Direct interaction of the ATP-sensitive K^+ channel by the tyrosine kinase inhibitors imatinib, sunitinib and nilotinib

Robin Fröbom[1,2], Erik Berglund[1,3], Craig A. Aspinwall[4], Weng-Onn Lui[5,], Inga-Lena Nilsson[1,2], Catharina Larsson[5, 6], Robert Bränström*[1,2]

[1]Department of Molecular Medicine and Surgery, Karolinska Institutet, [2]Endocrine and Sarcoma Surgery Unit, Karolinska University Hospital, [3]Department of Transplantation Surgery, Karolinska University Hospital [4]Department of Chemistry and Biochemistry and Department of Biomedical Engineering, University of Arizona, Tucson, AZ, USA [5]Department of Oncology–Pathology, Karolinska Institutet, [6]Medical Unit Clinical Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden,

* Address correspondence to:

Robert Bränström, robert.branstrom@ki.se

Department of Molecular Medicine and Surgery

Karolinska Institutet

Karolinska University Hospital, L1:03

SE-171 76 Stockholm, Sweden

Telephone: +46851770000

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Abstract

The ATP-regulated K^+ channel (K_{ATP}) plays an essential role in the control of many physiological processes, and contains a ATP-binding site. Tyrosine kinase inhibitors (TKI) are commonly used drugs, that primarily target ATP-binding sites in tyrosine kinases. Herein, we used the patch-clamp technique to examine the effects of three clinically established TKIs on K_{ATP} channel activity in isolated membrane patches, using a pancreatic ß-cell line as a K_{ATP} channel source. In excised inside-out patches, the activity of the K_{ATP} channel was dose-dependently inhibited by imatinib with half-maximal concentration of approximately 9.4 μM. The blocking effect of imatinib was slow and reversible. No effect of imatinib was observed on either the large (K_{BK}) or the small (K_{SK}) conductance, Ca^{2+}-regulated K^+ channel. In the presence of ATP/ADP (ratio 1) addition of imatinib increased channel activity approximately 1.5-fold. Sunitinib and nilotinib were also found to decrease K_{ATP} channel activity. These findings are compatible with the view that TKIs, designed to interact at the ATP-binding pocket on the tyrosine receptor, also interact at the ATP-binding site on the K_{ATP} channel. Possibly, this might explain some of the side effects seen with TKIs.

Introduction

Tyrosine kinases are major regulators of several important physiological processes [1, 2]. Mutations in genes coding for tyrosine kinases are frequently observed in cancers and other pathologies [3]. Therapeutic interventions with tyrosine kinase inhibitors (TKI) were introduced about two decades ago, and imatinib was the first TKI approved for clinical use in chronic myeloid leukemia (targeting bcr-abl) [4]. Imatinib also became the first targeted therapy of a solid tumor, the gastrointestinal stromal tumor (targeting c-kit or PDGFR-α receptor) [5]. From a precision medicine standpoint, targeting mutated oncogenic signaling pathways revolutionized the treatment of hematological malignancies, as well as solid tumors. However, due to the abundance of tyrosine kinases and their involvement in normal cellular processes, side effects of TKI treatment are common. Both hematological and non-hematological side effects are frequent, ranging across a wide variety of different symptoms [6].

Although the target specificity varies between different TKIs, both type I and type II TKI share a mechanism of action that involves occupancy of ATP-binding sites in tyrosine kinases, which leads to conformational changes that inactivate the enzymatic activity, with subsequent effects on cellular signaling [7]. Type I TKIs (e.g. sunitinib) bind to the ATP site, whereas type II TKIs (e.g. imatinib, nilotinib) bind the ATP binding cleft and an adjacent binding site [8, 9].

In many cell types, the ATP-sensitive K^+ channel (K_{ATP} channel) connects metabolism to electrical activity. The K_{ATP} channel is composed of the pore forming K^+ inwardly-rectifying channel (Kir6.x) and an ATP-binding cassette, sub-family C (SURx). In pancreatic β-cells, the K_{ATP} channel is composed of KCNJ11 (Kir6.2) and ABCC8

(SUR1). The regulation of the K_{ATP} channel is primarily by changes in intracellular concentrations of ATP and ADP, where increased ATP/ADP ratio inhibits and decreased ratio stimulates K_{ATP} channel activity [10]. Thus, stimulation of the β-cell with glucose leads to glucose metabolism and a corresponding increased ATP/ADP ratio resulting in inhibition of K_{ATP} channel activity, depolarization of the cell membrane and subsequent activation of voltage-dependent Ca^{2+} channels, which triggers insulin secretion. Thus, agents that are capable of modulating the K_{ATP} channel activity may also affect fuel-induced insulin secretion.

The ATP-binding motif is a shared feature between the K_{ATP} channel and tyrosine kinase receptors. A direct effect of TKIs on the K_{ATP} channel is not known, but is theoretically possible. Therefore, we investigated whether TKIs affects K_{ATP} channel activity using K_{ATP} channels from pancreatic β-cells as a channel donor, since its wildly expressed and well-studied in this cell system.

Materials and Methods

Cell line – The murine ß-cell line MIN6m9 was used as a K_{ATP} channel source. The cell line is derived from an insulinoma [11]. Cells were maintained as previously described [12].

Tyrosine kinase inhibitors (TKIs) – Imatinib and nilotinib were kindly provided by Novartis (Basel, Switzerland), and sunitinib was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Imatinib mesylate was dissolved in purified H_2O (Milli-Q purification system, Millipore, MA, USA). Sunitinib malate and nilotinib were both dissolved in dimethyl sulfoxide (DMSO). Stock solutions were aliquoted and stored at -20°C prior to use. The concentration used in this study was choosen from a study on intracellular concentration of imatinib in clinical specimens, thereby yielding clinically relevant concenntrations [13]. The same molar concentrations were used for sunitinib and nilotinib, respectively.

Solutions – The extracelllar and intracellular solutions used were according to our previous report [14]. The standard extracellular solution (i.e. pipette solution) contained 138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, and 5 mM HEPES-NaOH at pH 7.4. The intracellular solution (i.e. the bath solution) consisted of 125 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 25 mM KOH, and 5 mM HEPES-KOH at pH 7.4. As indicated in text and figures, ATP was added as the Mg^{2+} salt to the "intracellular" solution and ADP was added as Na^+ salt. Mg^{2+} was added to maintain excess concentrations of Mg^{2+}.

Electrophysiology – K_{ATP} channel activity was recorded using the patch-clamp technique (12). P-2000 laser pipette puller (Sutter Instrument, CA, USA) was used to pull pipettes from 1.6 mm o.d. boroscilate glass tubes. Pipettes were sized to obtain resistance values between 3-5 MΩ. Currents were recorded using an HEKA EPC-10 patch-clamp amplifier (HEKA Elektronik GmbH, Germany). Membrane patches were excised and maintained in a nucleotide-free solution. 100 μM ATP was first added to test for channel inhibition. With the specified solutions used, ion currents were directed outward, and channel records were displayed in accordance with the convention that upward deflections denote outward currents. Room temperature (20-24°C) was maintained throughout all experiments. Channel activity was measured at 0 mV unless otherwise stated.

Data analysis - Data analyses generally followed the procedures described previously [15]. In short, open time kinetics were determined using TAC software (Bruxton, WA, USA) by digitizing segments of the current records (60-s long) and forming histograms of baseline and open-level data points. Analysis of the distribution of K_{ATP} channel open times was restricted to segments containing no more than two active channels. Events were identified using a 50% amplitude criterion. The kinetic constants were derived by approximation of the data to exponential functions by the method of maximum likelihood [16]. Channel activity (NP_O, where N is the number of active channels and P_o is the open probability) was calculated as the mean current ($\bar{I}$) divided by the single K_{ATP} channel current amplitude (i_x), (see Equation 1).

Equation 1.

$$NP_o = \frac{\bar{I}}{i_x}$$

Results are expressed as mean ± S.D., and statistical significance was analyzed using paired or unpaired Student's t-tests, and ANOVA-test as appropriate.

Detailed analysis of the dose-inhibition relation for imatinib was performed similarily to that previously described [17]. Acquired data fitted to a modified Hill equation (Equation 2), where [*imatinib*] is the concentration of the TKI, L is the maximal inhibition caused by the compound, IC_{50} is the concentration of imatinib causing half-maximal inhibition and h is the slope parameter corresponding to the Hill co-efficient.

Equation 2.

$$NP_o = 1 - \frac{L}{1 + \left(\frac{[Imatinib]}{IC_{50}}\right)^{-h}}$$

Results

K_{ATP} channel activity is inhibited by imatinib

Possible effects of imatinib on K_{ATP} channel activity were studied by electrophysiology in MIN6m9 cells. Fig. 1A shows a typical trace obtained by exposing an excised patch to 30 μM imatinib. Upon exposure of membrane patches to 30 μM imatinib, channel activity (NP_O) decreased from 1.96±0.66 to 0.55±0.19 (n = 11; P<0.001). In Fig. 1B, we exposed isolated patches to increasing concentrations of imatinib, ranging from 1 to 10 μM. In addition, we constructed a dose response curve using five different concentrations of imatinib ranging from 1 to 100 μM (1, 3, 10, 30 and 100 μM). Compiled data of the concentration-inhibition relation are shown in Fig. 1C. Values are expressed as the channel activity as a function of imatinib concentration (NP_O), normalized to the activity in standard intracellular solution before the addition of imatinib (NP_O control). The mean values were fitted to the Hill equation (Equation 2), and the concentration causing a 50% of maximal inhibition of channel activity was found to be 9.4 μM, with a Hill coefficient (h) of 1.6, and the maximal inhibition (L) of 0.76. These findings demonstrate that imatinib decreases K_{ATP} channel activity in a dose-dependent manner.

Imatinib does not alter kinetic properties of the K_{ATP} channel

We further examined the effects of imatinib on kinetic properties of the K_{ATP} channel. In Fig. 2A, the current-voltage (i-V) relationship was investigated at different membrane potentials (V_m) ranging from -40 mV to +80 mV. The presence of imatinib (30 μM) did not alter the rectification properties and the i-V relation compared to control situation. We next analyzed the open-time distribution in the absence (Fig. 2B) and presence (Fig. 2C) of imatinib using patches containing no more than two simultaneously active channels. Examples of channel openings on an expanded time scale for each respective condition are shown in Fig. 2B and C. The mean duration of openings under control conditions was 20.9±4.8 ms (*n* = 6), which is similar to what has been reported earlier for the K_{ATP} channel [18]. Similar channel kinetics were observed in the presence of imatinib, with a mean open time of 18.8±5.2 ms (*n* = 4; *n.s.*).

Inhibition of the K_{ATP} channel activity by different types of TKI

Using the same protocol as in Fig. 1, we exposed a series of inside-out membrane patches to 30 μM sunitinib (Fig. 3A) and 30 μM nilotinib (Fig. 3B). For sunitinib, NP_O decreased from 2.3±1.5 to 1.3±0.85 (n = 7; $P < 0.01$), and for nilotinib NP_O decreased from 2.7±1.4 to 1.1±0.76 (n = 5; $P < 0.05$). A summary of the relative inhibitory effects of 100 μM ATP, 30 μM imatinib, 30 μM sunitinib and 30 μM nilotinib as compared to control is shown in Fig. 3C. In conclusion, all three TKIs, imatinib, sunitinib and nilotinib, decreased K_{ATP} channel activity.

The effect of imatinib exposure on large conductance Ca^{2+}-regulated K^+ (K_{BK}) channel showed no effect on channel activity (data not shown). Exposing inside-out patches to 30 μM imatinib did not affect the mean current of K_{BK} channel. K_{BK} channel activity (NP_O) was 0.49±0.2 during control solution, compared with 0.52±0.2 in the presence of imatinib (n = 3; *n.s.*). The activity of the small Ca^{2+}-regulated K^+ conductance channel (K_{SK}) was also unaffected after exposure to 30 μM imatinib (data not shown).

Imatinib activates the K_{ATP} channel in the presence of a low ATP/ADP ratio

Fig. 4A shows a recording of K_{ATP} channels in membrane excised in nucleotide-free solution, and sequently exposed to ATP, ATP + ADP, and ATP + ADP + imatinib. As expected, addition of 100 μM ATP potently blocked the channel activity. In the continuing presence of ATP, addition of 100 μM ADP restored the channel activity, and finally, in the presence of both nucleotide, addition of 30 μM imatinib resulted in a further increase of channel activity. Fig. 4B summarizes the data from multiple membrane patches using the same protocol.

Discussion

In the present study, we report that three TKIs established in clinical use inhibit the activity of K_{ATP} channels in membrane patches excised from murine β-cells. The effect is likely due to direct interaction with the K_{ATP} channel protein, since the measurements were performed on isolated membrane patches where cell metabolism is omitted. The effect of TKIs was reversible with the channel activity returning after TKI washout. However, the channel activity is not immediately recovered as seen after ATP removal [19]. It is likely that TKIs, known to interact on the ATP binding site on the tyrosine kinase, also interact with the ATP binding site on the K_{ATP} channel. Similar to ATP, imatinib does not alter the channel conductance or kinetics properties. This finding, combined with no observed effects on Ca^{2+} and voltage-activated K^+ channels (K_{BK} and K_{SK} channels), further supports the notion that imatinib, sunitinib and nilotinib interact with the K_{ATP} channel protein.

An intriguing finding, with unknown physiological impact *in vivo*, is that imatinib activates the K_{ATP} channel in the presence of ATP and ADP (100 μM each). Our hypothesis that imatinib interacts with the ATP binding site on the K_{ATP} channel in a competitive manner is supported by the notion that on molar basis, imatinib is a weaker channel blocker than ATP, and that stimulation of K_{ATP} channel requires ATP hydrolysis, since a non-metabolizable ATP analogue (ATP-γ-S) does not activate the channel in the presence of ADP [18].

The K_{ATP} channel is involved in several cellular functions and is present in multiple tissues including the central and peripheral nervous system, the pituitary gland, different types of muscles and pancreatic β-cells. Due to the abundant expression, interaction with the K_{ATP} channel could potentially explain some of the side effects seen during TKI treatment, such as gastrointestinal motility problems, cardiovascular, and neurological side effects [20]. To our knowledge, this is the first study to show a direct interaction with the K_{ATP} channel, even though it has been hypothesized to impact the K_{ATP} channel in some studies [21-23].

Type II TKI inhibitors have traditionally been thought to be more specific, due to binding an allosteric site in addition to the ATP-binding site [8]. This view has been challenged [24]. A broad analysis of different TKIs revealed that type II inhibitors generally exhibited a higher selectivity for their target kinase compared to type I, although it varied between individual TKIs [25]. According to our findings, both type I and type II inhibitors were observed to inihibit K_{ATP} channel activity, possibly suggesting their ability to act on ATP-binding sites of other proteins, in this case the K_{ATP} channel. It could be speculated that other inhibitors that target ATP-binding motifs, such as the ERK inhibitors, also affects the K_{ATP} channel but is not within the scope of the current study.

In gastrointestinal tumor, a *proof-of-concept* study found the intracellular concentration of imatinib to vary between 4.6-36.6 ng/mg wet tissue [13]. An estimate assuming that tumor density is close to fat (0.92 kg/L) would result in an intracellular concentration of 8.6-68.2 μM. In our view, the concentrations used in this study are likely in the range of the expected intracellular concentrations *in vivo* during per label imatinib use, further strengthening the current findings as clinically relevant.

Several studies have reported positive outcomes on diabetes upon imatinib and sunitinib treatment [26-28]. It is hypothesized that TKIs may affect insulin release or other unknown mechanisms to achieve the beneficial effects. Considering that pancreatic ß-cell insulin release is a fine-tuned process where small changes in K_{ATP} channel activity may cause membrane depolarization and affect down-stream hormone exocytosis [29], the present results warrants further investigation.

In summary, we present the first evidence of an immediate functional interaction between TKI and the K_{ATP} channel under physiologically relevant concentrations, which are concentrations also expected to be found in target tissues under per label TKI use. Our results may explain some of the commonly seen non-desirable off-target side effects with TKI treatment, as well as at least partly explain its potential anti-diabetogenic effects. This study adds further knowledge to these commonly used drugs in modern oncological treatment.

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References

1. Schlessinger, J. and A. Ullrich, Growth factor signaling by receptor tyrosine kinases. Neuron, 1992. 9(3): p. 383-91.
2. Manning, G., et al., The protein kinase complement of the human genome. Science, 2002. 298(5600): p. 1912-34.
3. Lemmon, M.A. and J. Schlessinger, Cell signaling by receptor tyrosine kinases. Cell, 2010. 141(7): p. 1117-34.
4. Druker, B.J., et al., Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med, 2001. 344(14): p. 1031-7.
5. Buchdunger, E., et al., Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther, 2000. 295(1): p. 139-45.
6. Hartmann, J.T., et al., Tyrosine kinase inhibitors - a review on pharmacology, metabolism and side effects. Curr Drug Metab, 2009. 10(5): p. 470-81.
7. Vijayan, R.S., et al., Conformational analysis of the DFG-out kinase motif and biochemical profiling of structurally validated type II inhibitors. J Med Chem, 2015. 58(1): p. 466-79.
8. Liu, Y. and N.S. Gray, Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol, 2006. 2(7): p. 358-64.
9. Mol, C.D., et al., Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem, 2004. 279(30): p. 31655-63.
10. Berggren, P.O. and O. Larsson, Ca2+ and pancreatic B-cell function. Biochem Soc Trans, 1994. 22(1): p. 12-8.
11. Miyazaki, J., et al., Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology, 1990. 127(1): p. 126-32.
12. Berglund, E., et al., Evidence for Ca(2+)-regulated ATP release in gastrointestinal stromal tumors. Exp Cell Res, 2013. 319(8): p. 1229-38.
13. Berglund, E., et al., Intracellular concentration of the tyrosine kinase inhibitor imatinib in gastrointestinal stromal tumor cells. Anticancer Drugs, 2014. 25(4): p. 415-22.
14. Bränström, R., et al., Long chain coenzyme A esters activate the pore-forming subunit (Kir6. 2) of the ATP-regulated potassium channel. J Biol Chem, 1998. 273(47): p. 31395-400.
15. Bränström, R., et al., Direct inhibition of the pancreatic beta-cell ATP-regulated potassium channel by alpha-ketoisocaproate. J Biol Chem, 1998. 273(23): p. 14113-8.
16. Colquhoun, D. and F.J. Sigworth, Single-Channel Recording, second ed., Springer, Boston, MA. 1983.
17. Berglund, E., et al., Functional role of the Ca(2)(+)-activated Cl(-) channel DOG1/TMEM16A in gastrointestinal stromal tumor cells. Exp Cell Res, 2014. 326(2): p. 315-25.
18. Larsson, O., et al., Stimulation of the KATP channel by ADP and diazoxide requires nucleotide hydrolysis in mouse pancreatic beta-cells. J Physiol, 1993. 463: p. 349-65.
19. Proks, P., et al., Running out of time: the decline of channel activity and nucleotide activation in adenosine triphosphate-sensitive K-channels. Philos Trans R Soc Lond B Biol Sci, 2016. 371(1700).

20. Jahangir, A. and A. Terzic, K(ATP) channel therapeutics at the bedside. J Mol Cell Cardiol, 2005. 39(1): p. 99-112.
21. Maihöfer, N.A., et al., Imatinib relaxes the pulmonary venous bed of guinea pigs. Respir Res, 2017. 18(1): p. 32.
22. Rieg, A.D., et al., Tyrosine kinase inhibitors relax pulmonary arteries in human and murine precision-cut lung slices. Respir Res, 2019. 20(1): p. 111.
23. Kim, B.J., et al., Effects of imatinib mesylate in interstitial cells of Cajal from murine small intestine. Biol Pharm Bull, 2010. 33(6): p. 993-7.
24. Zhao, Z., et al., Exploration of type II binding mode: A privileged approach for kinase inhibitor focused drug discovery? ACS Chem Biol, 2014. 9(6): p. 1230-41.
25. Davis, M.I., et al., Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol, 2011. 29(11): p. 1046-51.
26. Louvet, C., et al., Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice. Proc Natl Acad Sci U S A, 2008. 105(48): p. 18895-900.
27. Huda, M.S., et al., Tyrosine kinase inhibitor sunitinib allows insulin independence in long-standing type 1 diabetes. Diabetes Care, 2014. 37(5): p. e87-8.
28. Veneri, D., M. Franchini, and E. Bonora, Imatinib and regression of type 2 diabetes. N Engl J Med, 2005. 352(10): p. 1049-50.
29. Cook, D.L., et al., ATP-sensitive K+ channels in pancreatic beta-cells. Spare-channel hypothesis. Diabetes, 1988. 37(5): p. 495-8.

Figure Legends

Figure 1. **Effects of imatinib on K_{ATP} channel activity in inside-out membrane patches from the pancreatic ß-cell line MIN6m9.** ***A*** Example recording of K_{ATP} currents upon addition of 30 µM imatinib. In the presence of imatinib, the channel activity (NP_O) decreased approximately 4-fold, from 1.4 to 0.40. ***B*** Example of K_{ATP} currents upon increasing imatinib concentrations showed dose-dependent decrease of K_{ATP} channel activity, where NP_o decreased from 2.4 to 1.8 (1 µM imatinib), 1.5 (3 µM imatinib), and 0.9 (10 µM imatinib). **C** Dose-response curve for K_{ATP} channel activity after exposure to different concentrations of imatinib. The data were fitted to the *Hill*-equation, and results are presented as mean values ± S.D. for n = 6-11 observations. Arrowheads indicate the zero current level.

Figure 2. **Current-voltage (*i-V*) relationship and single channel kinetics of imatinib on K_{ATP} channel.** ***A*** Single K_{ATP} channel *i-V* relationship in the absence (filled circles) and presence (open circles) of 30 µM imatinib. Each value represents mean ± S.D., n = 3–5 recordings. Values were fitted to third order regression lines, yielding r^2 values >0.97 for both curves. ***B*** and ***C*** Frequency *versus* lifetime histograms of channel openings under control conditions (*B*) and in the presence of 30 µM imatinib (*C*). In control, the distribution of K_{ATP} channel open times could be described by a single exponential function with a time constant (τ_o) of 22.9 ms (n = 1509 events). In the presence of 30 µM imatinib, the distribution of open time could be fitted with a τ_o of 24.1 ms (n = 841 events). The insets show typical channel activity on an expanded time scale. Arrowheads indicate the current level when the channel is closed, and bin width was set to 10 ms.

Figure 3. **Effect of sunitinib and nilotinib on K_{ATP} channel activity in inside-out membrane patches.** Representative inside-out recording of K_{ATP} channel current in the presence of ***A*** 30 μM sunitinib (NP_O decreased approximately 2-fold, from 2.5 to 1.3) and ***B*** 30 μM nilotinib (NP_O decreased more than 3-fold, from 4.1 to 1.3). ***C*** Summary of K_{ATP} channel inhibition upon exposure to 100 μM ATP, 30 μM imatinib, 30 μM sunitinib, or 30 μM nilotinib. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *n.s.*, not significant. Arrowheads indicate the zero current level.

Figure 4. **Imatinib activates K_{ATP} channel current in the presence of ATP/ADP.** ***A*** Representative recording of K_{ATP} channel current in inside-out membrane patch isolated from MIN6m9 cells. Addition of 100 μM ATP rapidly decreased channel activity, which was restored upon addition of 100 μM ADP. Addition of 30 μM imatinib further increased channel activity. Addition of 100 μM ATP potently blocked the channel, and NP_O decreased from 2.0 to 0.1. In the continuing presence of ATP, addition of 100 μM ADP restored the channel activity and NP_O increased to 2.2. Finally, in the same recording and with both nucleotides present, addition of 30 μM imatinib resulted in a further increase of NP_O to 2.8. Arrowhead indicates the zero-current level. ***B*** Summary of consecutive exposures to ATP, ADP and imatinib. * $P<0.05$, *** $P < 0.001$.

Figure 1

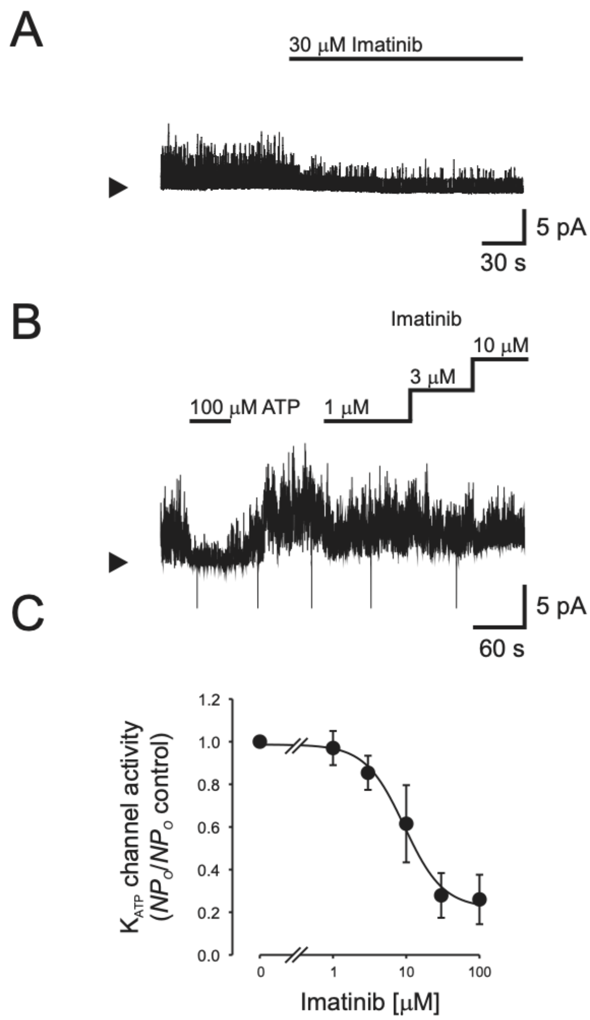

A
30 μM Imatinib
5 pA
30 s
B
Imatinib
10 μM
3 μM
100 μM ATP
1 μM
5 pA
60 s
C
1.2
1.0
0.8
0.6
0.4
0.2
0.0
K_{ATP} channel activity
(NP_o/NP_o control)
0
1
10
100
Imatinib [μM]

Figure 2

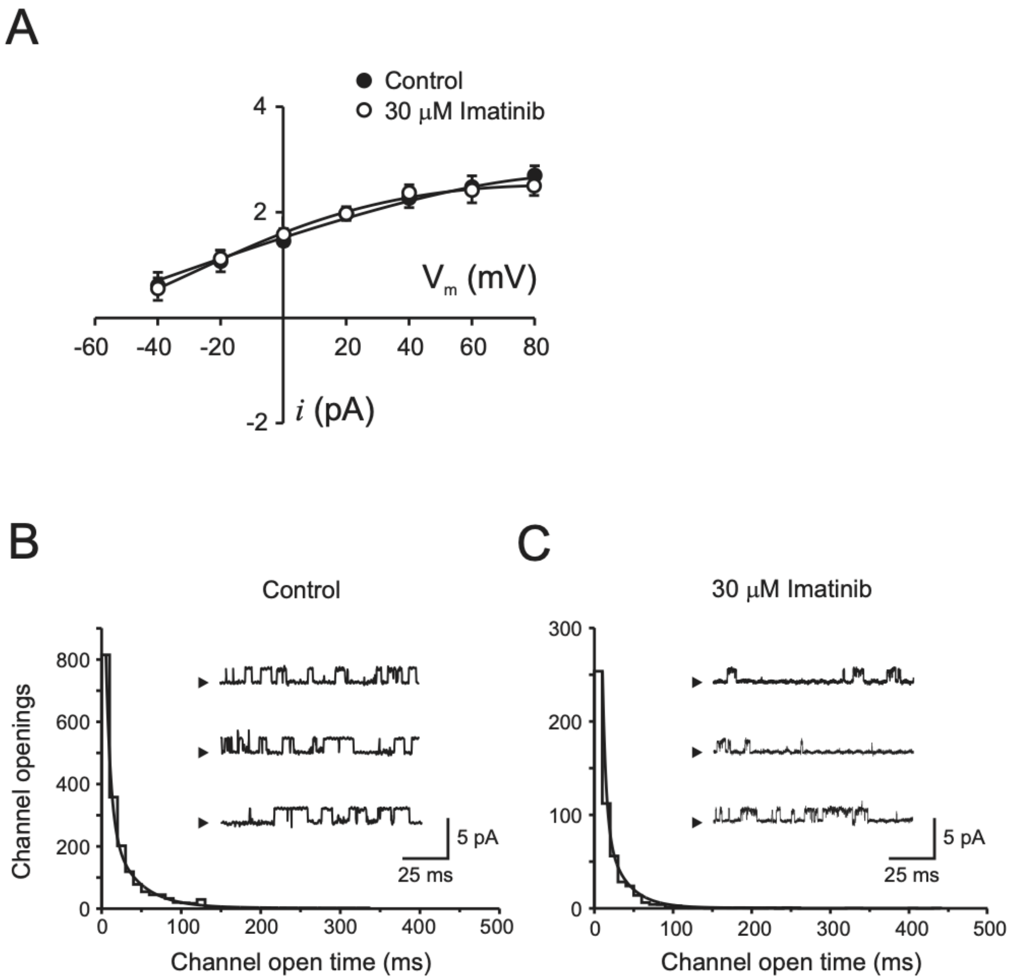
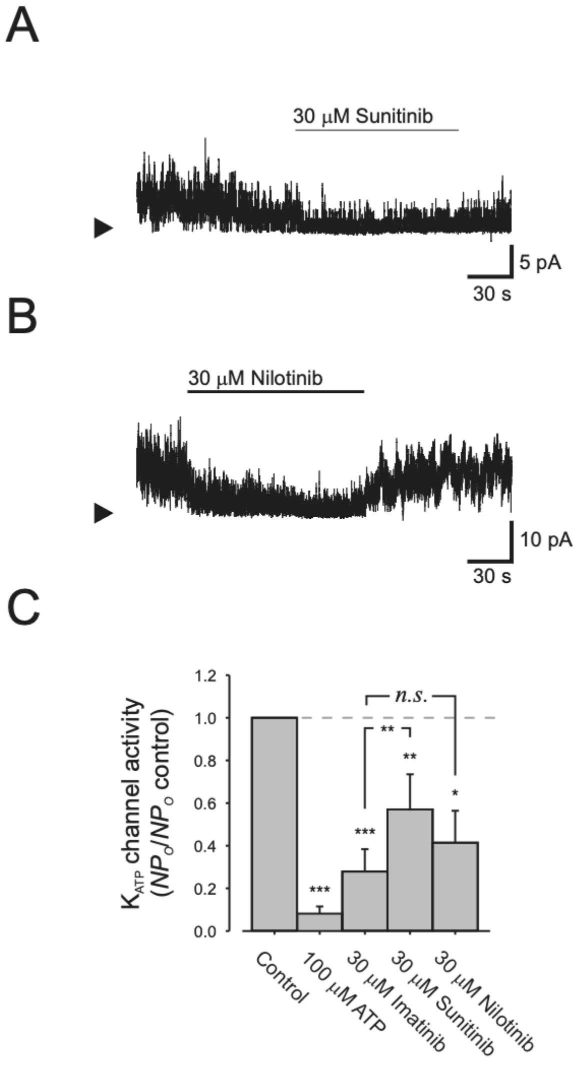
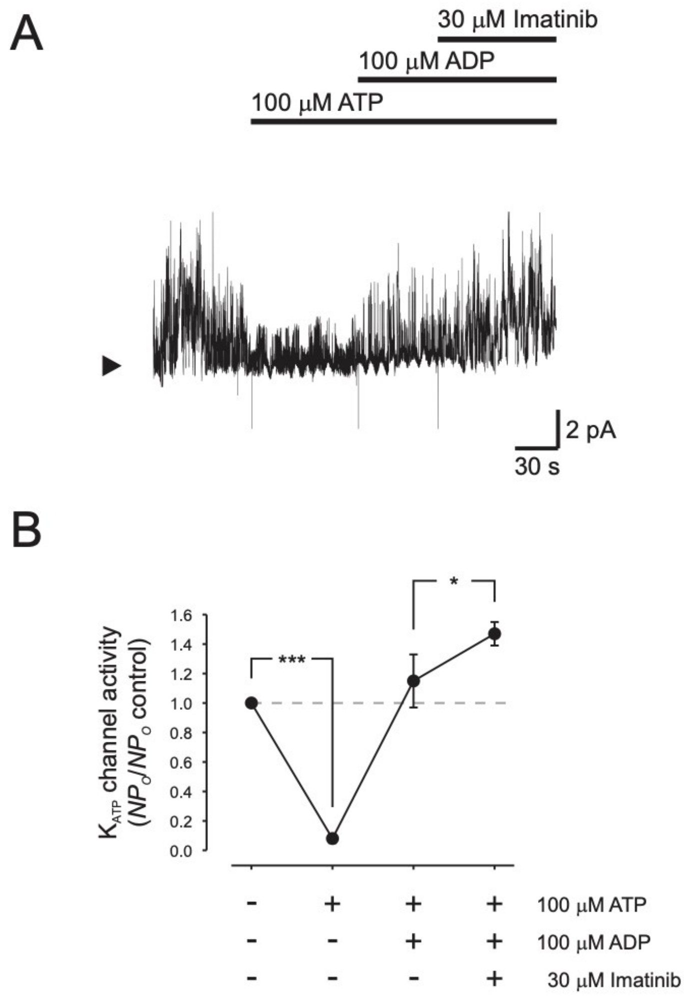

A
Control
30 μM Imatinib
4
2
-2
-60
-40
-20
20
40
60
80
V_m (mV)
i (pA)
B
Control
Channel openings
800
600
400
200
0
0
100
200
300
400
500
5 pA
25 ms
Channel open time (ms)
C
30 μM Imatinib
300
200
100
0
0
100
200
300
400
500
5 pA
25 ms
Channel open time (ms)

Figure 3



A
30 μM Sunitinib
5 pA
30 s
B
30 μM Nilotinib
10 pA
30 s
C
K_{ATP} channel activity
(NP_o/NP_o control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
n.s.
**
**
***
***
*
Control
100 μM ATP
30 μM Imatinib
30 μM Sunitinib
30 μM Nilotinib

Figure 4


A
30 μM Imatinib
100 μM ADP
100 μM ATP
2 pA
30 s
B
K_{ATP} channel activity
(NP_o/NP_o control)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
*
100 μM ATP
100 μM ADP
30 μM Imatinib