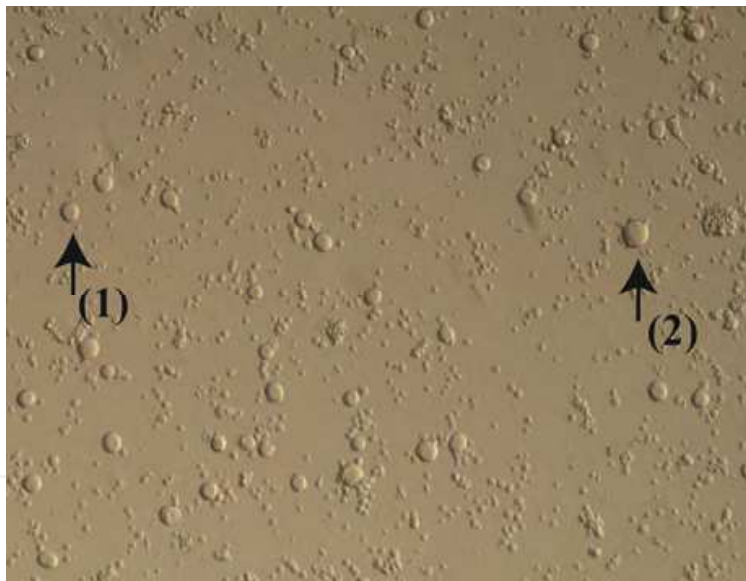


Fig. 3. Isolated DRG neurons. Neurons were viewed using Hoffman modulation optic systems under an inverted microscope (Nikon Diaphot 300). DRG neuronal somata observed after isolation varied in size about 15-50 μm in diameter, and were stratified by diameter into either large ($\geq 40 \mu\text{m}$) or small ($<30 \mu\text{m}$) neurons. These sizes correlates roughly with electrophysiological characteristics corresponding to either $A\beta$ or C fibers, respectively (Lawson, 2002; Harper & Lawson, 1985). Arrowhead (1) and (2) point to small (24 μm in diameter) and large (43 μm in diameter) neuron, respectively.

After centrifugation and removal of the supernatant, a second incubation at 37 °C followed for another 30 min in 0.2 ml trypsin (0.0625%) and deoxyribonuclease 1 (0.0125%) in 0.25 ml DMEM. Cells were then isolated by centrifugation (600 rpm for 5 min) after adding 0.25 ml

trypsin inhibitor (0.1%), and re-suspended in a medium consisting of 0.5 mM glutamine, 0.02 mg/ml gentamicin, 100 ng/ml nerve growth factor 7 S, 2% B27 supplement, and 98% neural basal medium A. Neurons were plated onto poly-L-lysine-coated 12-mm glass coverslips, and kept in a humidified incubator at 37 °C with 95% air and 5% CO₂. Patch-clamp experiments were performed within 3–8 h after the cell dissociation (Fig. 3).

3.2.2 Single DRG K_{ATP} channel currents from cell-attached patches

Biophysical and pharmacological characteristics of DRG K_{ATP} channels were first studied in cell-attached recordings at a sampling frequency of 5 kHz with 1 kHz low-pass filter. Cell-attached patch configuration is a non-invasive approach which is used to describe the endogenous properties of ion channels without disturbance of the intracellular milieu (Fig 4).

Both bath and pipette (extracellular) solutions were composed of the following (in mM): 140 KCl, 10 HEPES, 10 D-glucose, and 0.5 EGTA. The pH of all solutions was adjusted to 7.4 with KOH. Patch micropipettes were made from borosilicate glass capillaries using a Flaming/Brown micropipette puller, model P-97 (Sutter, San Rafael, CA) and flame polished with a microforge polisher (Narishige, Tokyo, Japan) prior to use. Their resistance ranged between 3 and 6 M Ω when filled with the internal solution, and placed into the recording solutions.

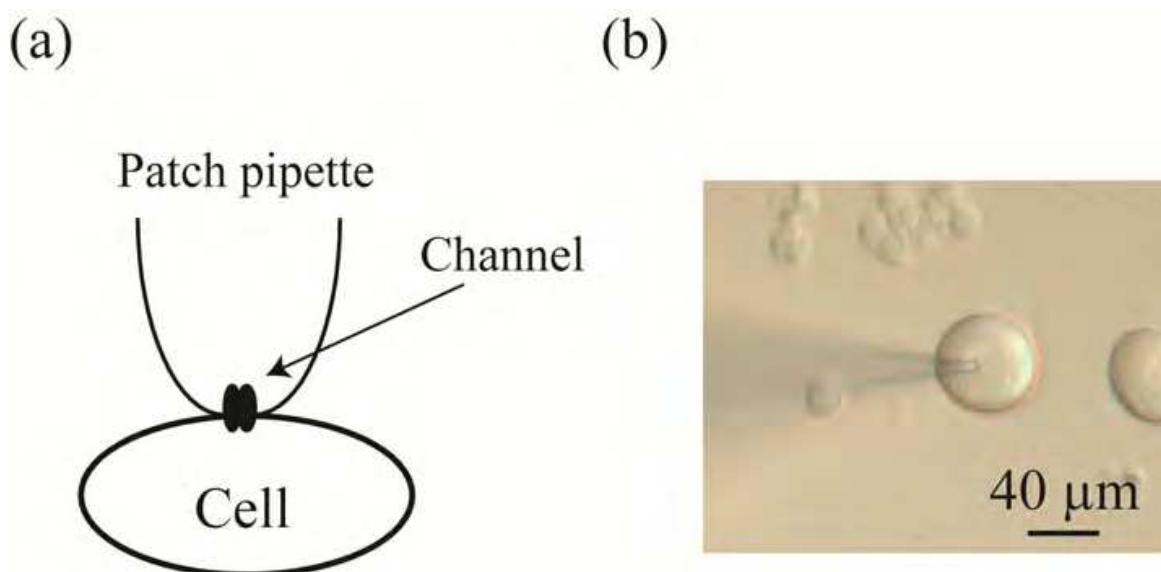


Fig. 4. Cell-attached patch clamp configuration. (a) Diagram illustrating the methods of making cell-attached patches. (b) Cell-attached pipette on a large DRG somata.

In cell-attached patches (Fig 5), infrequent but significant spontaneous channel activity was recorded. These basal channel activities were observed in cell-size independent manner. However, bath application of the uncoupler of mitochondrial ATP synthesis, 2,4-Dinitrophenol (DNP, 100 μM), gradually activated these baseline currents.

Subsequent bath application of glibenclamide 1 μM, a specific K_{ATP} channel inhibitor, completely blocked DNP-induced currents in both groups, indicating that these currents are conveyed via K_{ATP} channels.

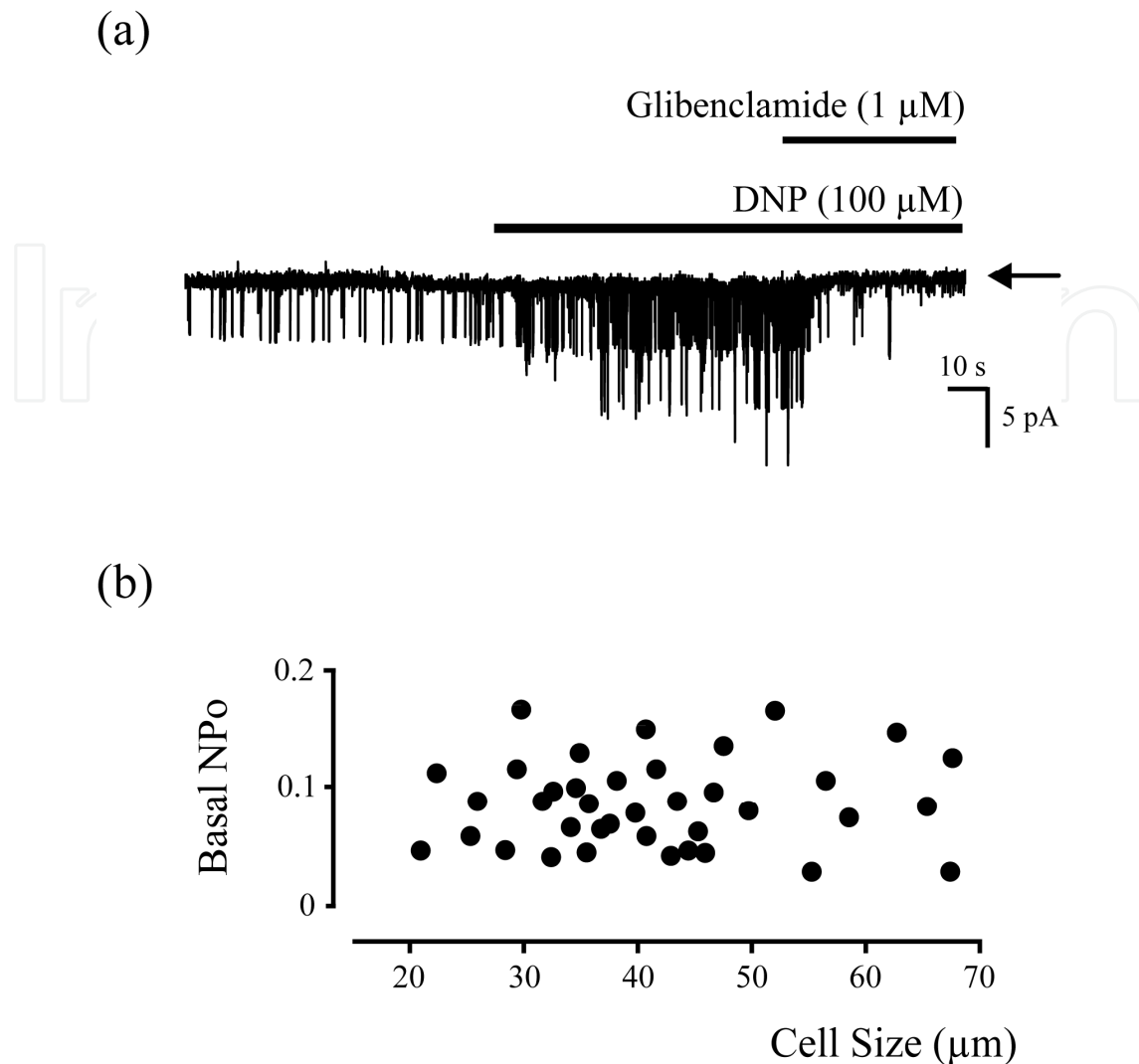


Fig. 5. Single-channel characteristics of K_{ATP} channel in DRG neurons from cell-attached patch clamp configuration. (a) Representative current trace of K_{ATP} channels in isolated DRG neurons recorded in cell-attached configuration at a holding potential of -60 mV. These patches typically showed one predominant channel-type at a holding potential of -60 mV, whereas a second channel-type was observed infrequently in 5-10% of all patches. Arrows indicate closed channel state. (b) Basal channel open probability (P_o) in individual DRG neurons. P_o was determined from the ratios of the area under the peaks in the amplitude histograms fitted by a Gaussian distribution. Channel activity was calculated as $N P_o$, where N is the number of observed channels in the patch.

3.2.3 Single DRG K_{ATP} channel currents from excised inside-out patches

In order to investigate the relative contribution of the intracellular milieu regulation of the intrinsic channel properties in DRG neurons, the K_{ATP} channel behavior was next examined in excised inside-out membrane patches. Inside-out patches were made by pulling the membrane patch off the cell into the bath solution.

The bath (intracellular) solution contained 140 mM KCl, 1.2 mM $MgCl_2$, 10 mM HEPES, 1.5 mM EGTA and 5.5 mM dextrose. The pipette (extracellular) solution was composed of the

following: 140 mM KCl, 10 mM HEPES, 5.5 mM dextrose, and 1 mM EGTA. The pH of all solutions was adjusted to 7.4 with KOH. Osmolality was adjusted approximately to 300 mOsm/l by adding sucrose if necessary.

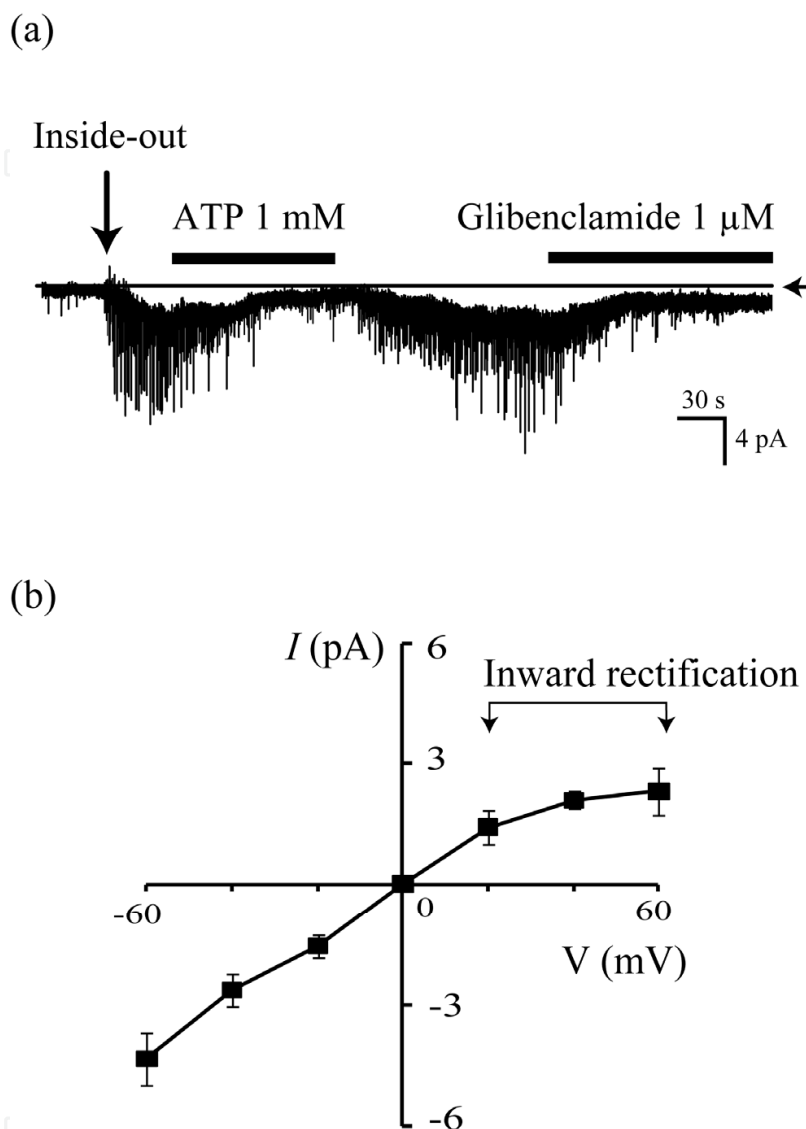


Fig. 6. Single-channel characteristics of K_{ATP} channel in DRG neurons from inside-out patch clamp configuration. (a) Representative trace of K_{ATP} channel activity recorded in cell-free patches excised from DRG neurons under symmetrical 140 mM K^+ conditions. Membrane potential was clamped at -60 mV. Upon patch excision (vertical arrow) into an ATP-free bath marked channel activity ensued. Horizontal arrows indicate closed channel state. (b) Current amplitude-voltage relationships, showing weak inward rectification. Means \pm SD are shown (n=10).

When inside-out patch recordings at a holding potential of -60 mV were obtained in ATP-free solution, intense channel activity was observed in all patches without any differences between groups of neurons classified by size. The current-voltage relationship showed weak inward rectification with single channel conductance of 70-80 pS without any differences between neuron sizes. This channel activity was reversibly blocked by 1 mM of ATP. In the

same recordings, subsequent superfusion with glibenclamide (1 μM) eliminated channel activity in ATP-free solution. Channel activation by ATP-free solution, as well as the inhibition by ATP and glibenclamide, rapidly occurred within a few seconds (Fig. 6).

Sensitivity to ATP and glibenclamide distinguishes these currents as conveyed through K_{ATP} channels (Babenko et al., 1998; Edwards & Weston, 1993).

4. Functional roles of K_{ATP} channel in primary afferent neurons

4.1 Basal K_{ATP} channel activity contributes to the resting membrane potential

To test the functional importance of K_{ATP} channel currents, their effect on resting membrane potential (RMP) in DRG neurons was examined using current-clamp recordings from β -escin perforated whole-cell patches.

The extracellular Tyrode's solution consisted of the following (in mM): 140 NaCl, 4 KCl, 2 CaCl_2 , 2 MgCl_2 , 10 D-glucose and 10 HEPES, at pH 7.4 and osmolality 300 mOsm/L. Internal pipette solution contained (in mM) 120 KCl, 20 HEPES, 5 EGTA, 5 MgCl_2 , 1 Na-ATP, and CaCl_2 2.25. pH was adjusted to 7.4 and osmolality to 300 mOsm/L. In this experiment, 50 μM β -escin was added into the pipette solution as a perforating agent (Sarantopoulos, et al., 2004) and recording was carried out when the access resistance was less than 15 $\text{M}\Omega$.

RMP was recorded at baseline for at least 1 min. After stability was confirmed, glibenclamide (1 μM) was superfused in the bath by a gravity dependent flow system, which depolarized the RMP in DRG neurons. These results imply that basal K_{ATP} channel opening physiologically regulates the RMP in DRG neurons (Fig 7).

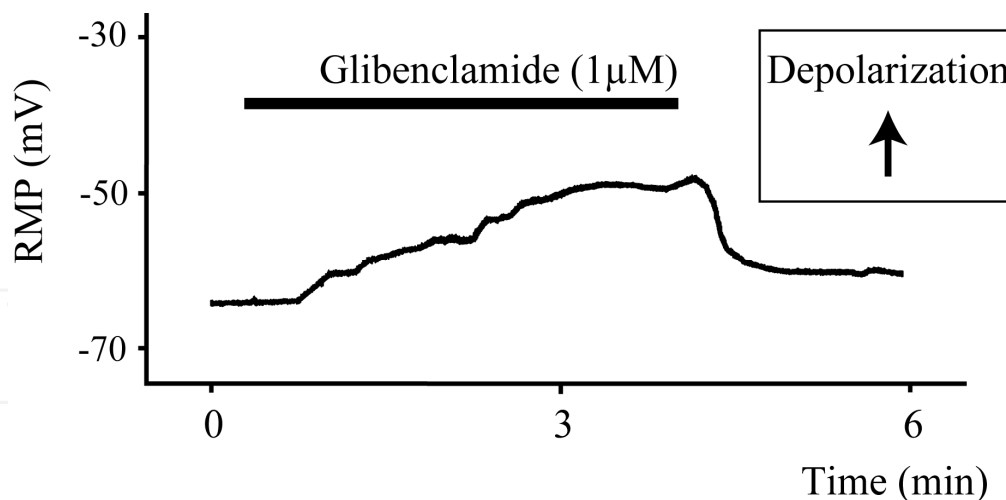


Fig. 7. Changes in resting membrane potential (RMP) induced by glibenclamide in DRG neurons. Representative RMP traces recorded in the current-clamp whole-cell patch configuration during the bath application of glibenclamide (1 μM).

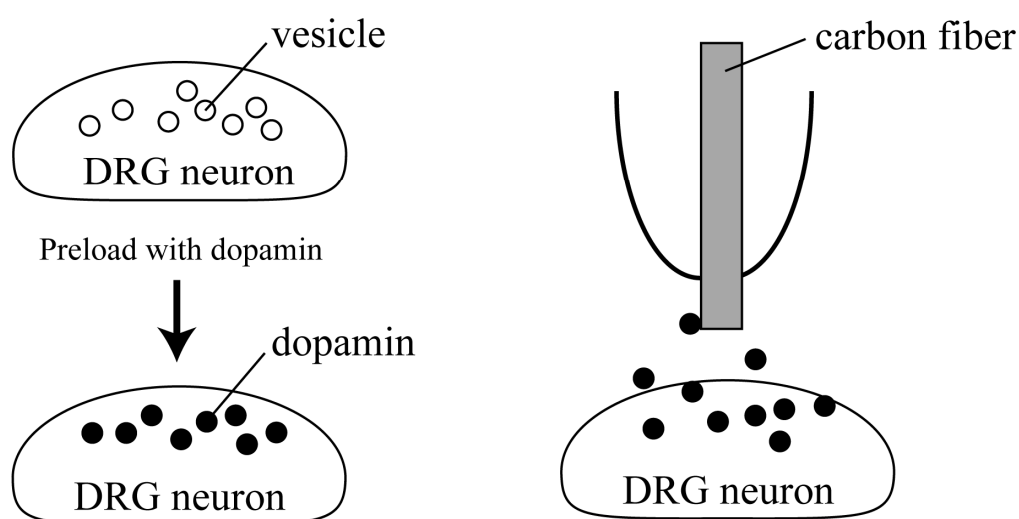
4.2 K_{ATP} channel activity regulates neurotransmitter release

K_{ATP} channel is known to modulate neurotransmission (Stefani & Gold, 2001; Steinkamp et al., 2007). To examine whether K_{ATP} channel in DRG neuron also modulate neurotransmitter

release, carbon-fiber amperometry was used to detect exocytosis from single DRG neuron in real time (Kawagoe, et al., 1993).

Amperometry provides high resolution to detect molecules released from single secretory vesicles. Amperometric measurements are normally limited to cells that package and secrete an endogenous oxidizable molecule such as catecholamines or serotonin; however, in some cases oxidizable molecules can be introduced artificially (Smith et al., 1995; Zhou & Mislser, 1996). So, amperometric analysis from DRG neurons was conducted by measured release of the pseudo-transmitter dopamine that had been loaded in DRG neurons (Fig. 8).

(a)



(b)

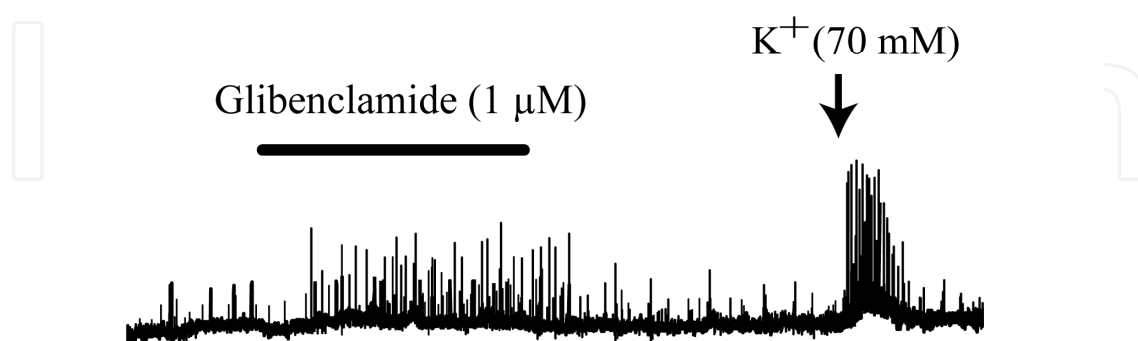


Fig. 8. Amperometric recordings from dopamine-loaded DRG neurons indicating the false transmitter release. Superfusion with glibenclamide ($1 \mu\text{M}$) enhanced basal transmitter release. Arrow indicates onset of massive transmitter release after depolarization with KCl (70 mM).

In this experiment, DRG neurons were incubated for 40 min in solution containing (in mM): 70 dopamine, 68 NaCl, 2.5 KCl, 2 CaCl_2 , 1 MgCl_2 , 10 D-glucose, and 10 HEPES at pH 7.4

with NaOH at 37°C. Recordings were performed at room temperature in amine-free external Tyrode's solution. To record amperometric events, 5 µm carbon fiber electrodes were backfilled with 3 M KCl. A carbon fiber electrode connected to a patch clamp amplifier was attached to the plasma membrane of the cell held at +800 mV for all experiments. The tip of the carbon fiber was manipulated onto the cell surface without disturbing cellular morphology. If infrequent response to high K⁺ (70 mM KCl) was observed, these data results were discarded. Currents were low-pass filtered at 5 kHz and sampled at 1 kHz by a Digidata 1440 Series interface (Axon Instruments).

As shown in Fig. 8b, carbon-fiber amperometry showed spikes (< 5 events/min) that were amplified by depolarization with external 70 mM K⁺ (200-300 events/min). Similarly, glibenclamide (1 µM) reversibly increased spike rate (20-50 events/min). These results indicate that K_{ATP} channels regulate vesicle release from DRG neurons.

4.3 Neuroprotection

Increased neuronal survival through activation of K_{ATP} channels has been demonstrated in association with membrane hyperpolarization, and reduction of excitability in response to hypoxia, ischemia or metabolic stress (Amoroso et al., 1990; Ballanyi, 2004; Sun et al., 2007; Yamada & Inagaki, 2005). K_{ATP} channels also act as transducers and effectors of neuronal preconditioning (Blondeau et al., 2000; Heurteaux et al., 1995). Pharmacological induced preconditioning with the K_{ATP} channel opener diazoxide may offer effective neuroprotection during hypothermic circulatory arrest (Shake et al., 2001). In addition, opening of K_{ATP} channels in the hippocampus or neocortex stabilizes the resting potential against anoxic stress, and protects neurons (Sun et al., 2006, 2007). In whole animals, the absence of Kir6.2 was associated with dramatically increased damage following ischemia induced by middle cerebral artery occlusion (Sun et al., 2006).

5. K_{ATP} channel in neuropathic pain

5.1 Pathophysiology of neuropathic pain

The specific cellular and molecular mechanisms underlying neuropathic pain remains largely unknown, but membrane hyperexcitability in those neurons that have lost their normal synaptic, physiological or electrical patterns is a common feature of most conditions leading to neuropathic pain (Gold, 2000; Woolf, 2010; Zimmermann, 2001).

Following nerve injury, damaged peripheral nerves become more excitable, with regards to their capacity to generate action potentials, leading to spontaneous, ongoing, ectopic electrical activity (Chung et al., 2002; Raouf et al., 2010; Woolf, 2010). In addition to the injury site, neuronal somata in the dorsal root ganglia are recognized as an important focus of ectopic electrical activity (Devor, 2009; Sapunar et al., 2005). This increase in primary afferent traffic to the dorsal horn is thought to induce raw pain signal (Devor, 2009) as well as central sensitization (Woolf, 2010). Substantial evidence indicates that altered expressions of ion channels on peripheral afferent neuronal somata contribute to abnormal sensory function following nerve injury (Raouf et al., 2010). Specifically, important changes have been noted in sodium (Amir et al., 2006), calcium (Gemes et al., 2011; McCallum et al., 2006) and potassium channels (Abdulla & Smith, 2001).

5.2 Animal models of neuropathic pain

Examination of the pathogenesis of neuropathic pain has been aided by the development of increasingly sophisticated rodent models of nerve injury that produce behavior indicative of on-going and evoked pain (Hogan, 2002).

A complete section of a nerve produces spontaneous pain, whereas they also lead to an anesthetic limb. On the other hand, partial injury retains a subset of afferent fibers and results in altered sensory function. Therefore, the latter is currently widely used for the study of neuropathic pain. These models involve: chronic constriction injury by loose ligation of the sciatic nerve (CCI) model (Bennett & Xie, 1988), tight ligation of the partial sciatic nerve (PSL) model (Shir & Seltzer, 1990), and tight ligation of spinal nerves (SNL) model (Kim & Chung, 1992).

5.2.1 CCI surgery

The right common sciatic nerve is exposed at the level of the middle of the thigh. Four loose ligatures of 4-0 chromic gut are placed around the sciatic nerve, and are loosely tied such that the diameter of the nerve was barely constricted.

5.2.2 PSL surgery

The right sciatic nerve was exposed near the trochanter. An 8-0 silk suture was inserted in the middle of the nerve, trapping in a tight ligation.

5.2.3 SNL surgery

The right paravertebral region was exposed via a lumbar incision, and the L6 transverse process was removed. The L5 and L6 spinal nerves were tightly ligated with a 6-0 silk suture and transected distal to the ligature.

5.3 Sensory testing

The purpose of behavioral sensory testing is to identify rats in which nerve injury has successfully produced behavior consistent with neuropathic pain. Operated rats are being tested for mechanical hyperalgesia, which provides a more consistent feature of the SNL model. Testing includes preoperative familiarization and acclimatization to testing environment, and subsequent repeated testing sessions.

Recently, Hogan et al. demonstrated the novel sensory testing that identifies hyperalgesia after SNL with high specificity (Hogan et al., 2004). Briefly, the plantar surface of each hind paw was touched with the tip of a Quincke 22-gauge spinal needle, which was applied with pressure adequate to indent but not penetrate the skin. Five needle applications were delivered in random order to each paw and repeated 5 min later for a total of 10 applications per session. These mechanical stimuli produced either a normal brief reflexive withdrawal or a hyperalgesia-type response that included sustained (> 1 s) paw lifting, shaking, and grooming (Fig. 9). The latter response occurs only after true SNL, and thus this may be accepted as an indication of a neuropathic pain. The intensity of hyperalgesia was assessed by the probability (%) of hyperalgesia-type responses out of ten trials of needle stimulation.

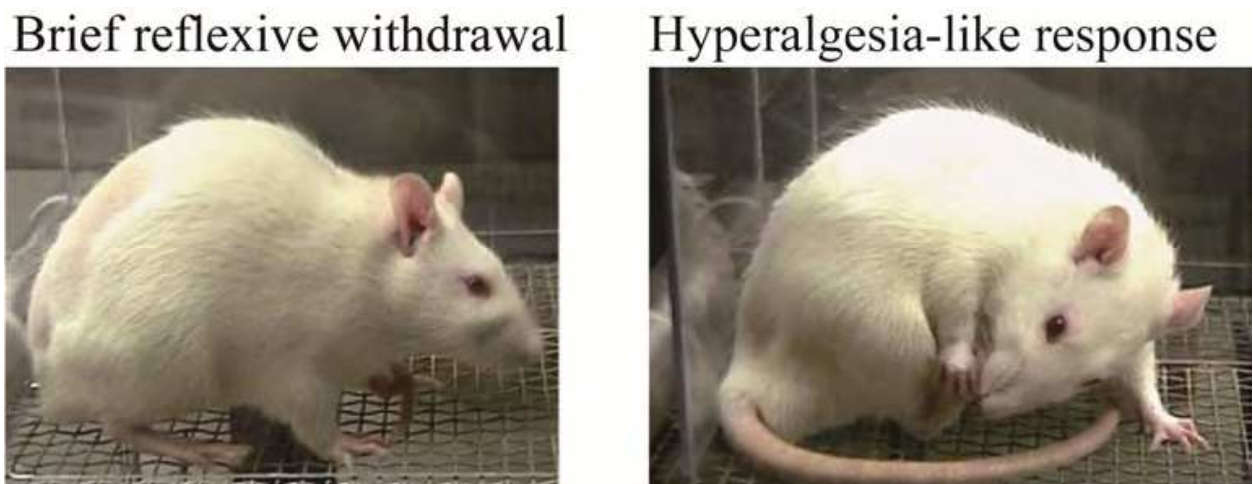


Fig. 9. Behavioral testing using a Quincke 22-gauge spinal needle. When a pin is applied to rat planter, the response is either a brief reflex withdrawal or a hyperalgesic reaction characterized by sustained lifting, shaking, and licking of the paw.

5.4 K_{ATP} channel activity in cell-attached patches depends on nerve injury status

To test whether K_{ATP} channels in primary afferent neuron contribute to the pathogenesis of neuropathic pain, basal K_{ATP} channel openings in either control (non-surgery) or SNL neurons were measured at -60 mV membrane holding potential using cell-attached patch clamp configurations.

Control



SNL



0.5 s
5 pA

Fig. 10. Basal K_{ATP} channel activity from cell-attached patches in control and SNL neurons. Representative cell-attached recording traces in control (non surgery) and SNL neurons. Basal single channel currents were recorded at -60 mV. Horizontal arrows indicate closed channel state.

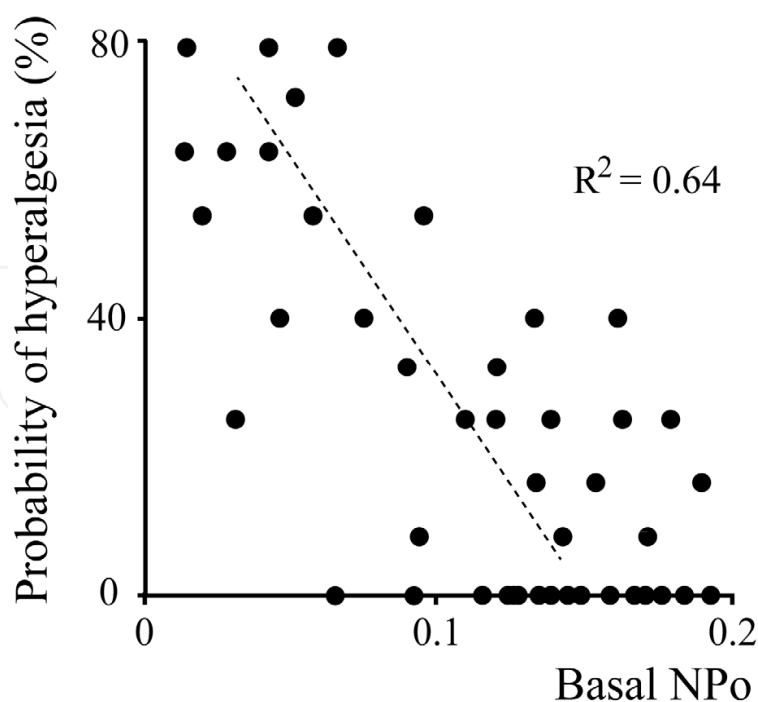


Fig. 11. Correlation of basal K_{ATP} channel NPo with each donor rat's probability of hyperalgesia, showing an inverse relationship. Simple linear regression curve and confidence intervals are shown.

In these recordings, basal channel opening was observed in both control and SNL neurons. In SNL neurons, however, basal K_{ATP} channel activity was diminished compared to controls (Fig. 10). NPo values in SNL neuron were also significantly reduced compared to control neurons. Analysis of single channel kinetics indicated that mean open time was shorter in SNL group compared to control group. Furthermore, basal K_{ATP} channel NPo correlated inversely with the probability of the donor animal responding to punctuate mechanical stimulus with a sustained, complex hyperalgesia-type behavior (Fig. 11.)

These results suggest that loss of current through these channels contributes to the pathogenesis of neuropathic pain. This hypothesis is further supported by a previous report that non-specific K^+ channel blockade evokes spontaneous firing in large $A\beta$ fibers after SNL (Liu et al., 2001). In addition, other study reported that hyperexcitability following peripheral nerve injury is mediated by loss of various K^+ currents (Chung & Chung, 2002).

5.5 K_{ATP} channel activity in inside-out patches are not altered by nerve injury

To examine whether unitary K_{ATP} channel currents is altered by axotomy, single channel properties in cell-free patches in either control (non-surgery) or SNL neurons were measured at -60 mV membrane holding potential.

In these recordings, marked current activity was observed in inside-out patches excised from either control or SNL neurons into ATP-free solution with symmetrical 140 mM K^+ condition (Fig. 12). In both groups, inside-out patches showed only one type of K^+ channel current, especially at negative potentials. In addition, single-channel conductance was the same in control and SNL neurons (70-80 pS). Furthermore, sensitivity to ATP and diazoxide, a selective SUR-1 containing K_{ATP} channel opener, also did not differ between groups (Fig. 12).

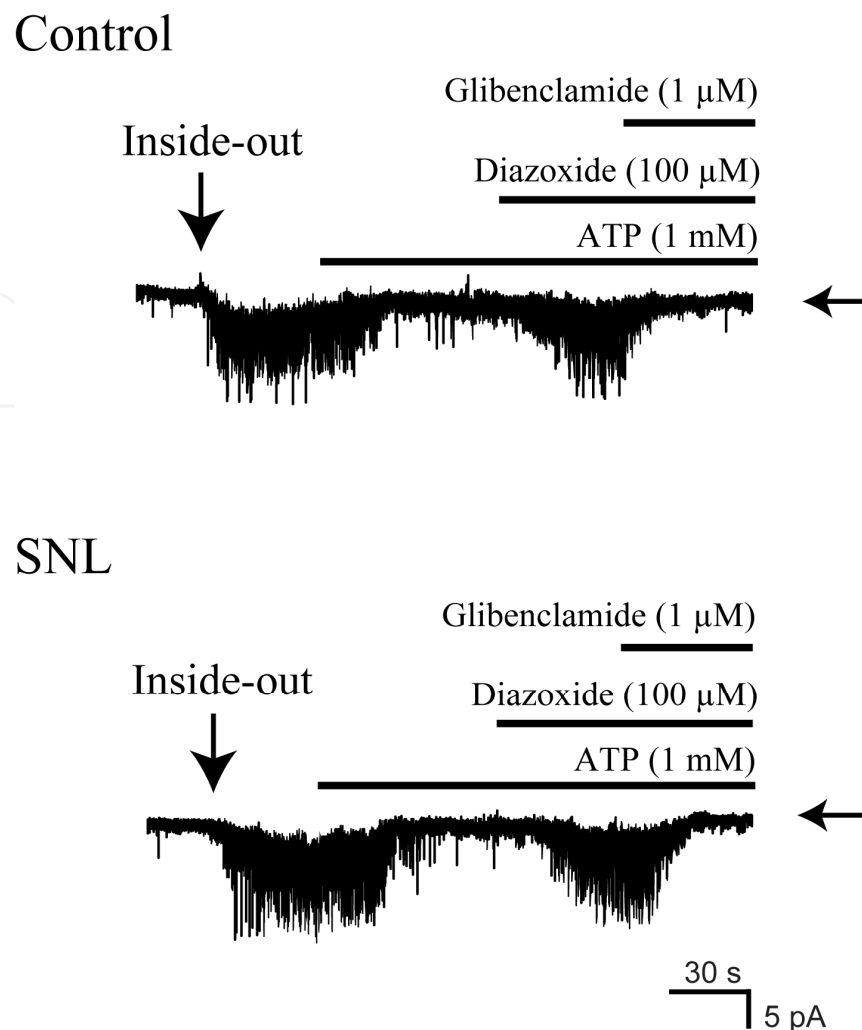


Fig. 12. Single-channel characteristics of K_{ATP} channels from inside-out recordings from control or SNL neurons. Membrane potential was clamped at -60 mV. Upon patch excision (vertical arrow) into an ATP-free bath marked channel activity ensued. ATP (1 mM), diazoxide (100 μ M), and glibenclamide (1 μ M) was added to the intracellular (bath) solution as indicated by the horizontal solid bar. Horizontal arrows indicate closed channel state.

These results suggest that SNL does not affect the K_{ATP} channel per se or any associated membrane-resident regulatory proteins. Our findings further imply that the molecular composition of the K_{ATP} channels is not affected by axotomy.

Therefore, the suppressed K_{ATP} channel activity observed from cell attached recordings may be attributed to alterations in the cytosolic signaling following painful nerve injury.

6. Conclusion

K_{ATP} channels couple cellular electrical activity to cytosolic metabolic status in various excitable tissues. These channels are widely expressed in central neurons, wherein they regulate membrane excitability and neurotransmitter release, and they provide neuroprotection. In addition to the functional K_{ATP} channels in the central nervous system, we have identified these channels in rat primary afferent neurons, dissociated from the rat DRG.

Altered sensory function contributes to the pathogenesis of neuropathic pain via hyperexcitability in injured axons and the corresponding somata in the DRG, increased synaptic transmission at the dorsal horns, and loss of DRG neurons. We have identified loss of K_{ATP} currents in DRG somata from rats that demonstrated sustained hyperalgesia-type response to nociceptive stimulation after axotomy. Thus, reduced K_{ATP} currents may be a factor in generating neuropathic pain through increased excitability, amplified excitatory neurotransmission, and enhanced susceptibility to neuronal cell death. In addition, intrinsic single-K_{ATP} channel characteristics are preserved even after painful nerve injury. Therefore, intact biophysical and pharmacological properties provide opportunities for therapeutic targeting with K_{ATP} channel openers against neuropathic pain.

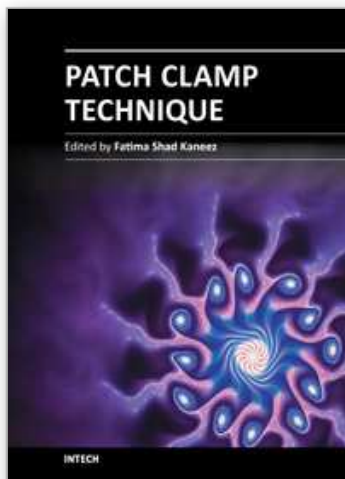
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Patch Clamp Technique

Edited by Prof. Fatima Shad Kaneez

ISBN 978-953-51-0406-3

Hard cover, 356 pages

Publisher InTech

Published online 23, March, 2012

Published in print edition March, 2012

This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

How to reference

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Takashi Kawano (2012). Single-Channel Properties and Pharmacological Characteristics of KATP Channels in Primary Afferent Neurons, Patch Clamp Technique, Prof. Fatima Shad Kaneez (Ed.), ISBN: 978-953-51-0406-3, InTech, Available from: <http://www.intechopen.com/books/patch-clamp-technique/single-channel-properties-and-pharmacological-characteristics-of-katp-channels-in-primary-afferent-n>

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