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The Sodium-Activated Potassium Channel Slack (Slo 2.2) is Modulated by Hypercapnia and Acidosis

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Abstract

Slack (Slo 2.2), a member of the Slo potassium channel family, is activated by both voltage and cytosolic factors, such as Na^+ ($[Na^+]_i$) and Cl^- ($[Cl^-]_i$). Since Slo family is known to play a role in hypoxia, and since hypoxia/ischemia is associated with an increase in H^+ and CO_2 intracellularly, we hypothesized that the Slack channel may be affected by changes in intracellular concentrations of CO_2 and H^+. To examine this, we expressed the Slack channel in *Xenopus* oocytes and the Slo 2.2 protein was allowed to be inserted into the plasma membrane. Inside-out patch recordings were performed to examine the response of Slack to different CO_2 concentrations (0.038%, 5%, 12%) and to different pH levels (6.3, 6.8, 7.3, 7.8, 8.3). In the presence of low ($[Na^+]_i$ (5 mM), the Slack channel open probability decreased when exposed to decreased pH or increased CO_2 in a dose-dependent fashion (from 0.28±0.03, n=3, at pH 7.3 to 0.006±0.005, n=3, p=0.0004, at pH 6.8; and from 0.65±0.17, n=3, at 0.038% CO_2 to 0.22±0.07, n=3, p=0.04 at 12% CO_2). In the presence of high $[Na^+]_i$ (45 mM), Slack open probability increased (from 0.03±0.01 at 5 mM $[Na^+]_i$, n=3, to 0.11±0.01, n=3, p=0.01) even in the presence of decreased pH (6.3). Since Slack activity increases significantly when exposed to increased $[Na^+]_i$, even in presence of increased H^+, we propose that Slack may play an important role in pathological conditions during which there is an increase in the intracellular concentrations of both acid and Na^+, such as in ischemia/hypoxia.

Keywords

Na^+-activated K^+ channel; brain; hypoxia/ischemia

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Introduction

Potassium (K^+) channels belong to many families of channels and are important regulators of cell excitability in normal and abnormal conditions (Hille, 2001). More recently, for example, Na^+-activated K^+ channels (K_{Na}) have been identified in the heart (Kameyama et al., 1984; Niu and Meech, 2000) and central nervous system (CNS) of avians (Dryer, 1991) and mammals (Bhattacharjee et al., 2002; Franceschetti et al., 2003). K_{Na} channels are thought to play a role in terminating neuronal excitability when activated by an increase in $[Na^+]_i$ (Kameyama et al., 1984; Dryer, 2003; Franceschetti et al., 2003). The **Slack** (**s**equence **l**ike **a** **C**a^{2+}- activated **K**$^+$ channel) channel is a newly recognized K_{Na} channel in the Slo family (though Slack and its ortholog in C. elegans are only 7% (Joiner et al. 1998) identical to the voltage- and Calcium-dependent Slo and the voltage-dependent but calcium-insensitive Slo3 (see reviews by Gribkoff et al., 2001; Bhattacharjee and Kaczmarek, 2005). In mammals, Slack has been identified in the CNS (Bhattacharjee et al., 2002) and hypothesized to be important in regulating neuronal excitation in hypoxia/ischemia due particularly to its sensitivity to both Na^+ and Cl^- (Bhattacharjee and Kaczmarek, 2005). The Slack channel cloned first by Joiner et al. (1998) and then by Yuan et al. (2003) shares most of the characteristics as those of the native Slack channels, with the exception of cooperative activation by Na^+ and Cl^- (Yuan et al. 2003 and Dryer 2003).

During brain or heart hypoxia/ischemia, there is decreased tissue perfusion which decreases O_2 delivery and CO_2 removal. As a result of the low tissue pO_2, a shift to anaerobic metabolism occurs leading to lactic acidosis (Nedergaard and Goldman, 1993). In addition, as a result of CO_2 accumulation, respiratory acidosis develops to promote a further drop in pH. The switch in metabolism decreases the amount of ATP available to support ATP-dependent exchangers (e. g. the Na^+-K^+ ATPase), which usually help maintain ionic gradients (Mitani and Shattock, 1992; Dobrota et al., 1999; Lipton, 1999). In order to examine the properties of these channels during pathophysiological conditions, we studied the Slack channel in an isolated environment where each variable can be manipulated independently. Hence, we studied Slack in an oocyte system to focus on its properties and the responses to changes in CO_2 and pH, both of which change in a major way in brain or heart hypoxia/ischemia.

Methods

Preparation of cRNA

The Slack α (Slo 2.2) cDNA plasmid was inserted into a Bluescript vector (kindly provided by W. Joiner and L. Kaczmarek, Yale University School of Medicine, New Haven, CT) and transformed using ampicillin selection in Top Ten Competent Cells (Invitrogen, Carlsbad, CA). The Slack cDNA was amplified, purified using Qiaprep Spin Miniprep Kit (Qiagen, Valencia, CA), and then linearized by the Not I restriction enzyme (Invitrogen, Carlsbad, CA). The Slack cDNA (0.24 μg/μl) was *in-vitro* transcribed using the mMessage mMachine T3 (Ambion, Austin, TX), polymerase to generate a cRNA transcript (1.0–2.0 μg/μl) that when translated *in vivo*. The cRNA was purified using the RNeasy Mini kit (Qiagen, Valencia, CA).

Oocyte injection

The African claw frog (*Xenopus laevis*, Nasco, Fort Atkinson, WI) was anesthetized using 3-benzoic acid and the oocytes were removed surgically. Seventy nl of cRNA of Slack α (mSlo-2.2) subunit was injected into isolated oocytes and allowed to be translated into the protein product, and inserted into the oocyte plasma membrane. Oocytes were recorded from three to ten days after the injection.

Solutions

A symmetrical potassium solution was applied to both sides of the oocyte membrane when in the inside-out patch configuration consisting of (mM): 130 KCl, 5 Na- d-gluconate, 10 HEPES, 5 EGTA, and 29 Glucose, and pH was adjusted to 7.3 with KOH and glacial acetic acid. Chemicals were purchased from Sigma-Aldrich (Saint Louis, MO). Osmolarity of the solution was adjusted to around 300 mOsm.

To test the effect of Cl^- on Slack channel activity, a solution made up of the following (in mM) 3 KCl, 127 K-d-gluconate, 5 Na-d-gluconate, 10 HEPES, 5 EGTA and 29 glucose, was applied to both sides of the membrane. The bath solution (cytosolic side) was later replaced with a solution (in mM) composed of 130 KCl, 5 Na-d-gluconate, 10 HEPES, 5 EGTA and 29 glucose to examine the response of Slack to increased $[Cl^-]_i$. Both solutions were adjusted to 293–299 mOsm and a pH of 7.3.

To test the effect of Na^+ on Slack channel activity, a solution made up of the following (in mM) 30 KCl, 10 K-d-gluconate, 5 Na-d-gluconate, 85 n-methyl-d-glucamine, 10 HEPES, 5 EGTA and 10 glucose, was applied to both sides of the membrane. The bath solution (cytosolic side) was later replaced with a solution (in mM) composed of 30 KCl, 10 K-d-gluconate, 90 Na-d-gluconate, 10 HEPES, 5 EGTA and 10 glucose to examine the response of Slack to increased intracellular $[Na^+]_i$. Both solutions were adjusted to 275–290 mOsm and a pH of 7.3.

To determine the effect of varying pH on Slack activity, the bath solution was first adjusted to pH 8.3 with KOH to maintain equal concentrations of K^+ and then glacial acetic acid was added to achieve more acidic pH values. The inside-out patches of oocytes were perfused with the bath solution bubbled with either 5% or 12% CO_2 to test the effect of higher CO_2 on Slack channel properties.. The perfusion solution was bubbled with either 5% or 12% CO_2 for >2 hours before use to allow for adequate equilibration time.

To ensure quick and efficient solution exchange, some experiments were performed with a “microchamber” perfusion system. In this system, the tip of the recording pipette was deeply inserted into the microchamber made by a glass ball from the tip of a thin wall capillary glass tubing (1.5 mm OD, Warner Instruments, Hamden, CT). The solution exchange was almost instantaneous without any delay. The flow rate was around 0.5–1.0 ml/min, controlled by gravity. To make sure that there are no technical errors related to the order by which we bathed the patches, the same solution exchange was performed in both directions: from a low to a high concentration and from a high to a low concentration of a particular ion, with some experiments repeatedly performed several times with different concentrations of one particular ion (e.g. Na^+ or Cl^-).

Electrophysiology

Patch-clamp experiments were performed using the PC-501 amplifier (Warner Instruments, Hamden, CT). Recordings were made in inside-out configuration with borosilicate glass pipettes (World Precision Instruments, Serasota, FL) pulled with a Sutter puller (P-87, Sutter Instruments, Navato, FL), with a resistance of 5–10 MΩ. The signal was low-pass filtered at 2–5 kHz, sampled at 5–10 kHz and stored in a PC-DOS based computer using pClamp 5 and 6 software (Axon Instruments, Foster City, CA). Data were then analyzed using pClamp 9 software. Voltages in the text are all given comparing the internal side of the oocyte membrane to the external side. The open probability was defined as the time a particular channel (P_o) or channels (NP_o) in the patch spend in the open versus the total recording time.

Results

Very few endogenous channels were present in the oocyte and the Slack channel was not present. Three to ten days after the injection of the Slack α subunit cRNA into *Xenopus oocytes*, recordings were done. A current could be detected using the inside-out configuration of the patch clamp technique (Figure 1A).

The transfection of Slack (Slo 2.2) induced a detectable macroscopic K^+ current that was not present in the water injected oocyte (Figure 1B). In response to the voltage protocol shown in Figure 1B, an outwardly rectifying current was observed (Figure 1C). The Slack channel was voltage-dependent, increasing single channel amplitude in response to increasing changes in V_m (Figure 2A, B). Furthermore, an increase in NP_o was obtained at positive V_m (Figure 2C). The single channel I-V relationship could be fitted with a linear regression, yielding a single channel slope conductance of 120 ± 5 pS (n=3) (Figure 2A). In addition, this single channel conductance is within the range of published conductances for the Slack α subunit in symmetrical K^+ solutions (from 88 to 160 pS) (Yuan et al., 2003).

In addition to being voltage-dependent, Slack, being a member of the Slo family, is also modulated by intracellular factors. Single channel (inside-out patch) experiments were performed to examine the sensitivity of the Slack channel to the $[Cl^-]_i$. In low $[Cl^-]_i$ solutions (3 mM), the open probability of single Slack channels was very low (Fig. 3A, D) and there seems to be no voltage dependency of NP_o (Fig. 3D). Single Slack channel activity increased remarkably when the intracellular side of the membrane was exposed to 130 mM Cl^- at all voltages from −140 to 140 mV. In high Cl^- solutions, there is a voltage dependency of NP_o with a low open probability in negative voltage ranges. NP_o increased with an increase in V_m from 40 to 100 mV and the NPo reached a plateau at around 100 mV. Shifting the internal solution from 3 mM Cl^- to 130 mM Cl^- did not shift the single channel reversal potential (Fig. 3C, and A, B traces at 0 mV), which confirms that the currents obtained from these experiments are indeed K^+ currents (symmetrical K^+ in both intracellular and extracellular sides of the membrane in both low and high Cl^- solutions) and not Cl^- currents. Shifting internal solutions from 3 mM Cl^- to 130 mM Cl^- did not change single Slack channel amplitude (Fig. 3C). There is no difference of single channel amplitudes in the two Cl^- solutions with the exception of currents at +140 mV. This Cl^-

sensitivity has been also found in a previous publication on Slack (Bhattacharjee and Kaczmarek, 2005).

We also examined the sensitivity of the Slack channel to $[Na^+]_i$. Single channel (inside-out patch) experiments were performed to examine the sensitivity of the Slack channel to the $[Na^+]_i$. In low $[Na^+]_i$ solutions (5 mM), the open probability of single Slack channels was low (Fig. 3A, D). Single Slack channel activity significantly increased when the intracellular side of the membrane was exposed to 90 mM Na^+ at all voltages from −140 to 140 mV (with the exception at +60 mV). The normalized relationship between NP_o and V_m for both low and high Na^+ experiments indicates that the Na^+ concentration does not change the voltage dependency of the Slack single channel open probability. Shifting internal solutions from 5 mM to 90 mM Na^+ did not alter the single channel reversal potential (Fig. 4C, and A, B traces at 0 mV), which confirms that the currents are carried by K^+ ions. Changing the internal solutions from 5 mM to 90 mM Na^+ significantly increased single Slack channel amplitude (Fig. 4C).

We then tested the effect of a decrease in intracellular pH (pH_i) and an increase in CO_2 on the activity of the Slack channel. Inside-out patches were held at V_m equal to −100 mV and perfused with different solutions of varying pH to examine the effects of pH_i changes on channel activity. There was a dose-dependent inhibition of channel activity when the Slack channel was exposed to acidic pH, reflected by a decrease in NP_o ($p<0.05$, n=3, for pH 6.8 vs. pH 7.3 and $p<0.05$, n=3, for pH6.3 vs. pH 7.3) (Figure 5A). There was an increase in channel NP_o when Slack was exposed to basic pH values ($p<0.05$, n=3, for pH 8.3 vs. pH 7.3) (Figure 5B), though at pH 7.8, NP_o was not significantly different from that at pH 7.3 ($p>0.05$, n=3). Insets in this figure show channel opening in an expanded time frame at the indicated points. The effect of pH on the channel was partially reversible. The NP_o was restored close to its original level in most cases when pH was returned to 7.3. The relationship of NP_o versus pH could be fitted by the Boltzmann equation and yielded a middle point 0.17 of NP_o at pH 7.2.

To examine the effect of $[CO_2]_i$, inside-out patches (cytosolic side facing the bath solution) recordings were performed at V_m = −100 mV and the bath was perfused with solutions of varying CO_2 concentrations. pH of the bath solution was decreased with increased CO_2. In room air, pH of the bath solution was 7.29±0.01 (n=3), With 5% CO2, pH was 6.42±0.06 (n=3) and with 12% CO_2, pH was 6.14±0.06 (n=4). There was a dose- dependent inhibition of channel activity when the Slack channel was exposed to 5% as reflected by a decreased NP_o ($p>0.05$, n=3, for 0.038% vs. 5% and $p<0.05$, n=3, for 0.038% vs. 12%) (Figure 6A, B). Insets show channel opening in an expanded time frame at the indicated points. Based on our data, we estimated that the NP_o was decreased by about 41%, when 5% CO_2 was in bath solution. With increased CO_2 (12%), NP_o was decreased further to about 66%.

The Slack channel pH sensitivity was examined when the $[Na^+]_i$ was increased to 45 mM. Insets show clear channel opening at different pH values when exposed to 45 mM Na^+. The Slack channel increased its activity significantly ($p<0.05$), especially at the higher pHs, when exposed to increased $[Na^+]_i$ even in the presence of decreased pH_i (Figure 7, n=3).

Discussion

Based on our experimental data, the channel we expressed in oocytes is the Slack (Slo 2.2) channel. The current is a voltage-dependent, outwardly rectifying current with a slope conductance in the range reported in the literature for this channel (Yuan et al., 2003). Our work has demonstrated that 1) the Slack current from this study is a K^+ current since single Slack currents reversed at 0 mV with symmetrical K^+ solutions (Fig. 3C and 4C); 2) the Slack current from this study is voltage-dependent and outwardly rectifying (Fig. 1C); 3) the Slack current is sensitive to intracellular Cl^- with physiological intracellular Cl^- concentrations (130 mM) increasing open probability (Fig. 3D) of single Slack channel but does not modify slope conductance (Fig. 3C); 4) the Slack current is sensitive to intracellular Na^+, with physiological concentrations (e.g. 5 mM) keeping this channel in moderate open status. Higher concentrations of Na^+ increase not only single channel open probability (Fig. 4D) but also single channel slope conductance, keeping the driving force the same (Fig. 4C); 5) Slack channel has a decreased open probability when exposed to decreased pH or increased CO_2 and 6) Slack channel activity is modulated by $[Na^+]_i$ in the presence of decreased pH.

The Slack channel is widely expressed throughout the CNS (Bhattacharjee et al., 2002) and has been hypothesized to be important in neuronal function, especially because of its known $[Na^+]_i$ sensitivity (Bhattacharjee et al., 2003; Yuan et al., 2003; Bhattacharjee and Kaczmarek, 2005; Santi et al., 2006). Since we have shown in this study that the Slack channel is active in 5 mM $[Na^+]_i$, which is within the range of the resting neuronal $[Na^+]_i$ (Rose, 2002), we believe that Slack is most likely active in neurons under basal conditions and contributes to setting the resting membrane potential. Our data show that the Slack channel increases its activity when $[Na^+]_i$ is increased, resulting in increased K^+ efflux and promoting membrane repolarization. Therefore, the increased neuronal $[Na^+]_i$ can be considered to be a “second messenger” feedback ion that terminates cell excitation by activating Slack.

We and others have also shown that hypoxia and ischemia are associated with a substantial increase in $[Na^+]_i$, primarily as a result of the inhibition of the Na^+-K^+ ATPase (as suggested by Friedman and Haddad, 1994), Na^+ influx through voltage-gated Na^+ channels (Hammarstrom and Gage, 2002; see a review by Banasiak et al., 2004), glutamate receptors (Muller and Somjen, 2000), Na^+/H^+, Na^+/Ca^{2+} exchanger (as suggested by Friedman and Haddad, 1994), or mainly NMDA channel minorly through AMPA or voltage-gated Na^+ channels (Rose and Konnerth, 2001; see a review by Rose 2002). As expected from our current results, this increase in $[Na^+]_i$ enhances the open probability of Slack. However, clearly there are a variety of intracellular factors and other ion channels which would determine ionic gradients and shape neuronal excitability and the relative role of Slack depends on its expression and temporal activation in specific neurons in the CNS.

In ischemia or hypoxia there is usually a build-up of CO_2 and metabolic by- products, which would decrease pH_i (Yao and Haddad, 2004). We have shown in this work that Slack activity is dose-dependently decreased by acidic pH_i (see Figure 5) or by increased CO_2 (see Figure 6). Either pH_i or CO_2 or both could contribute to the inhibitory effect on Slack activity. Acid

sensitive channels have been reported (K_{ir}: Wu et al., 2004; ASIC (acid sensitive ion channel-1, Na channel): Zhang et al., 2006). One characteristic of acid-sensitive K channels is in its regulation by hyper- or hypocapnia (Jiang et al., 2004). However, what was particularly interesting to us was that high $[Na^+]_i$ attenuated the acid- induced inhibition of Slack activity (see Figure 7). We propose that, during pathological conditions such as hypoxia or ischemia, Slack channel activity is under the influence of at least resultant opposing factors: increased $[Na^+]_i$ and decreased pH_i, which produce a net modulatory signal to regulate Slack channel activity.

We and others have shown that hypoxia decreases the open probability of BK channels in either acutely dissociated (Liu et al., 1999) or membrane delimited preparations (Lewis et al., 2002). The mechanism behind this inhibition of K^+ channels has not been fully resolved. It is thought that CO_2 exerts its inhibitory effect in the form of H^+ ions intracellularly ($[H^+]_i$). The increased $[H^+]_i$ may alter charges on the residues that line the mouth of the pore through which the K^+ ions must pass, thereby affecting the sensitivity of the voltage sensor of the channel. Ruppersberg et al. (1991) reported changes in pH dependency of inactivation of K_A channel mutation of cysteine with serine residue in the ball domain of RCK4 in the N-terminus suggesting that a change in structure would affect channel gating kinetics. Furthermore, the increased $[H^+]_i$ may cause protons to line the inner membrane surface altering the voltage difference across the membrane which may affect the threshold (for example, Ca threshold, Haddad and Jiang, 1997) for K^+ channel activation.

In summary, we have shown that the Slack channel is active even when $[Na^+]_i$ is low and that its opening probability decreases when exposed to decreased pH or hypercapnia. In the presence of increased $[Na^+]_i$, the Slack channel opening probability increases, even when exposed to decreased pH_i. The Slack channel may play an important role in pathological conditions such as in ischemia/hypoxia.

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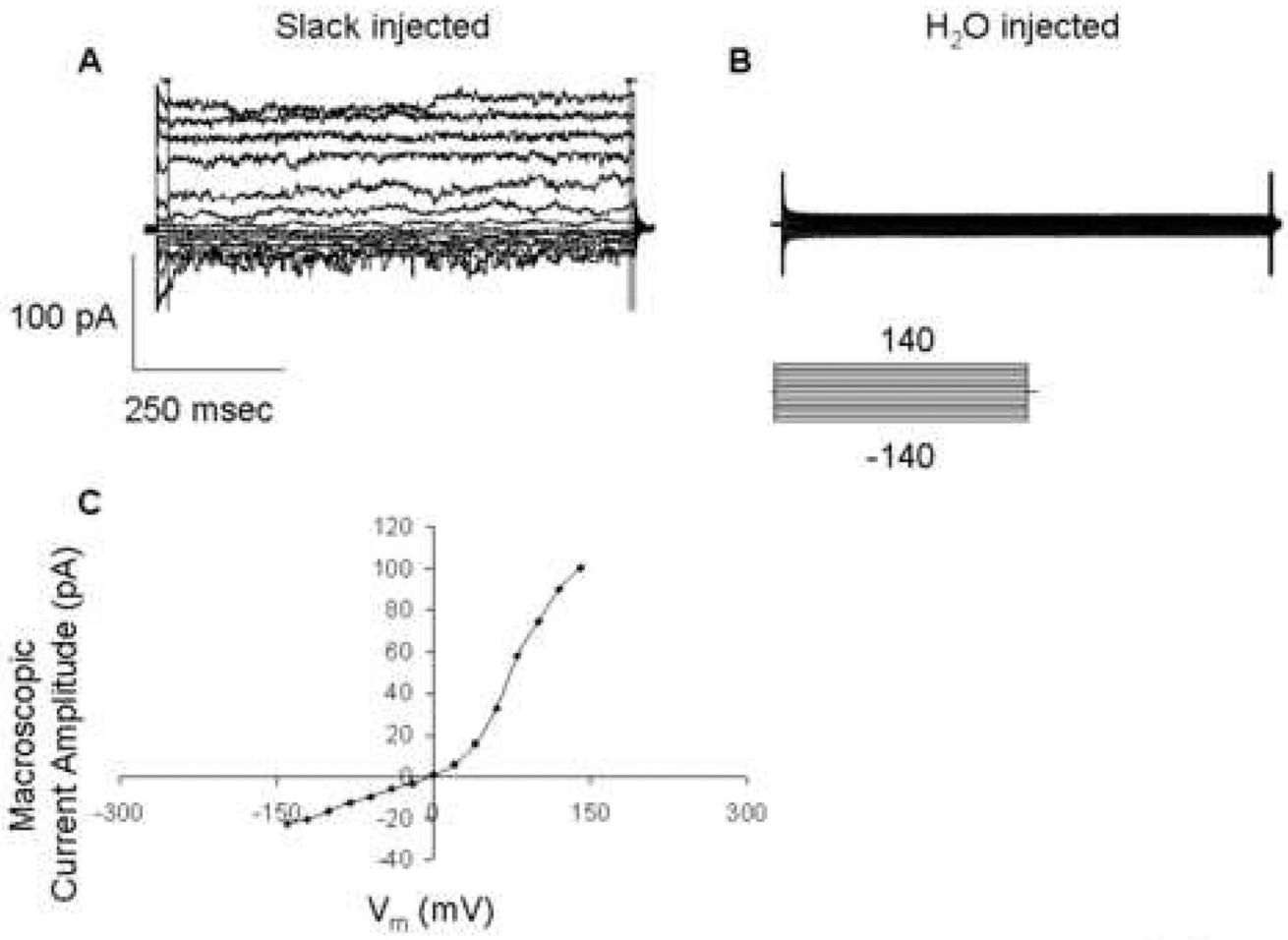

Figure 1.

Slack channel activity is present in the Slack cRNA-transfected oocyte (A) and not in the H_2O-injected oocyte (B). IV relationship in expressed Slack channel (C). Recordings were performed with a protocol under (B). Symmetrical 130 mM KCl and 5 mM Na-gluconate solutions were used in both pipette and bath for the macro-patch recordings (inside-out).

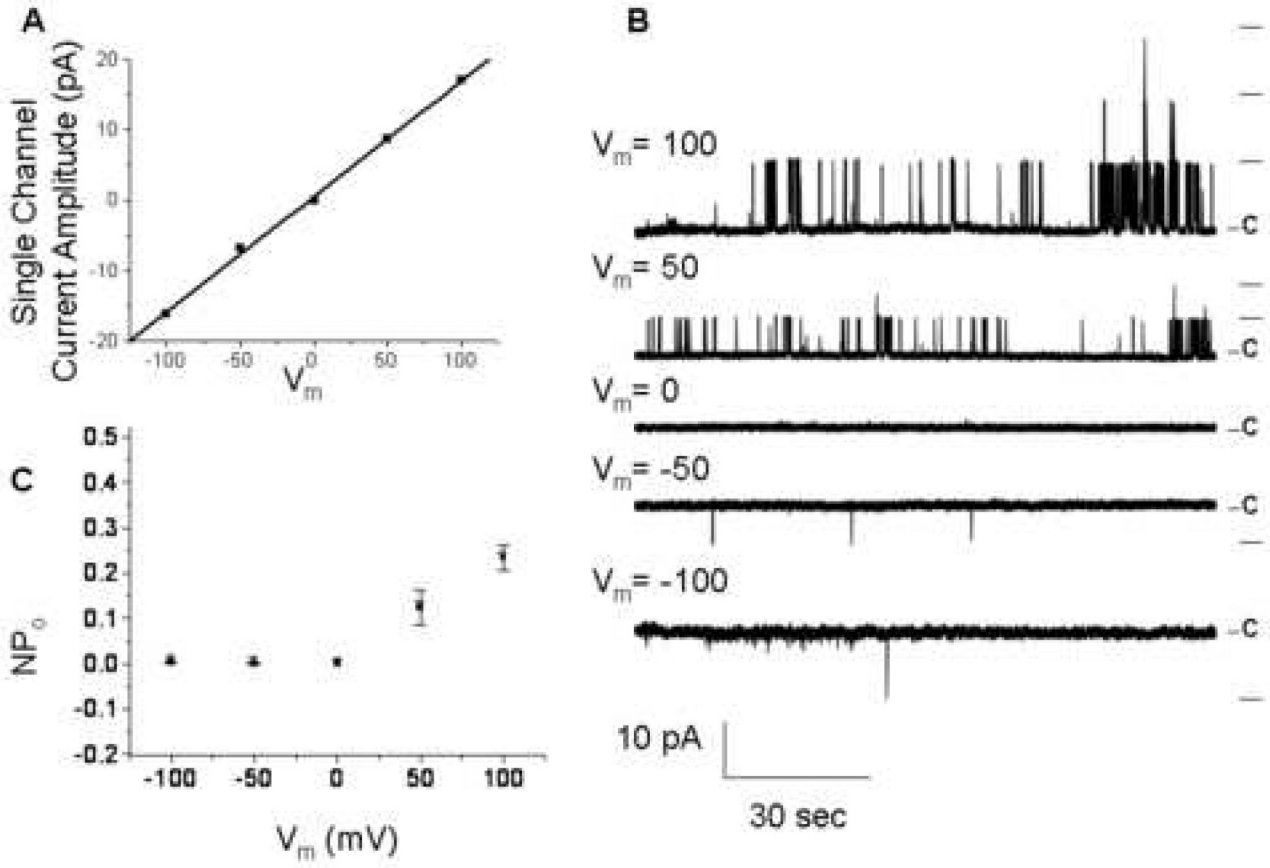

Figure 2.

Voltage dependency of Slack single channel opening. Slack single channel activities were recorded using symmetrical solutions (see Fig. 1) in both pipette and bath at different V_m (B) and single Slack channel amplitudes were plotted against different V_m (A). Open probability (NP_o) of Slack single channel increases with increased V_m (C) (n=3).

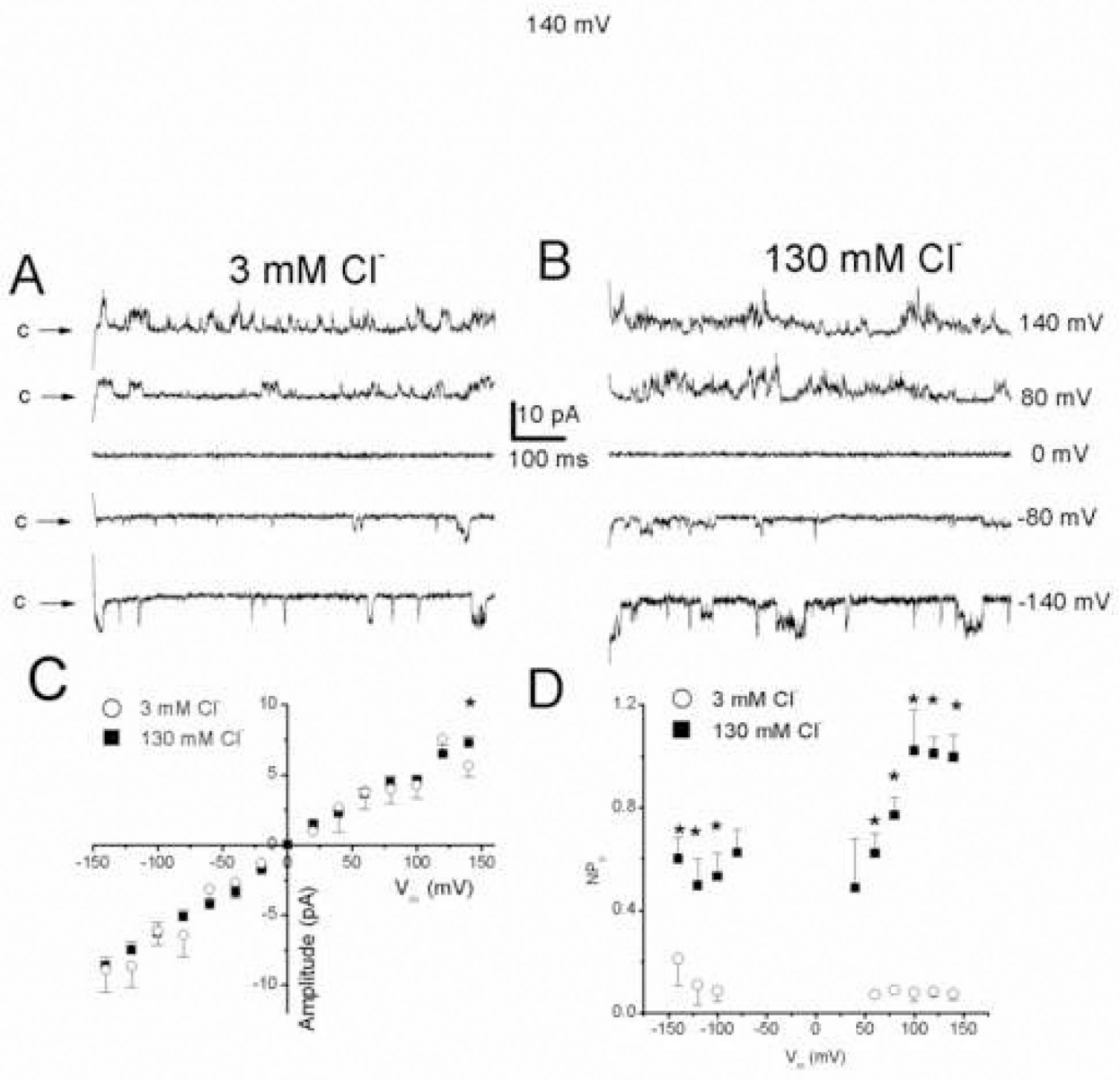

Figure 3.

Single Slack channel activity is sensitive to intracellular chloride. Slack channel activity increases when the $[Cl^-]_i$ increased from 3 (Fig. 3A) to 130 mM (Fig. 3B). Single channel currents were evoked at V_m −140 to 140 mV with a 20 mV increment from V_m 0 mV. Only currents from selected voltages listed on the right side of the Fig. 3B are shown for simplicity. The experiment started with symmetrical solutions (3 mM KCl and 127 mM K-d-gluconate) in both pipette and bath and then bath solutions (intracellular side) were replaced with solutions containing130 mM KCl. Traces in A and B are from two different patches. C. Current-voltage relationship of single Slack channel. Each current in is an average of 2 to 39 (n=11.6±2.2 for 3 mM Cl^- experiments; n=22.6±2.0 for 130 mM Cl^- experiments) measurements from 6 patches of 3 mM Cl^- experiments and 7 patches of 130 mM Cl^-

experiments. D. Relationship between V_m and NP_o. Each point in Fig. 3D is from 1 to 5 (n=2.9±0.5 for 3 mM Cl^- experiments; n=3.5±0.3 for 130 mM) measurements.
* shows statistical difference between 3 and 130 mM Cl^- experiments ($p<0.05$).

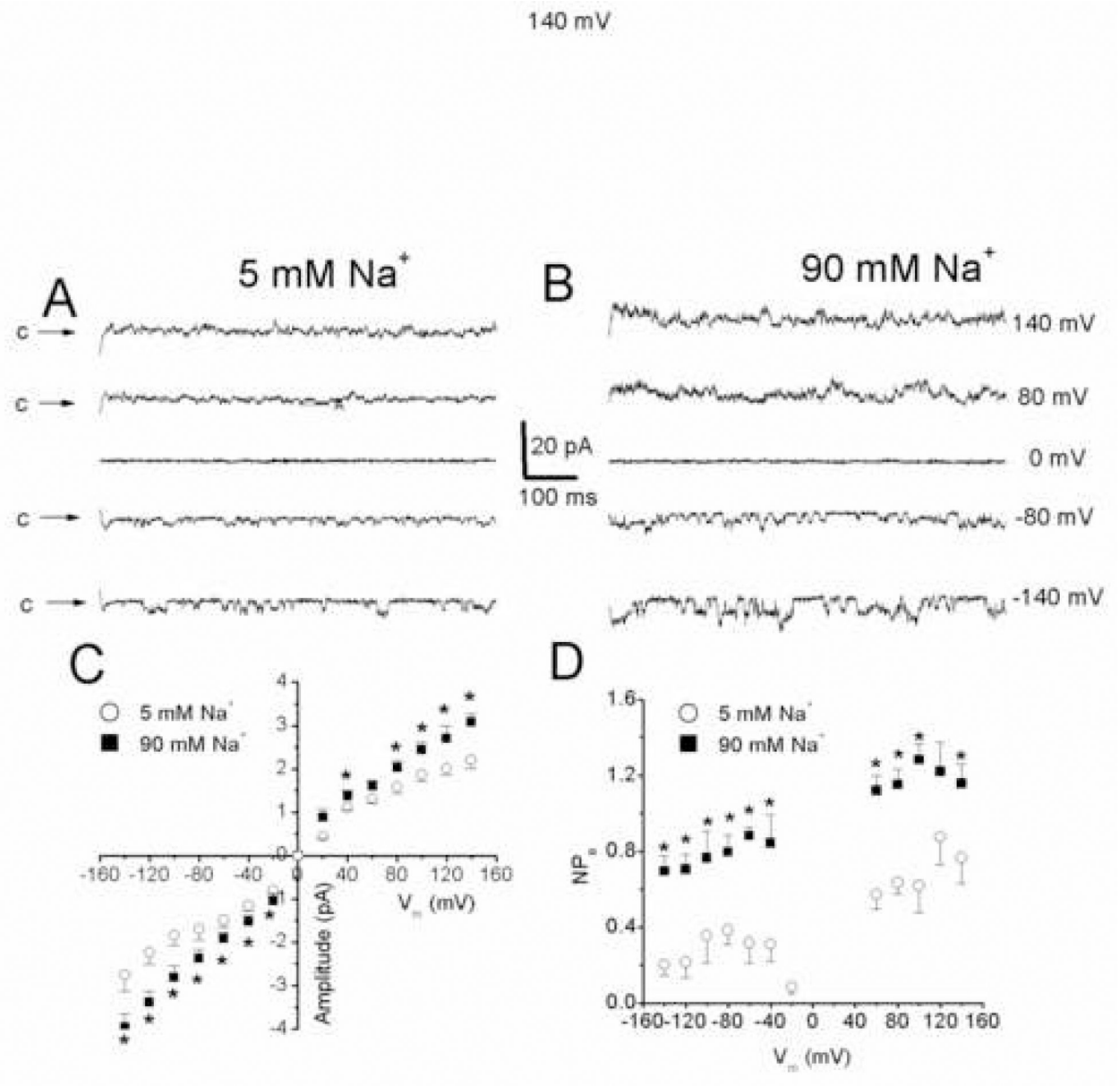

Figure 4.

Single Slack channel activity is sensitive to intracellular sodium. Slack channel activity increases when the $[Na^+]_i$ increased from 5 (Fig. 4A) to 90 mM (Fig. 4B). Single channel currents were evoked at V_m −140 to 140 mV with a 20 mV increment from V_m 0 mV. Only currents from selected voltages listed on the right side of the Fig. 4B are shown for simplicity. The experiment started with symmetrical solutions (30 mM KCl, 10 mM K-d-gluconate and 5 mM Na-d- gluconate and 85 n-methyl-d-glucamine) in both pipette and bath and then bath solutions (intracellular side) were replaced with solutions containing 30 mM KCl, 10 K-d-gluconate and 90 mM Na-d-gluconate. Traces in A and B are from the same patch and the order of the experiment was 90 mM Na^+ experiment first and 5 mM Na^+ experiment second. C. Current-voltage relationship of single Slack channel. Each current in Fig. 4C is an average of 2 to 18 (n=12.1±1.0 for 5 mM Na^+ experiments; n=13.5±1.1 for 90

mM Na^+ experiments) measurements from 4 patches of 5 mM Na^+ experiments and 4 patches of 90 mM Na^+ experiments. D. Relationship between V_m and NP_o. Each point in Fig. 3D is from 2 to 7 (n=5.4±0.4 for 5 mM Na^+ experiments; n=4.3±0.4 for 90 mM Na^+ experiments) measurements.

* shows statistical difference between 5 and 90 mM Na^+ experiments ($p<0.05$).

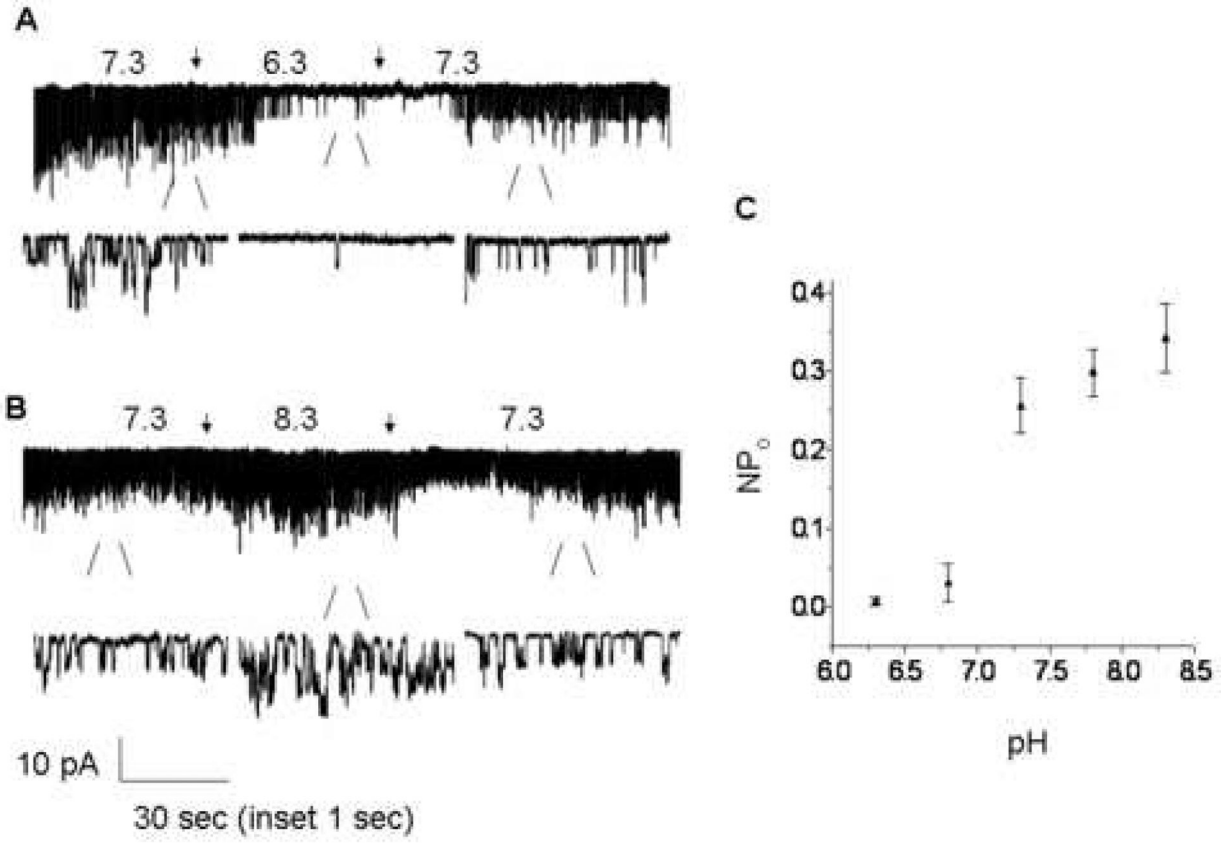

Figure 5.

Slack single channel activity is modulated by intracellular pH. Slack single channel activities were initially recorded using symmetrical solutions (see Fig. 1) in both pipette and bath at V_m = −100 mV. Intracellular pH was then increased or decreased by replacing the original bath solution with a solution having the same chemical composition but for pH which was adjusted by acetic acid or KOH. When exposed to acidic $[pH]_i$, Slack channel activity decreases (A). When exposed to basic $[pH]_i$, Slack channel activity increases (B). Insets show channel opening in higher resolution. Slack NP_o increases with increased pH (C) (n=3, V_m = −100mV).

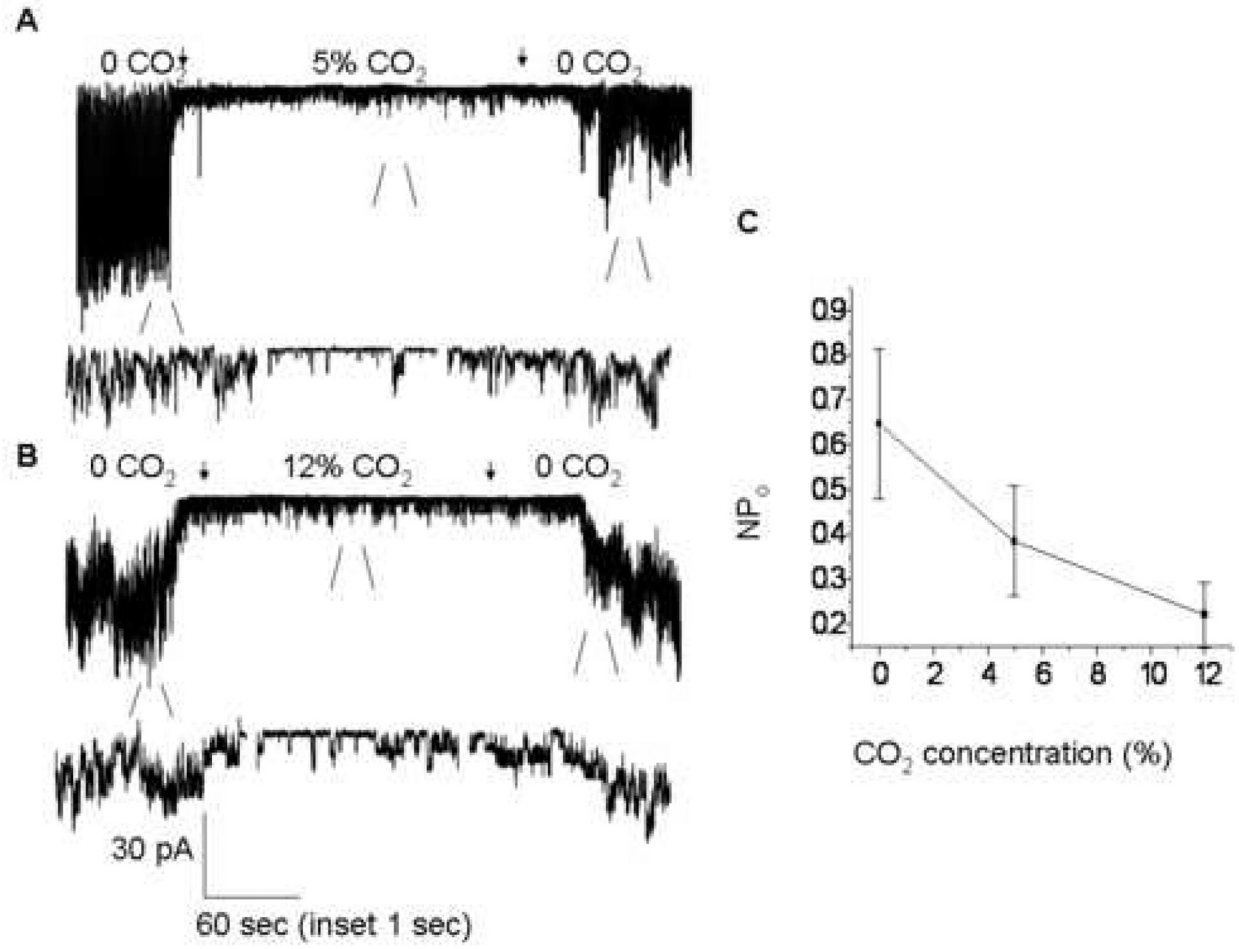

Figure 6.

Slack single channel activity is modulated by hypercapnia. Slack single channel activities were initially recorded using symmetrical solutions (see Fig. 1) in both pipette and bath at V_m = −100 mV. Bath (intracellular side) solution was replaced with a solution bubbled with either 5% (A) or 12% (B) CO_2. Insets show channel opening in higher resolution. Slack NP_o decreases with increased CO_2 concentration (C) (n=3, V_m = −100mV).

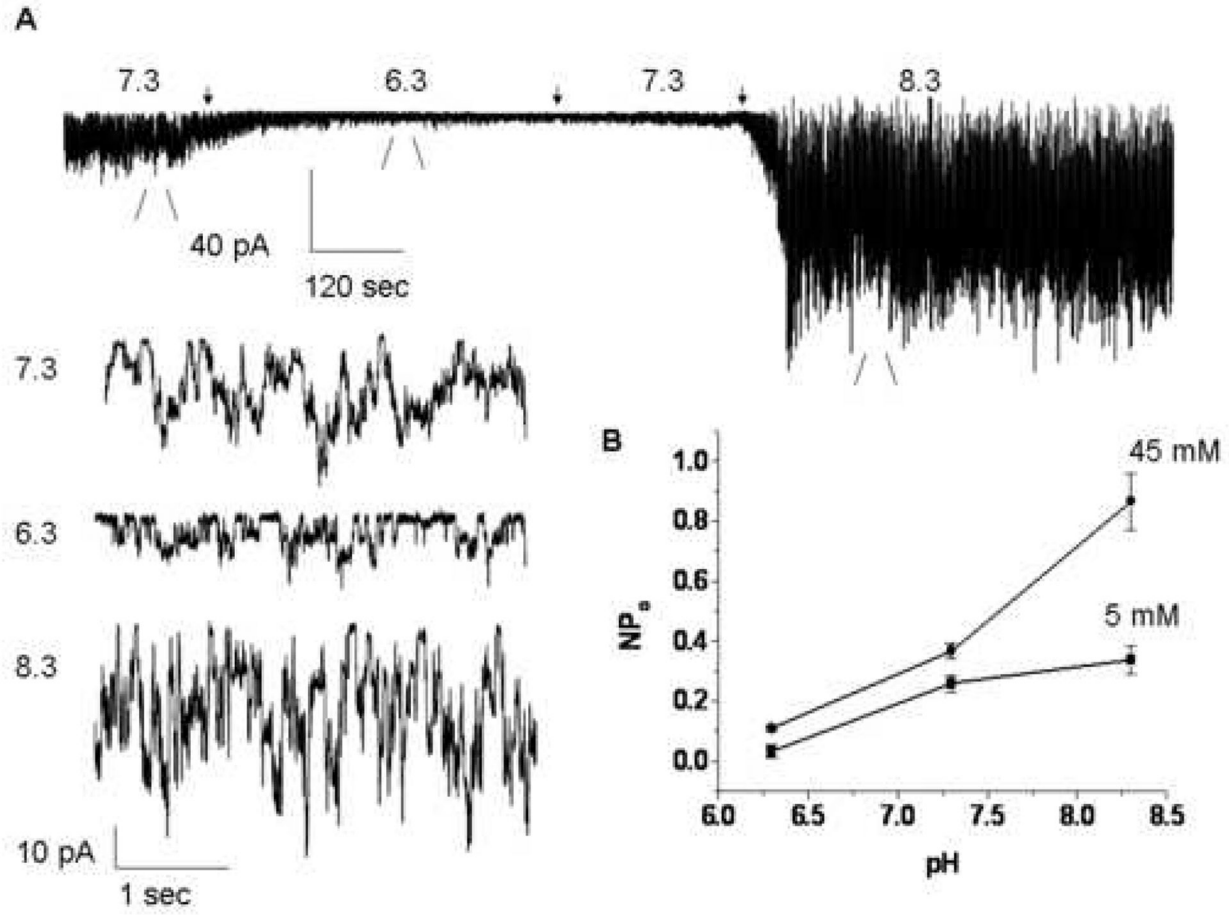

Figure 7.

Slack single channel activity is increased when exposed to increased $[Na^+]_i$ even in the presence of decreased $[pH]_i$. Slack channel activity was initially recorded using symmetrical solutions (130 mM KCl, 5 mM Na-d-gluconate, 85 mM n-methy-d-glucamine) in both pipette and bath at V_m = −100 mV and later the bath (intracellular side) was replaced with a solution containing 130 mM KCl, 45 mM Na-d-gluconate and 45 n-methy-d-glucamine. Slack single channel activity decreased when the bath was changed from 7.3 to 6.3 even when the bath contained high $[Na^+]_i$ (45 mM) (A). Slack single channel activity increased dramatically when exposed to $[pH]_i$ 8.3 (A). Insets show channel opening at different $[pH]_i$ in higher resolution. Slack NP_o is significantly increased with increased $[Na^+]_i$ at all pH values ($p < 0.05$) (B) (n=3, V_m= −100mV).