Modulation of sulphonylurea block of K_{ATP} channels by adenosine nucleotides

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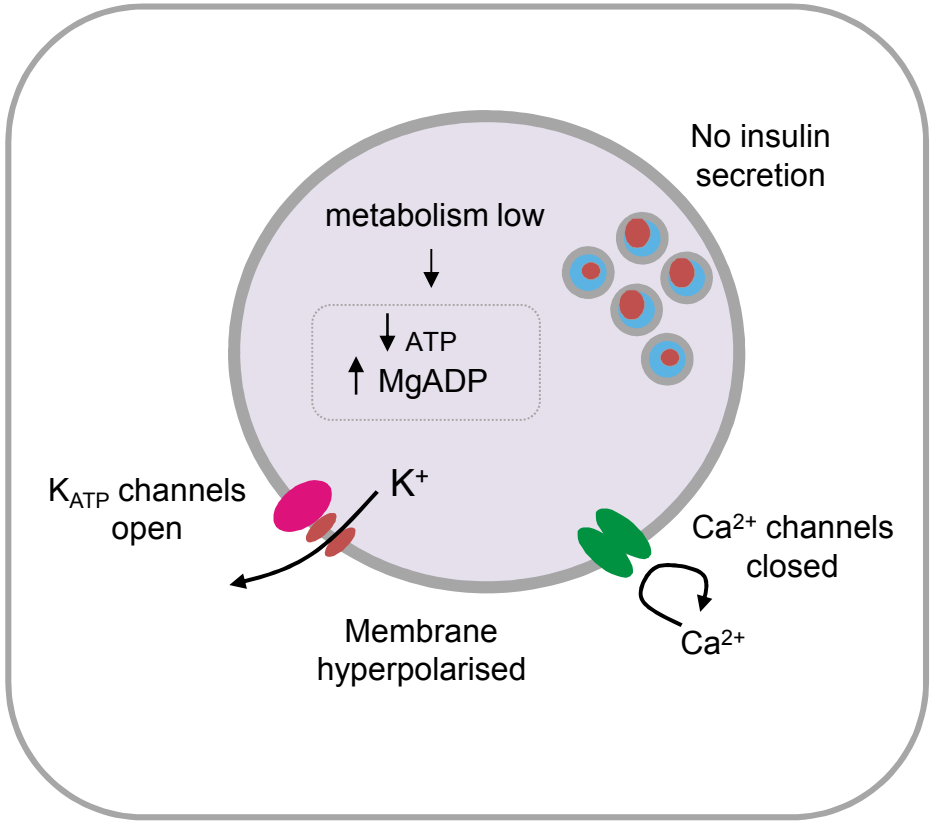
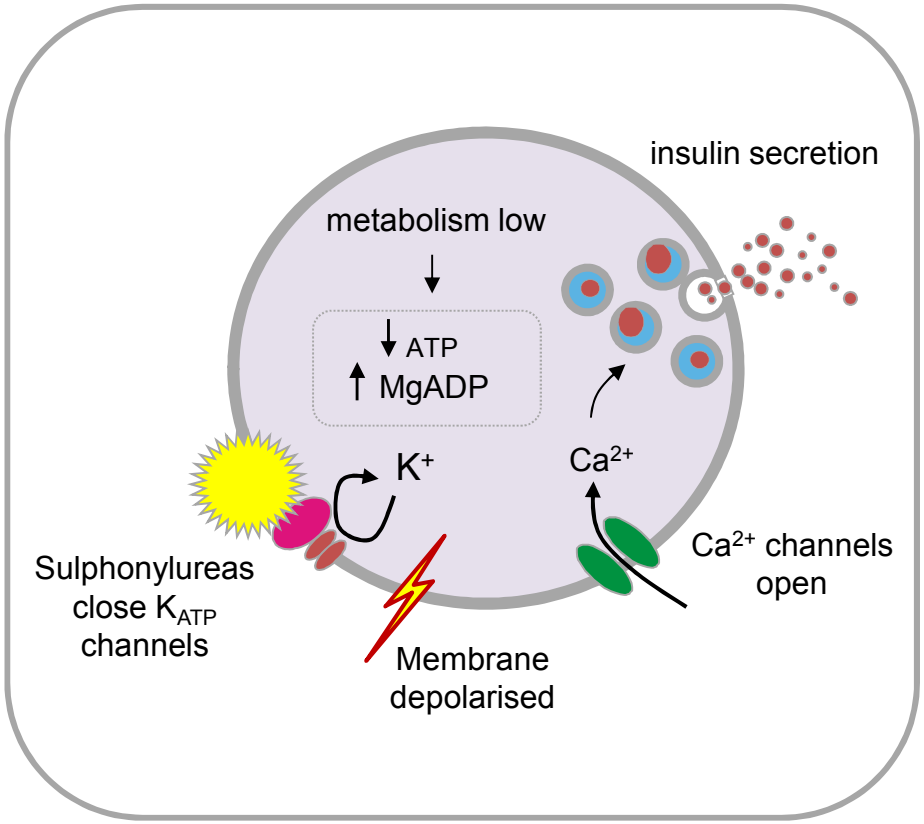
Stimulation of insulin secretion by sulphonylureas

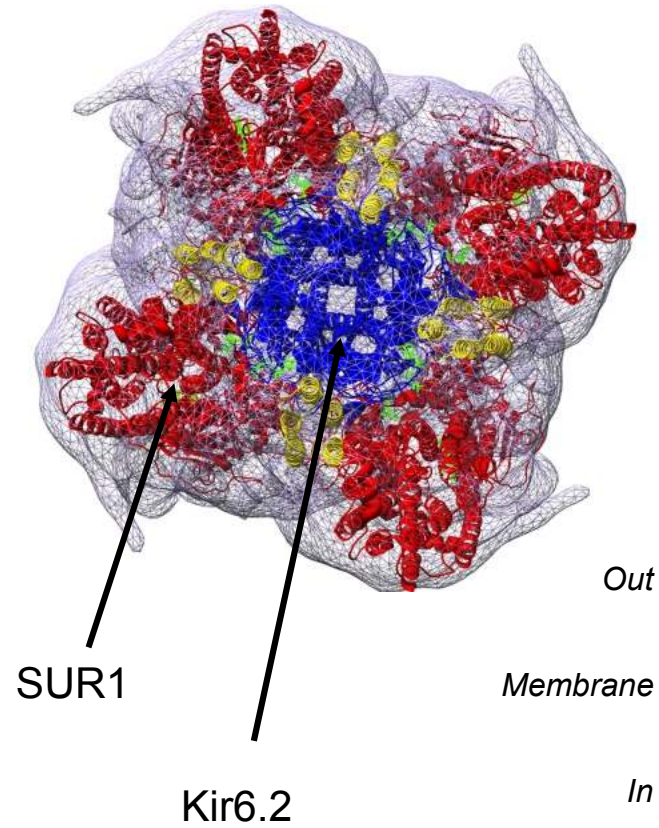


Metabolism LOW
K_{ATP} channels open
No insulin secreted

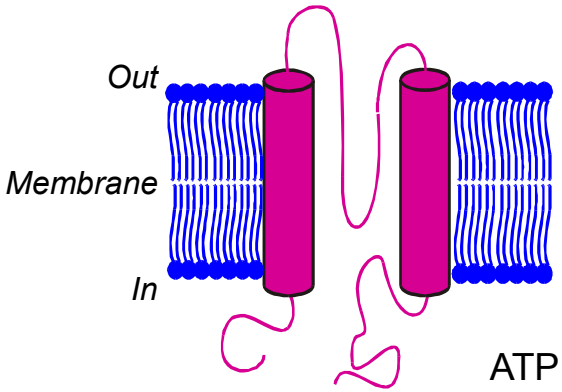


Metabolism LOW
K_{ATP} channels closed
Insulin secretion

The β-cell K_{ATP} channel



Mikhailov et al (2005) EMBO J 24, 4166

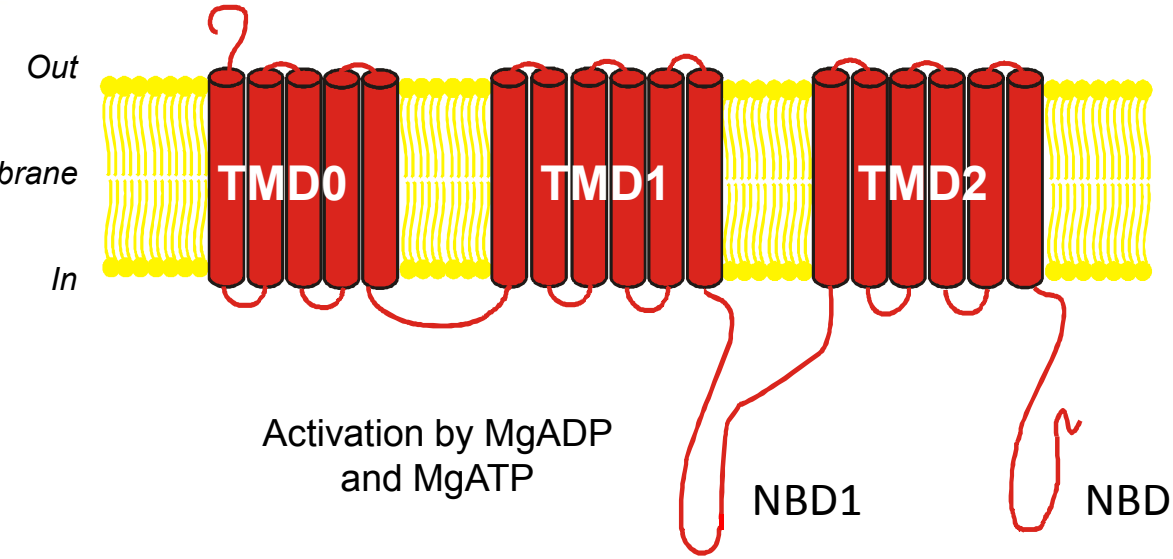


Pore subunit

(Kir6.2)

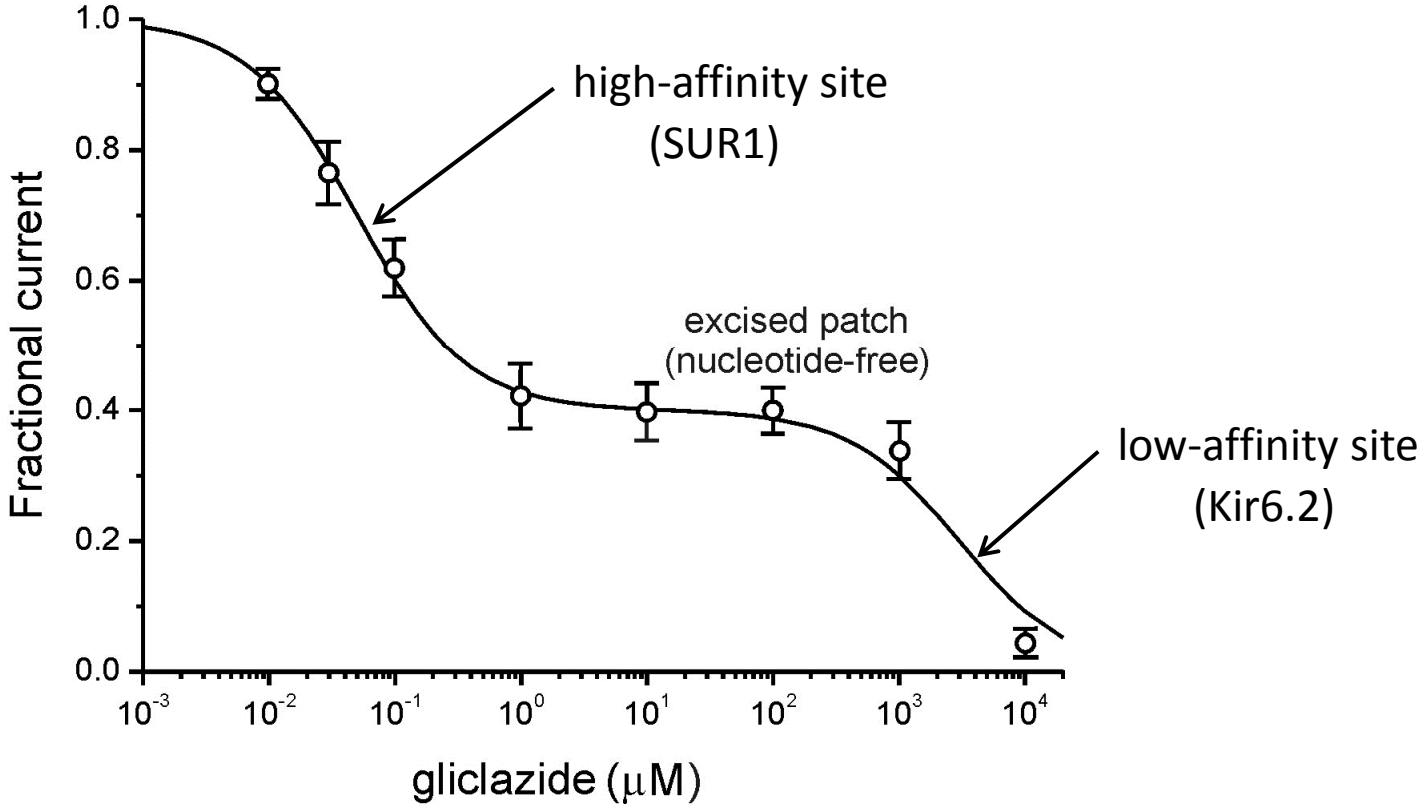
ATP inhibitory site

Sulphonylurea receptor (SUR1)



Activation by MgADP
and MgATP

Dual action of sulphonylureas:
1.Direct block



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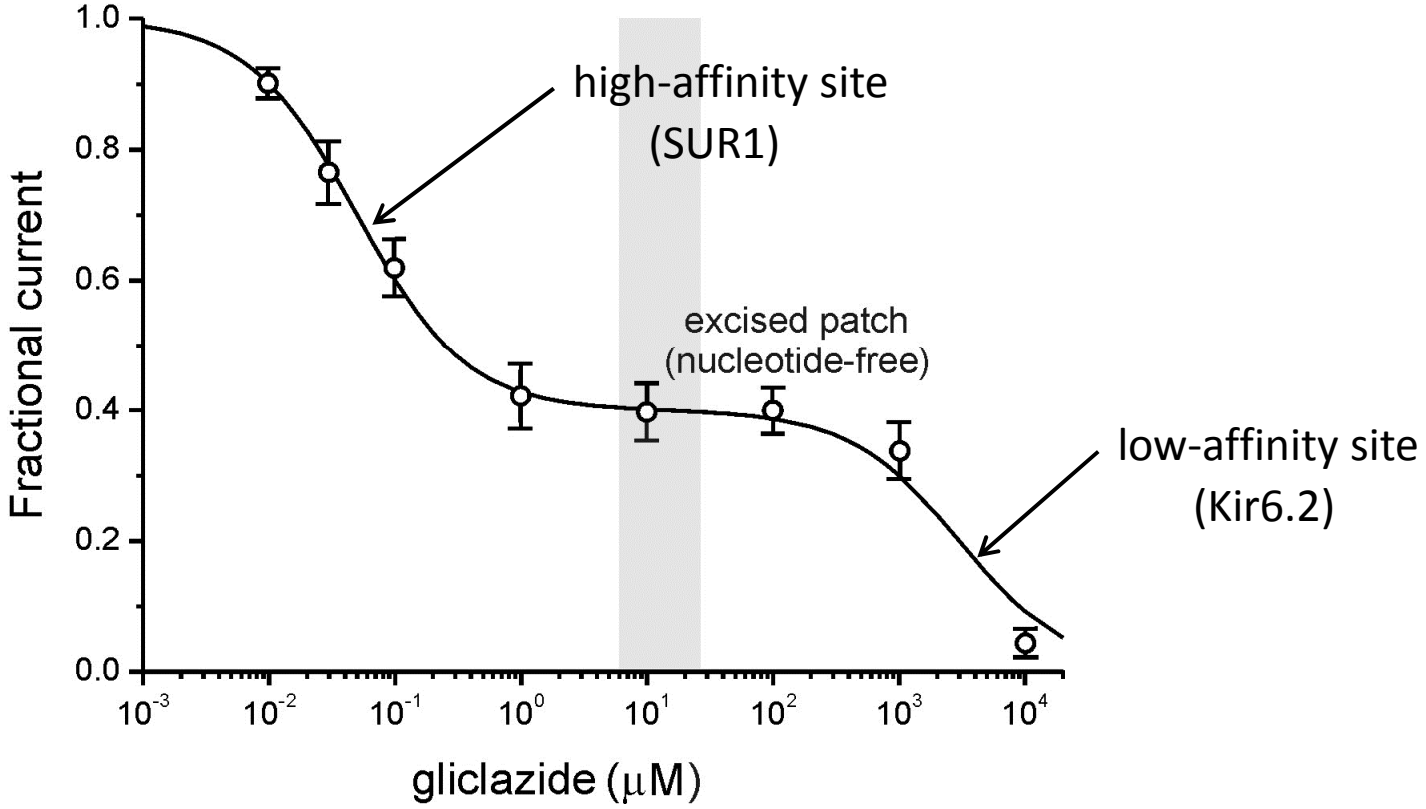
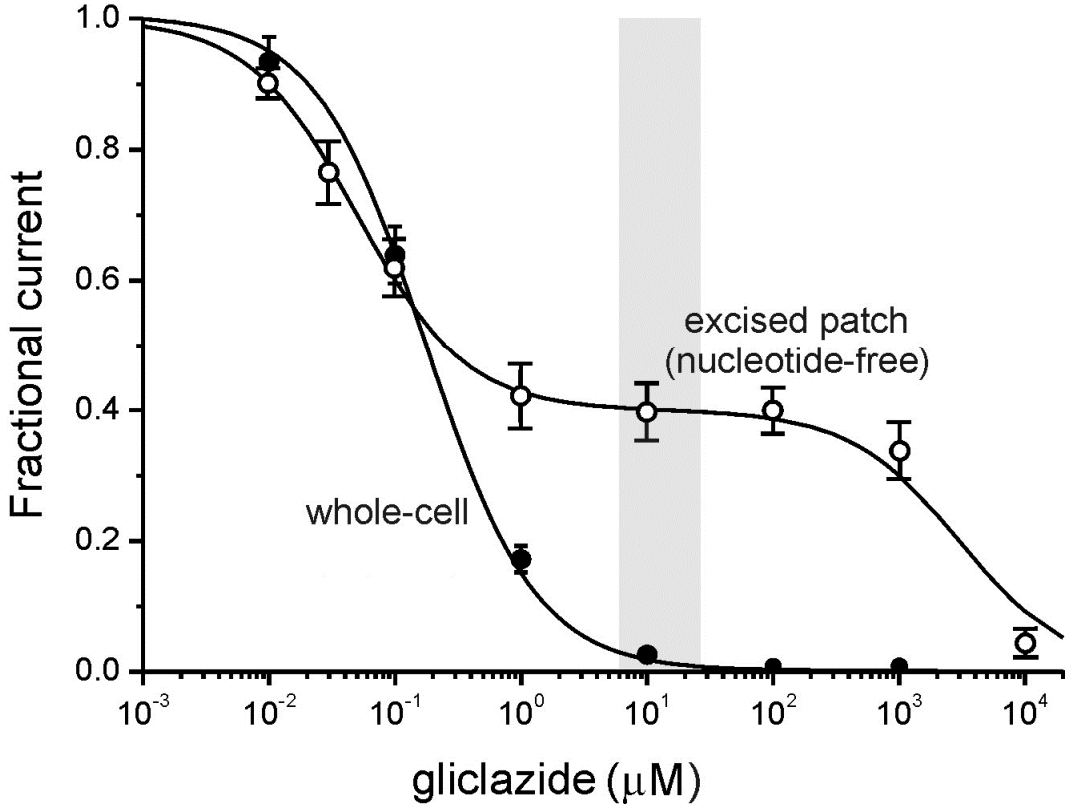
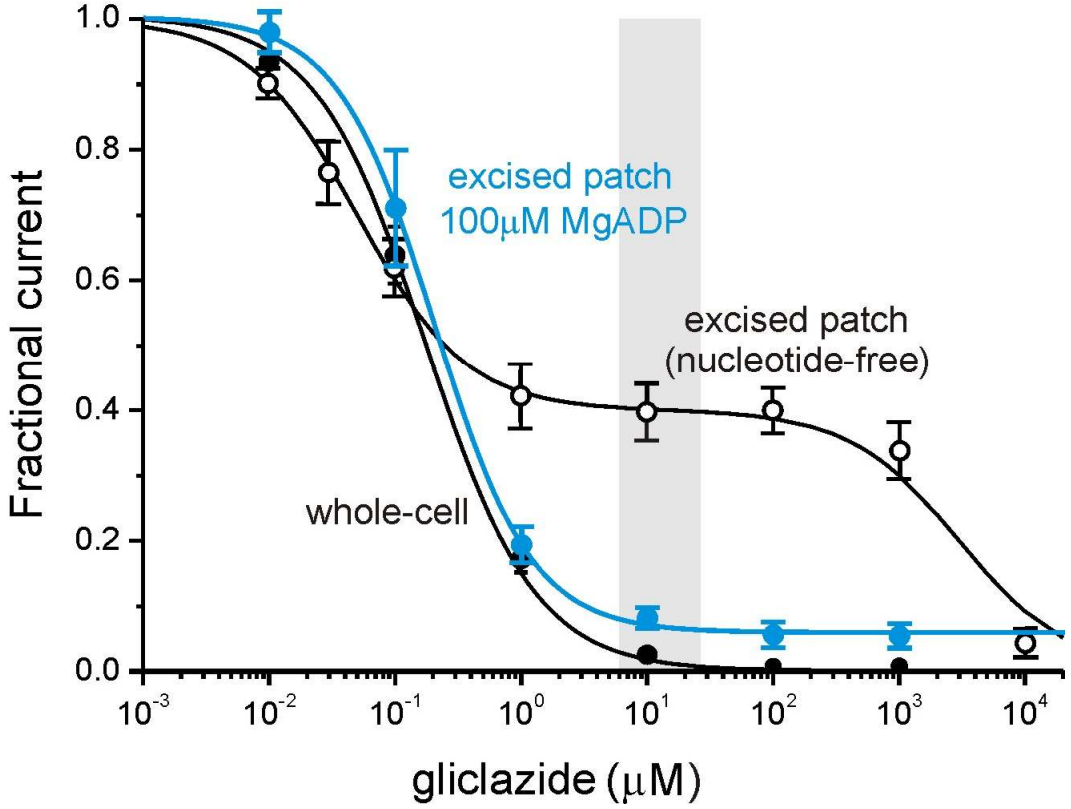


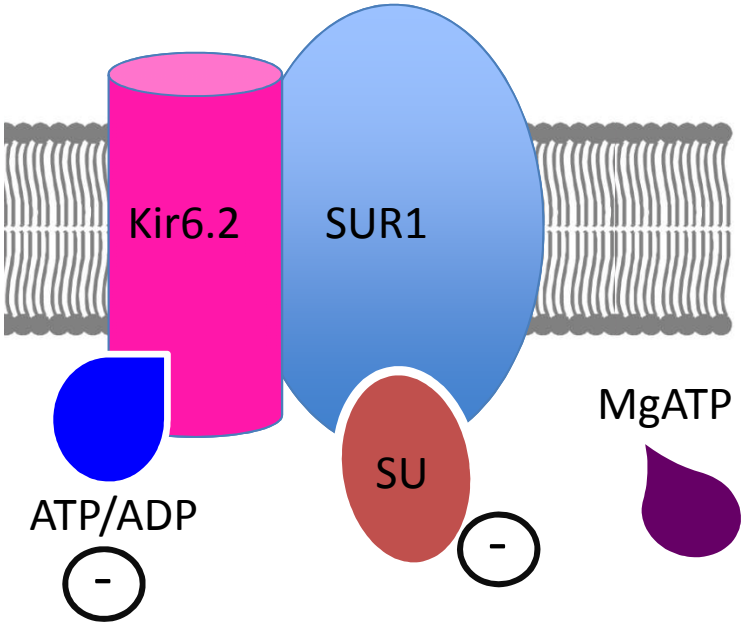
Dual action of sulphonylureas:



Dual action of sulphonylureas:



Dual action of sulphonylureas:
2. Suppression of activation by Mg-nucleotides



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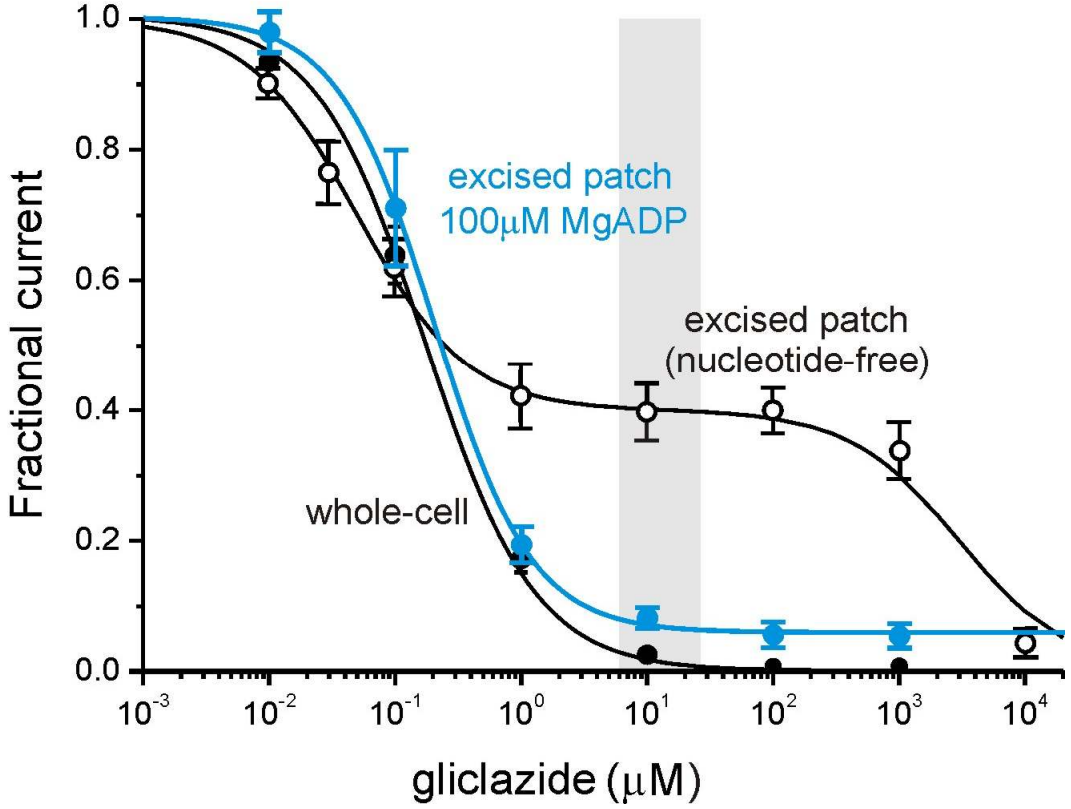
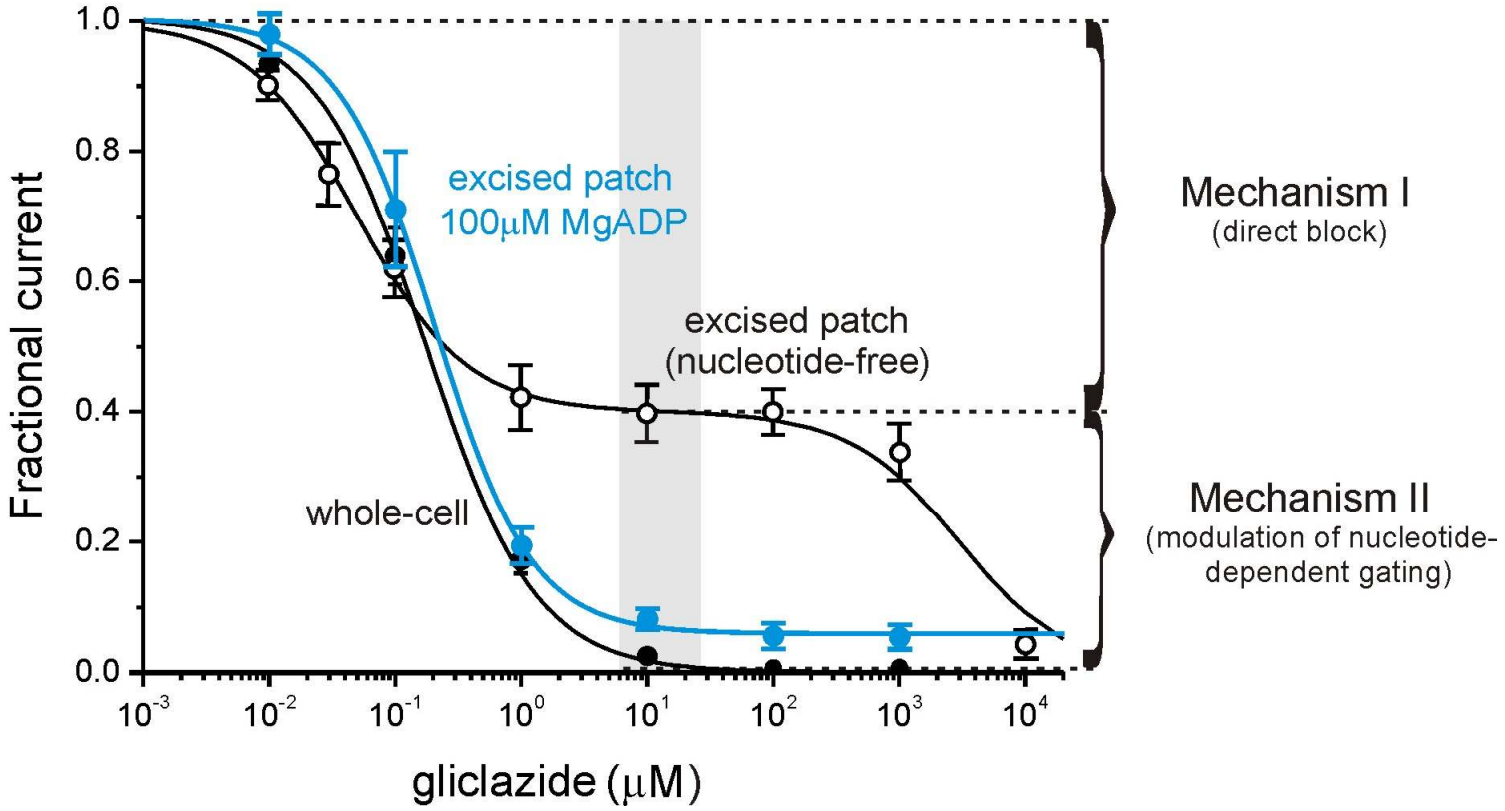


Dual action of sulphonylureas:

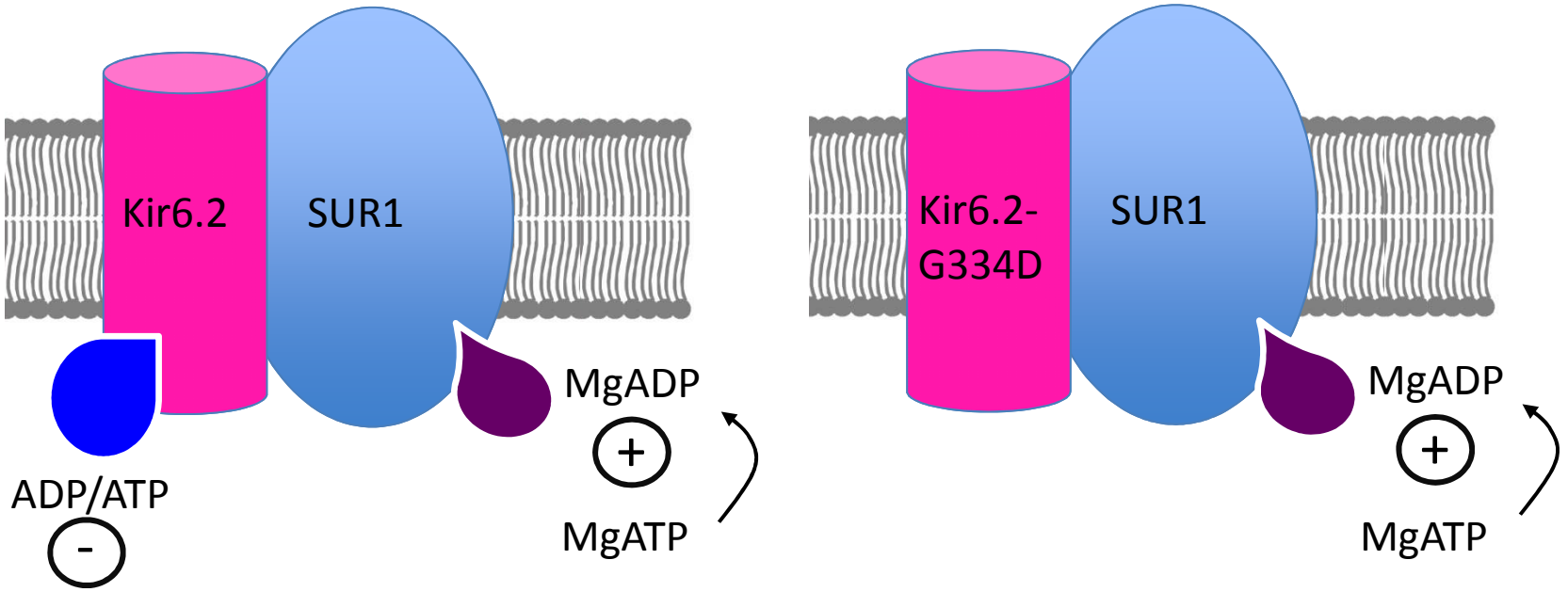


Questions:

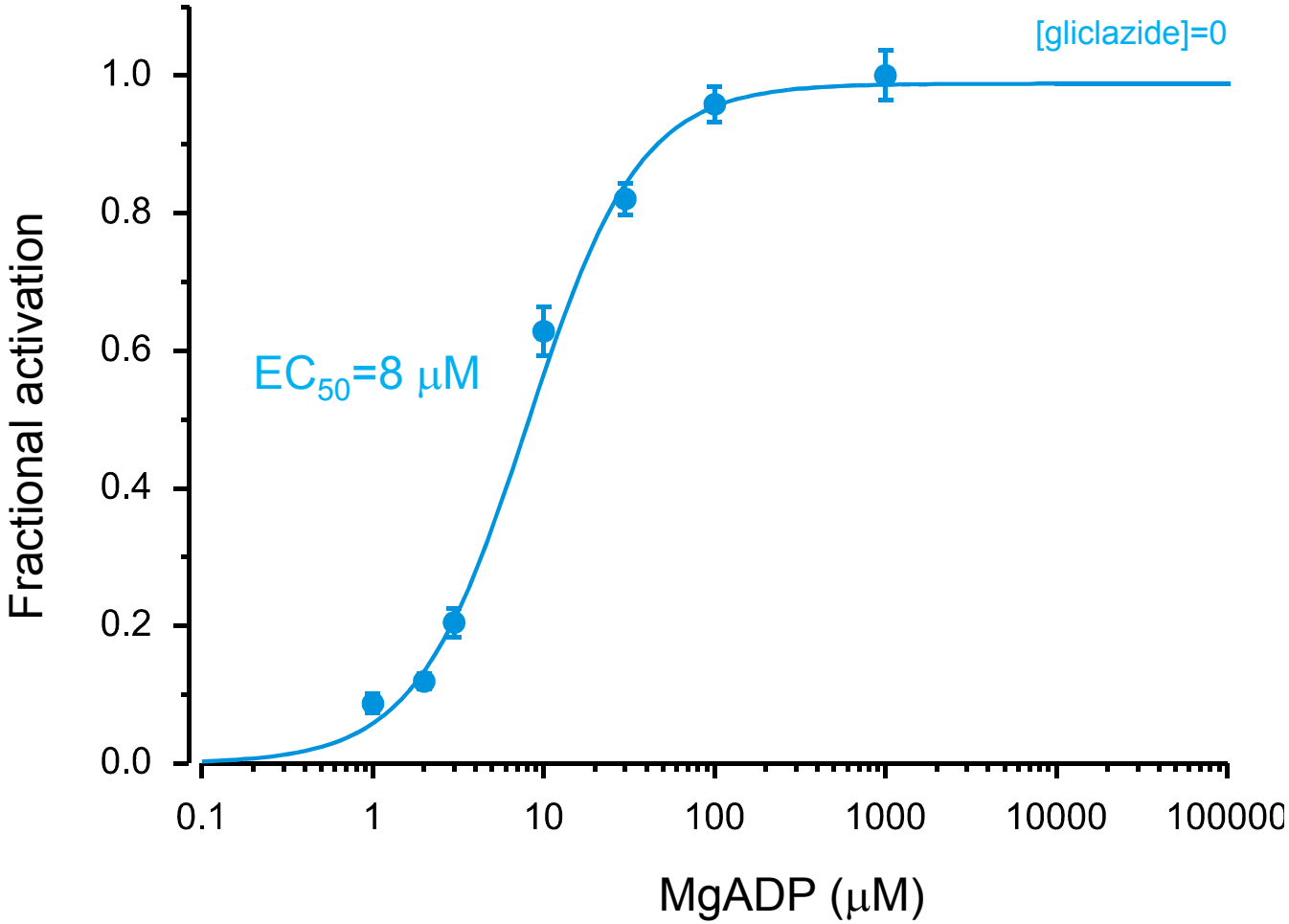
- Suppression of nucleotide activation by sulphonylureas was investigated only for MgADP, not for MgATP
- What is the molecular basis for mechanism II ?
- Can sulphonylureas also affect the inhibitory effects of nucleotides?
- How is sulphonylurea efficacy affected in patients with K_{ATP} channel mutations ?
- Why patients with neonatal diabetes require higher doses of sulphonylureas compared to patients with type 2 diabetes?

1.Molecular mechanism for suppression of Mg-nucleotide activation of K_{ATP} channels by sulphonylureas

ATP-insensitive mutant (Kir6.2-G334D/SUR1) to study Mg-nucleotide activation in isolation from nucleotide inhibition



Suppression of Mg-nucleotide activation of K_{ATP} channels by gliclazide



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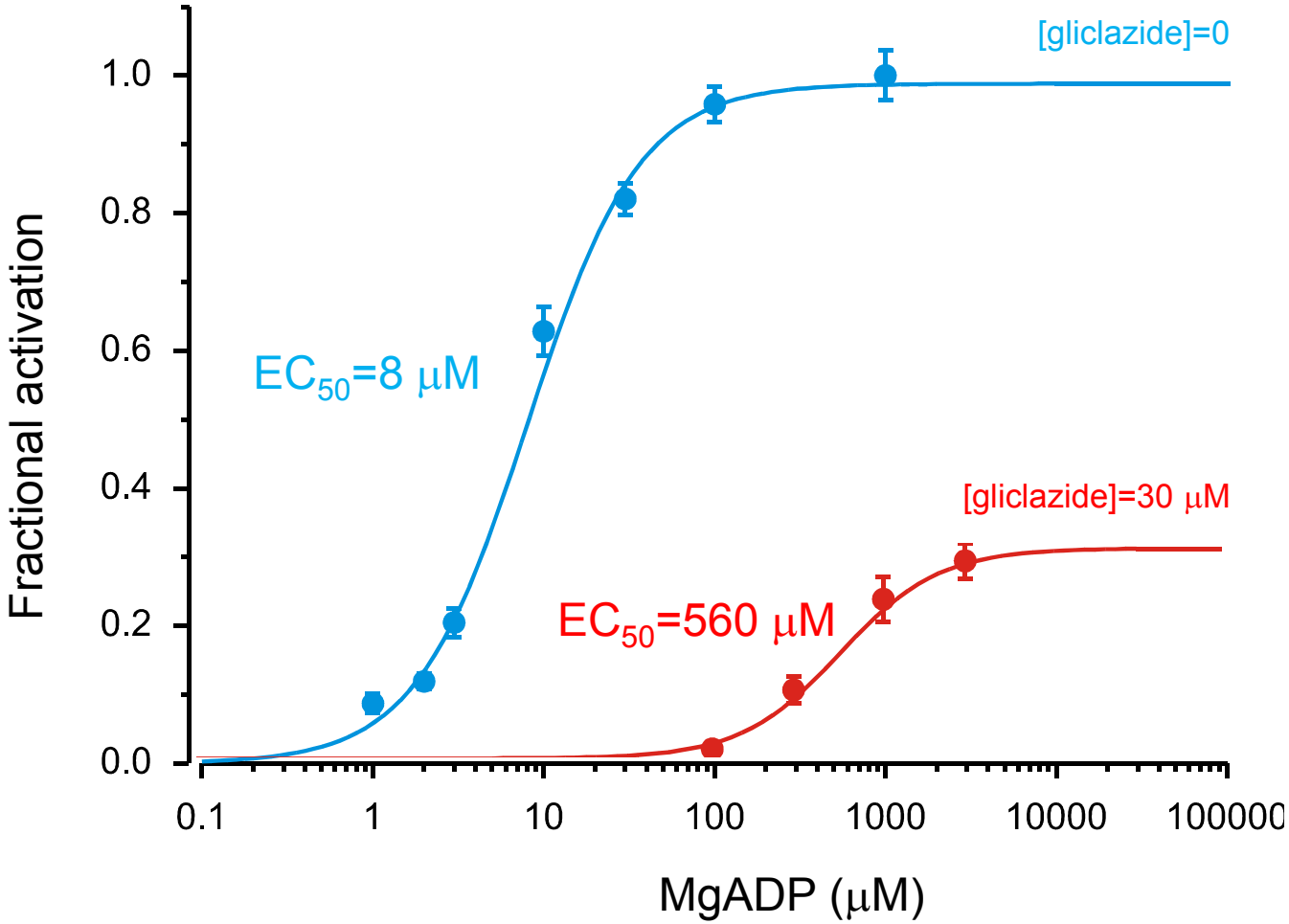

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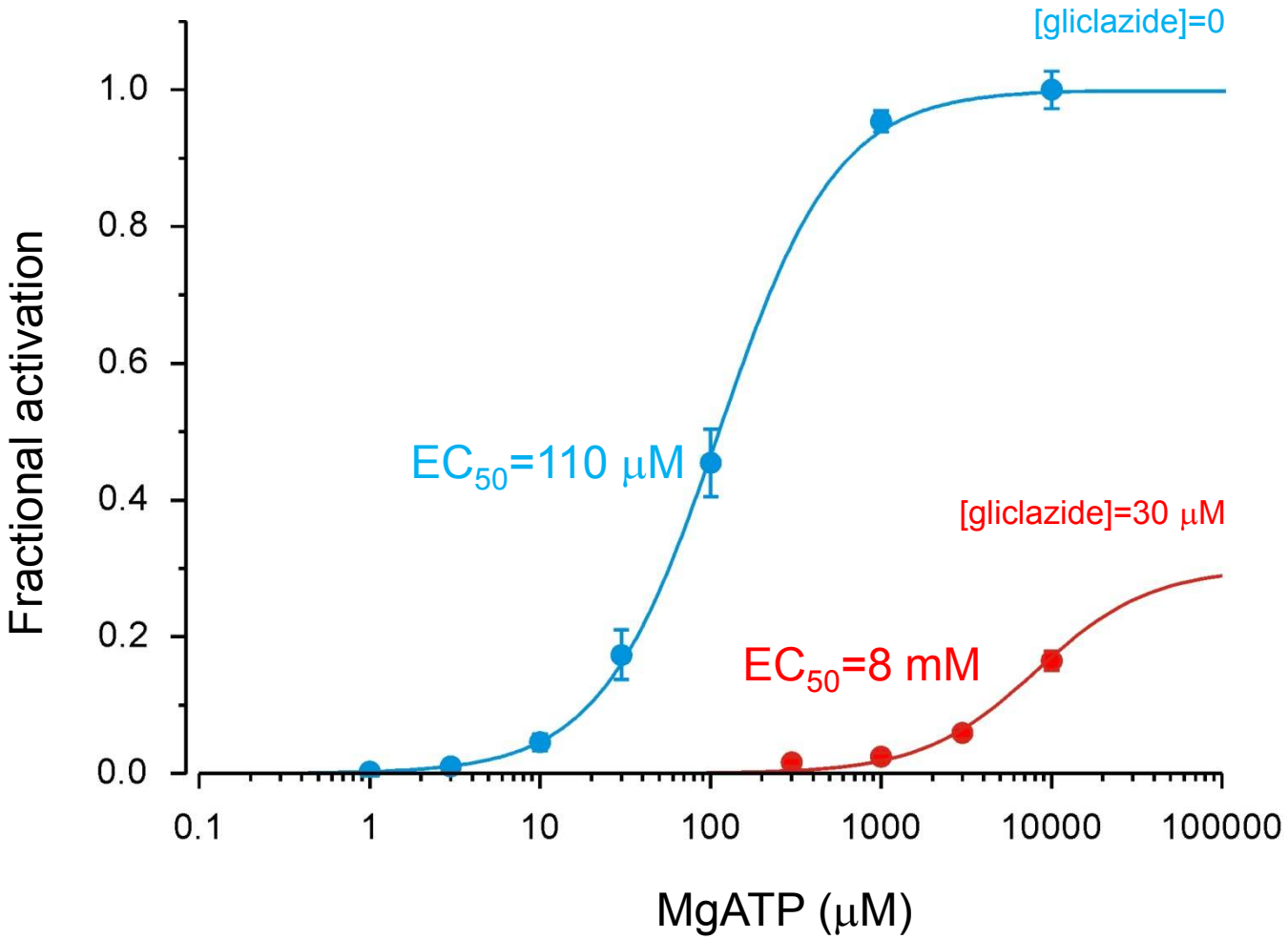

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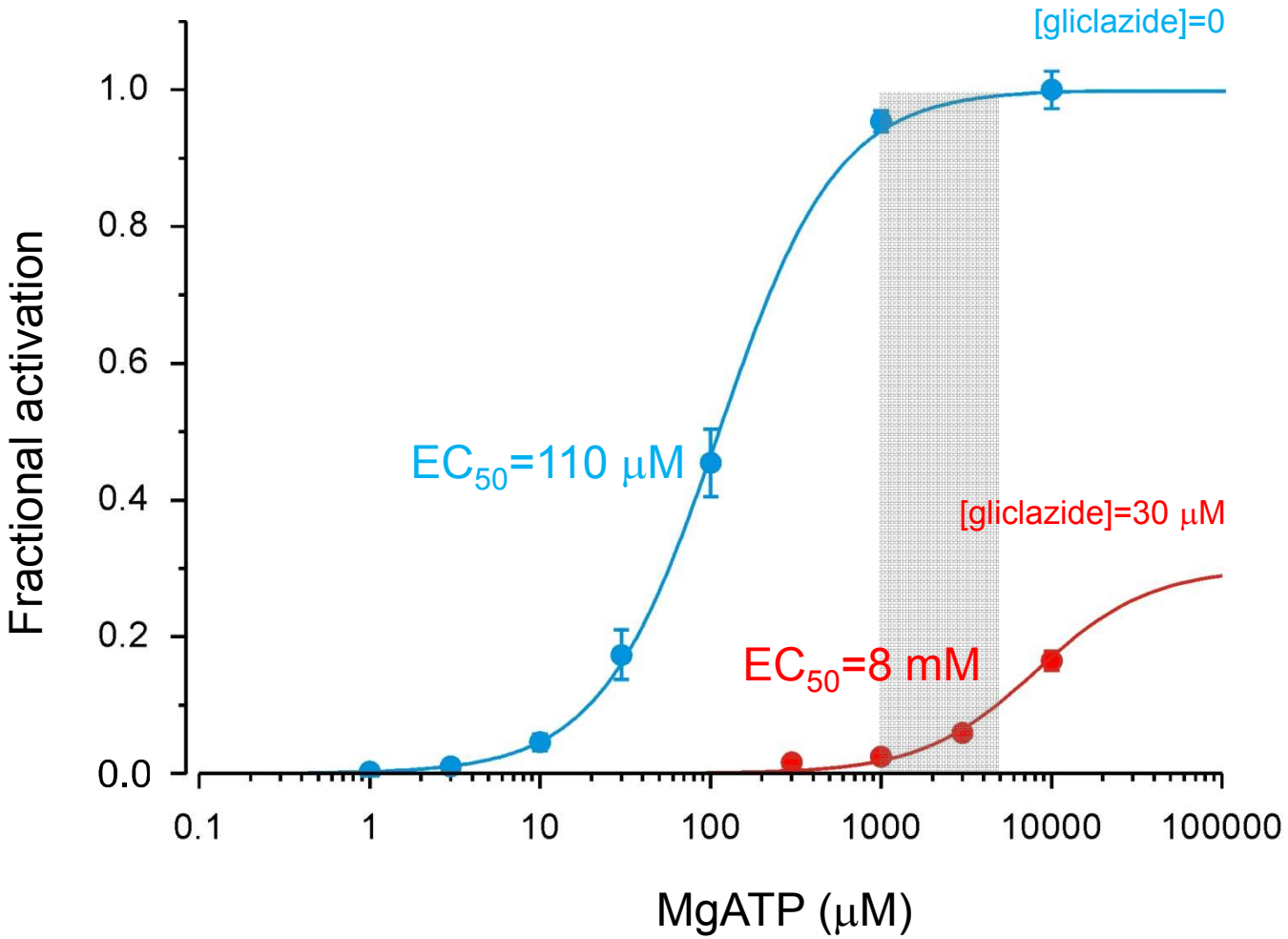
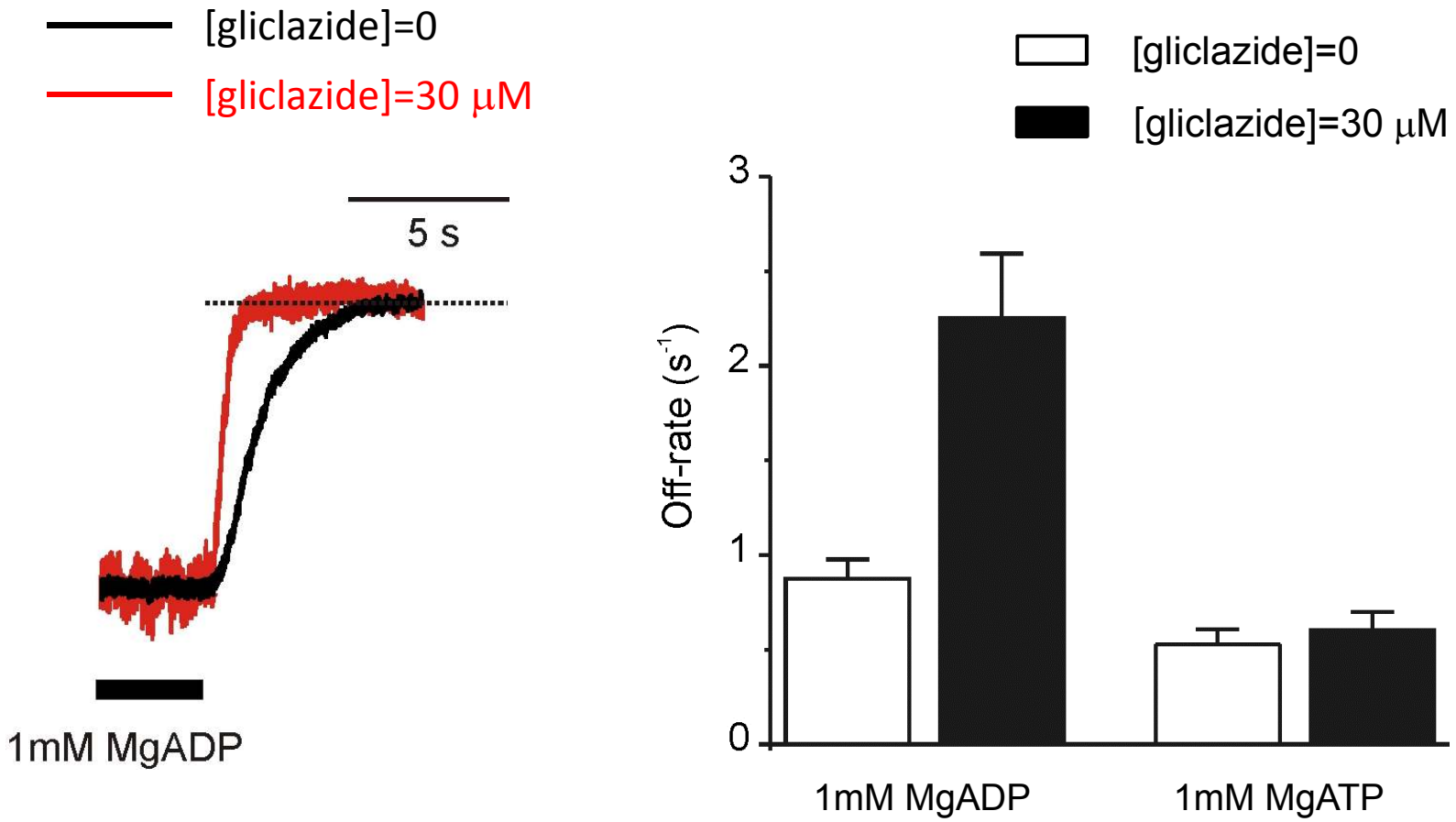
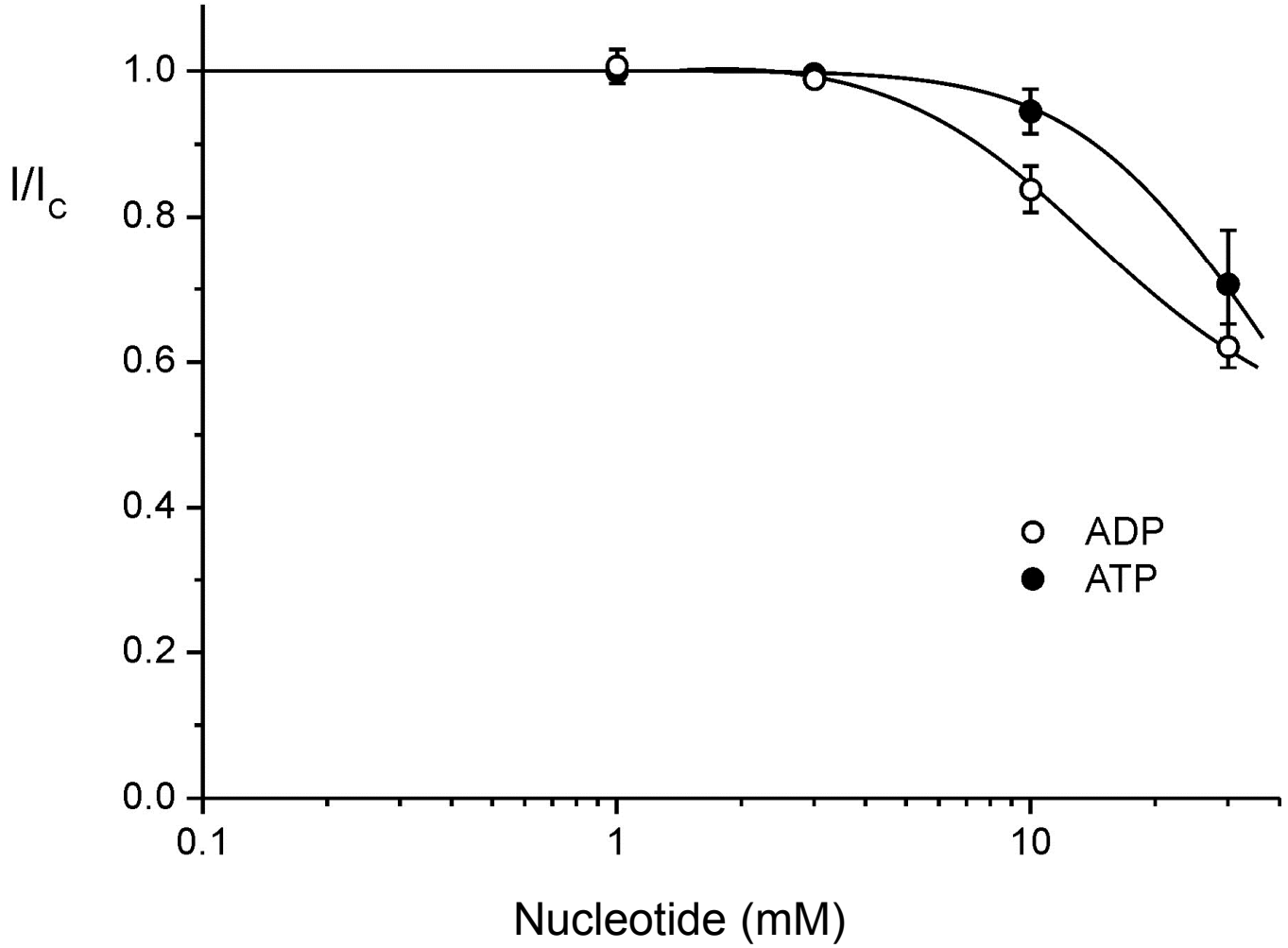


Gliclazide effect on the off-rates for Mg-nucleotide activation

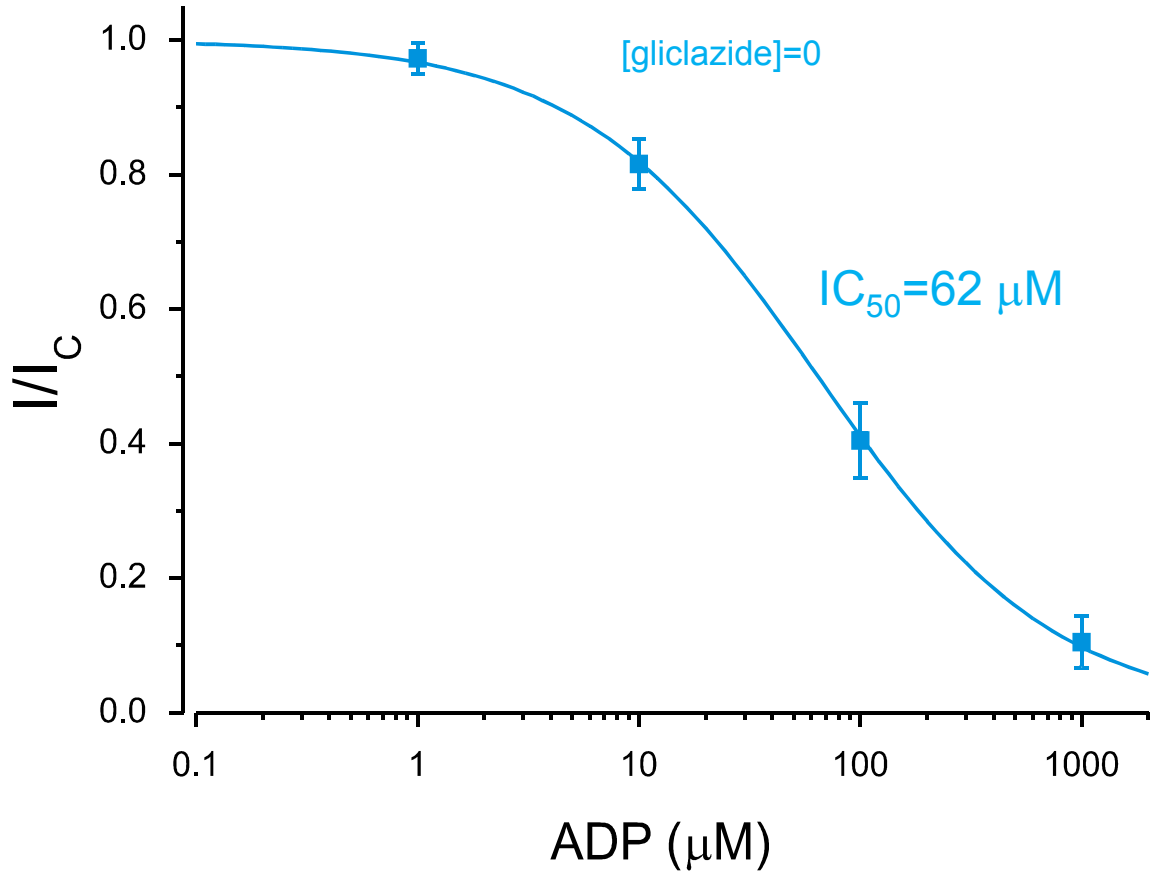


Activatory effects of nucleotides on Kir6.2-G334D/SUR1 channels are absent in Mg^{2+}-free solutions



2. Sulphonylurea modification of wild-type K_{ATP} channel gating by adenosine nucleotides

Gliclazide does not affect inhibition of wild-type K_{ATP} channels by ADP



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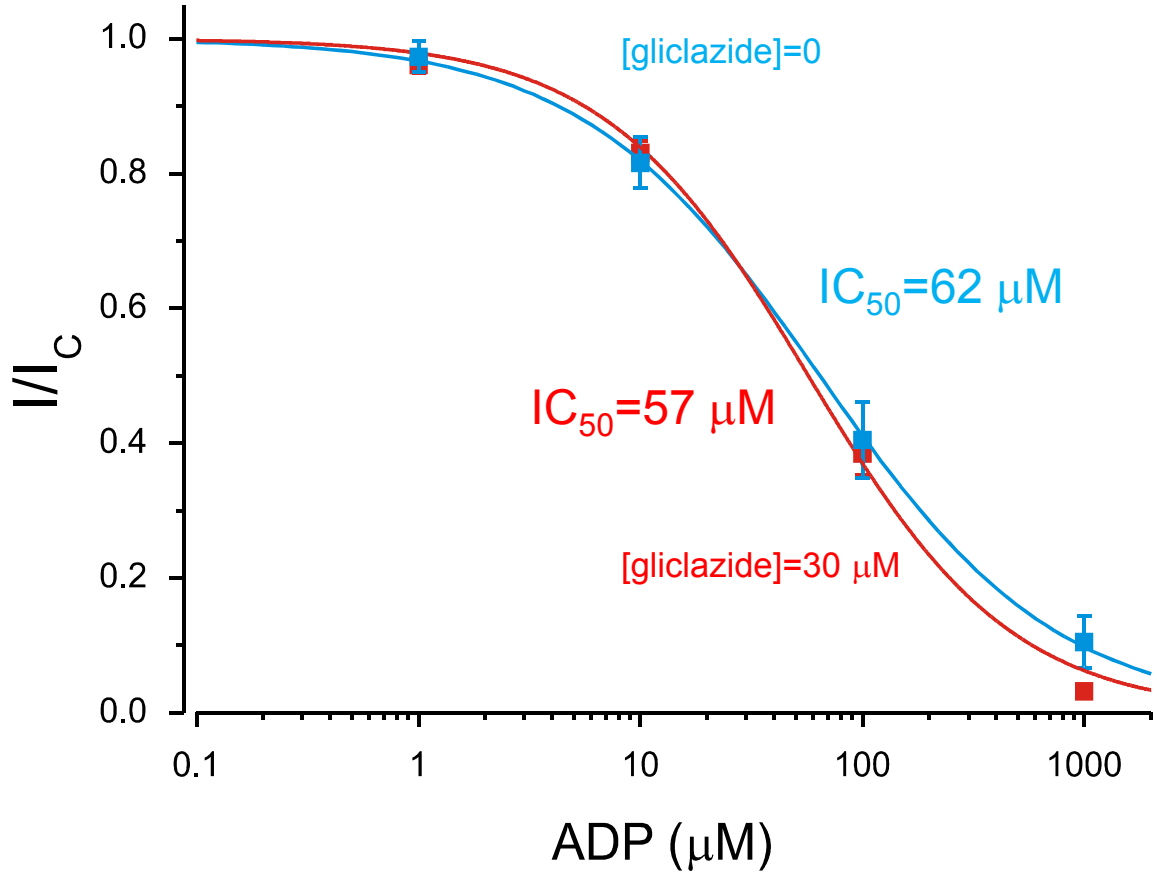
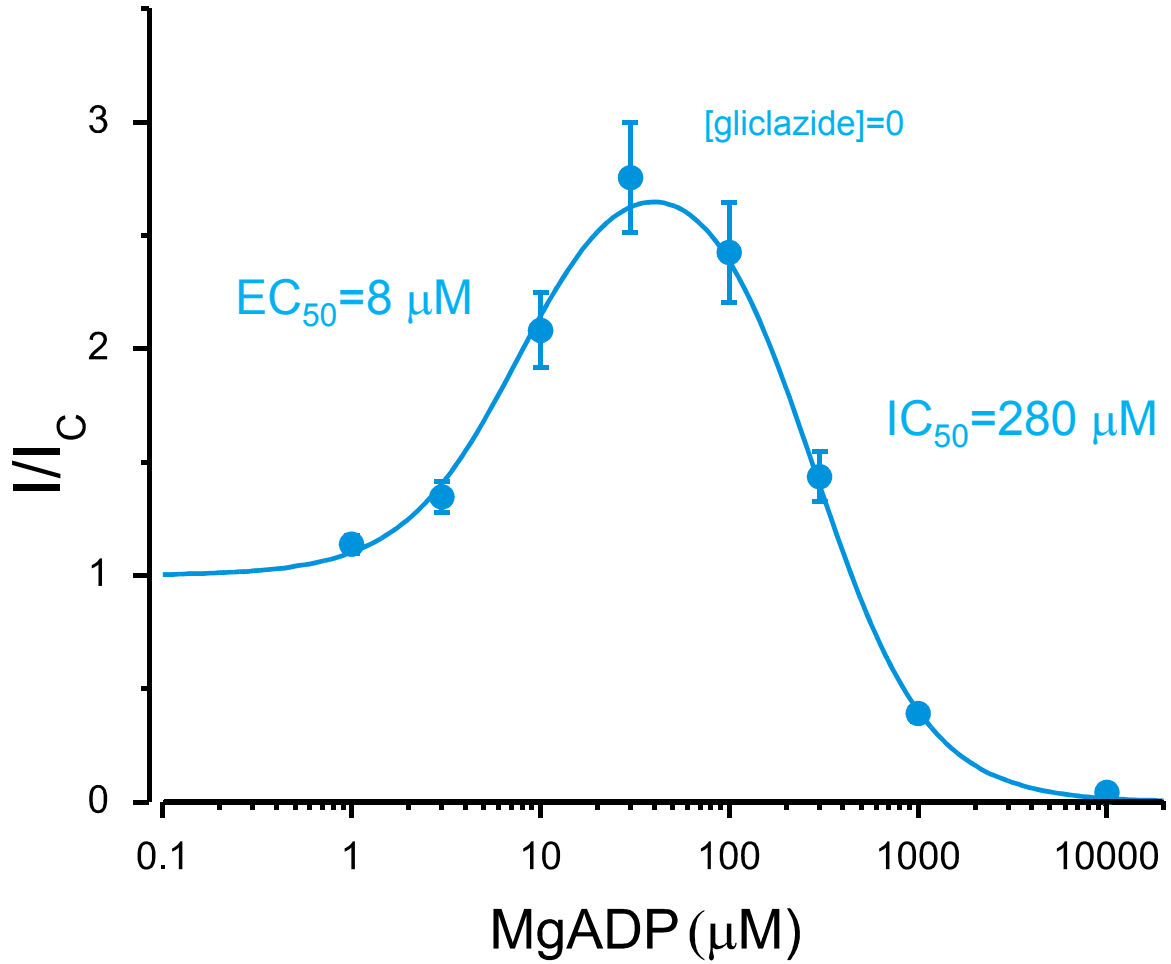


Gliclazide fully abolishes activation of wild-type K_{ATP} channels by MgADP



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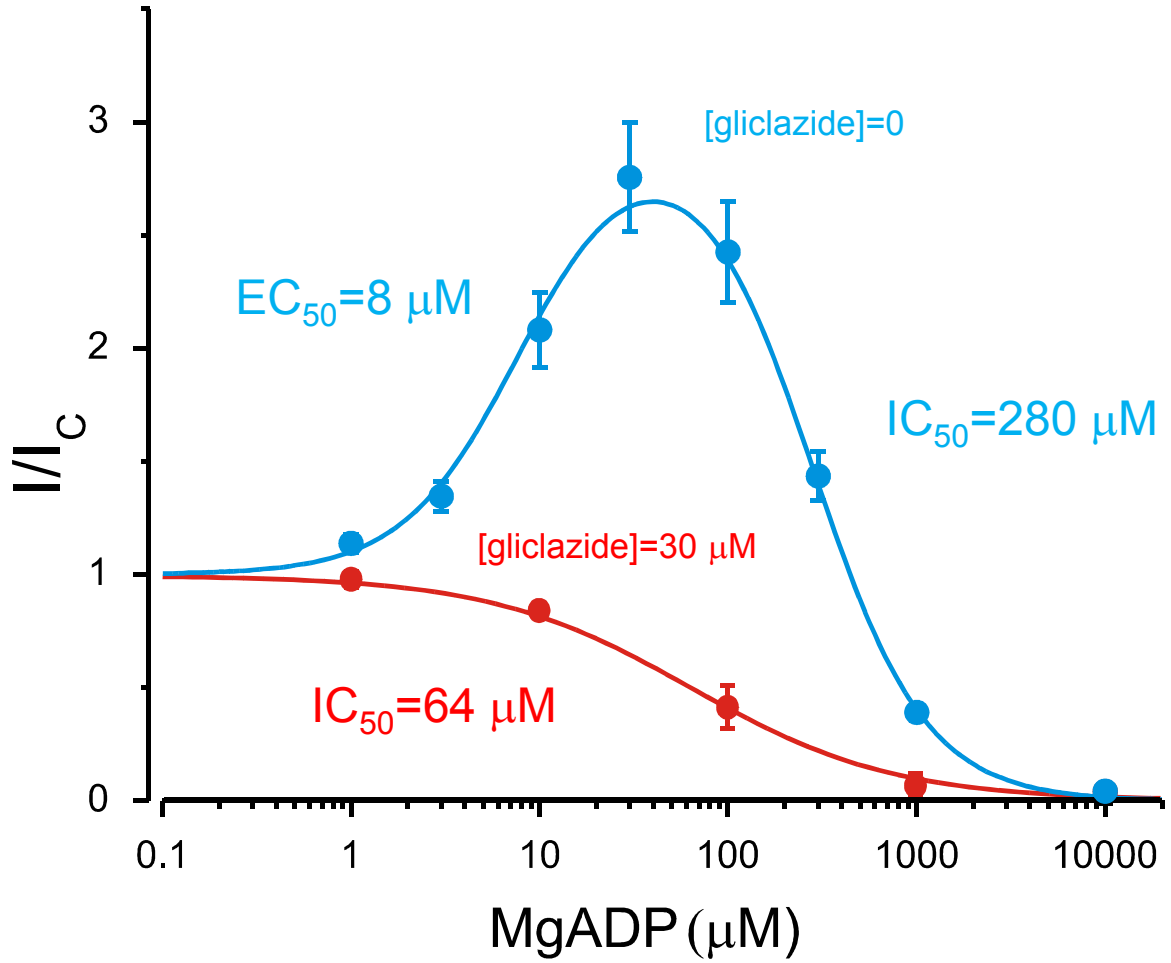
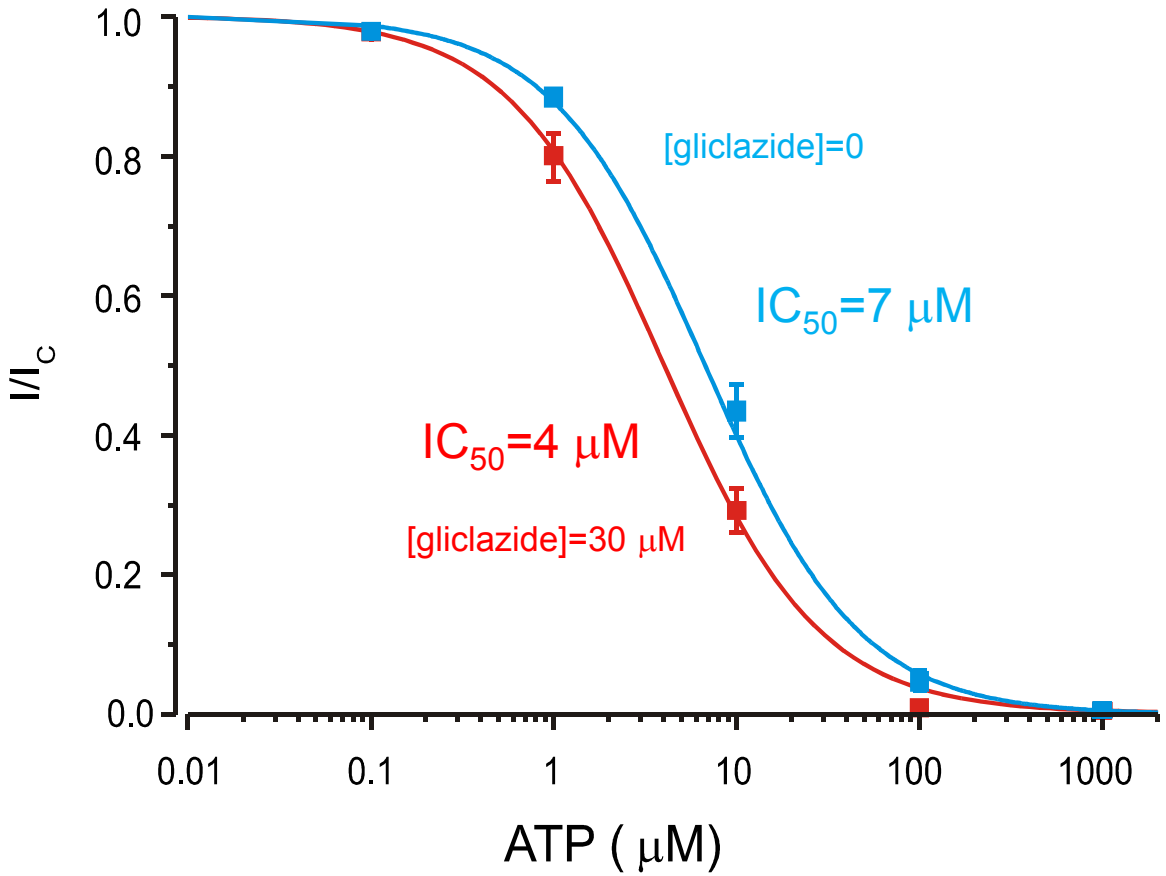
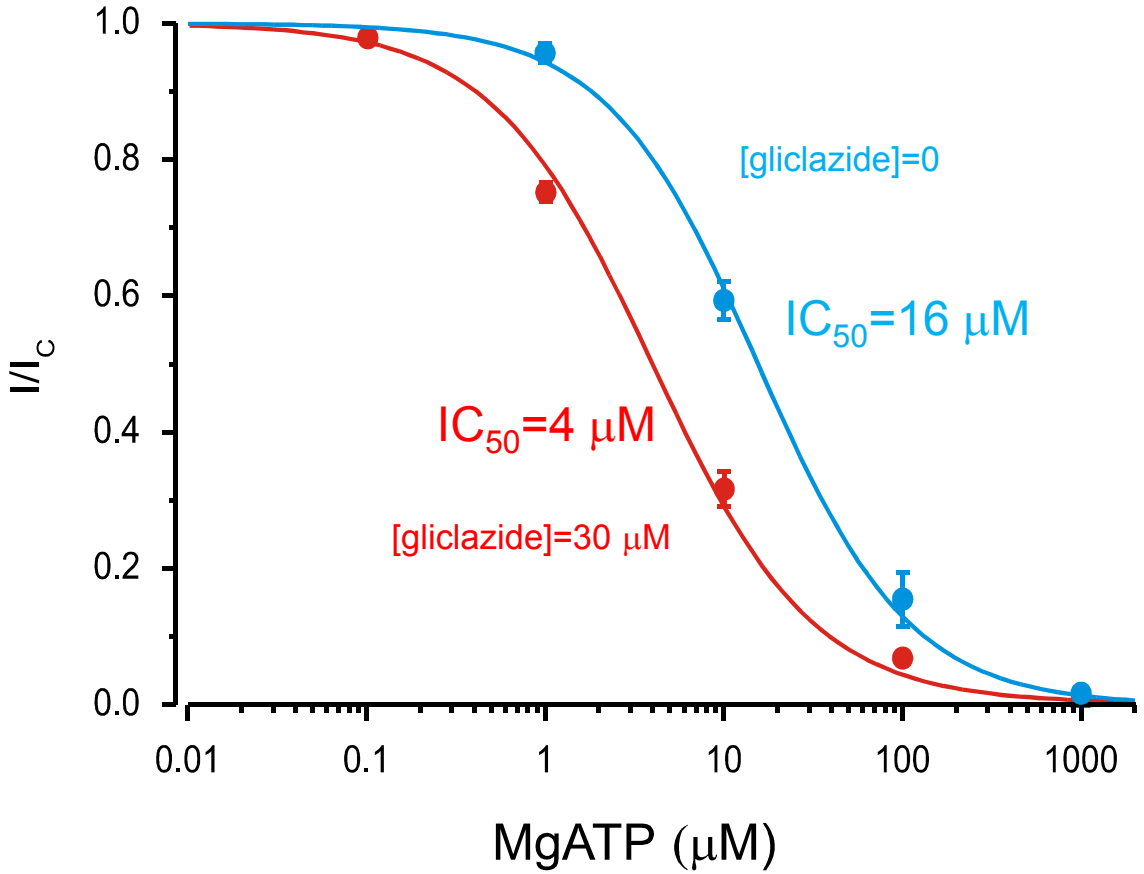


Gliclazide enhances inhibition of wild-type K_{ATP} channels by ATP in the absence of Mg^{2+}



Gliclazide enhances inhibition of wild-type K_{ATP} channels by ATP in the presence of Mg^{2+}



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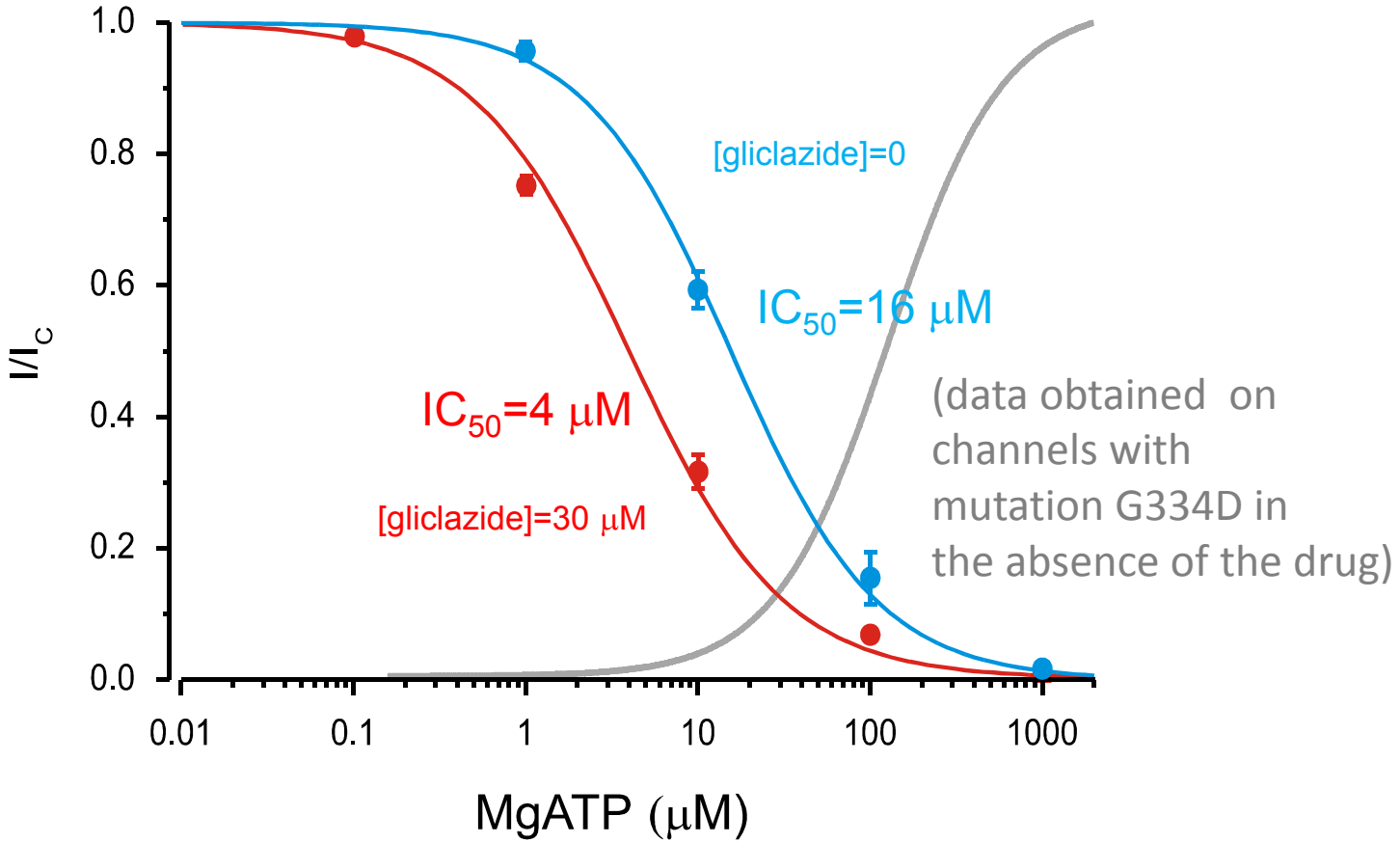
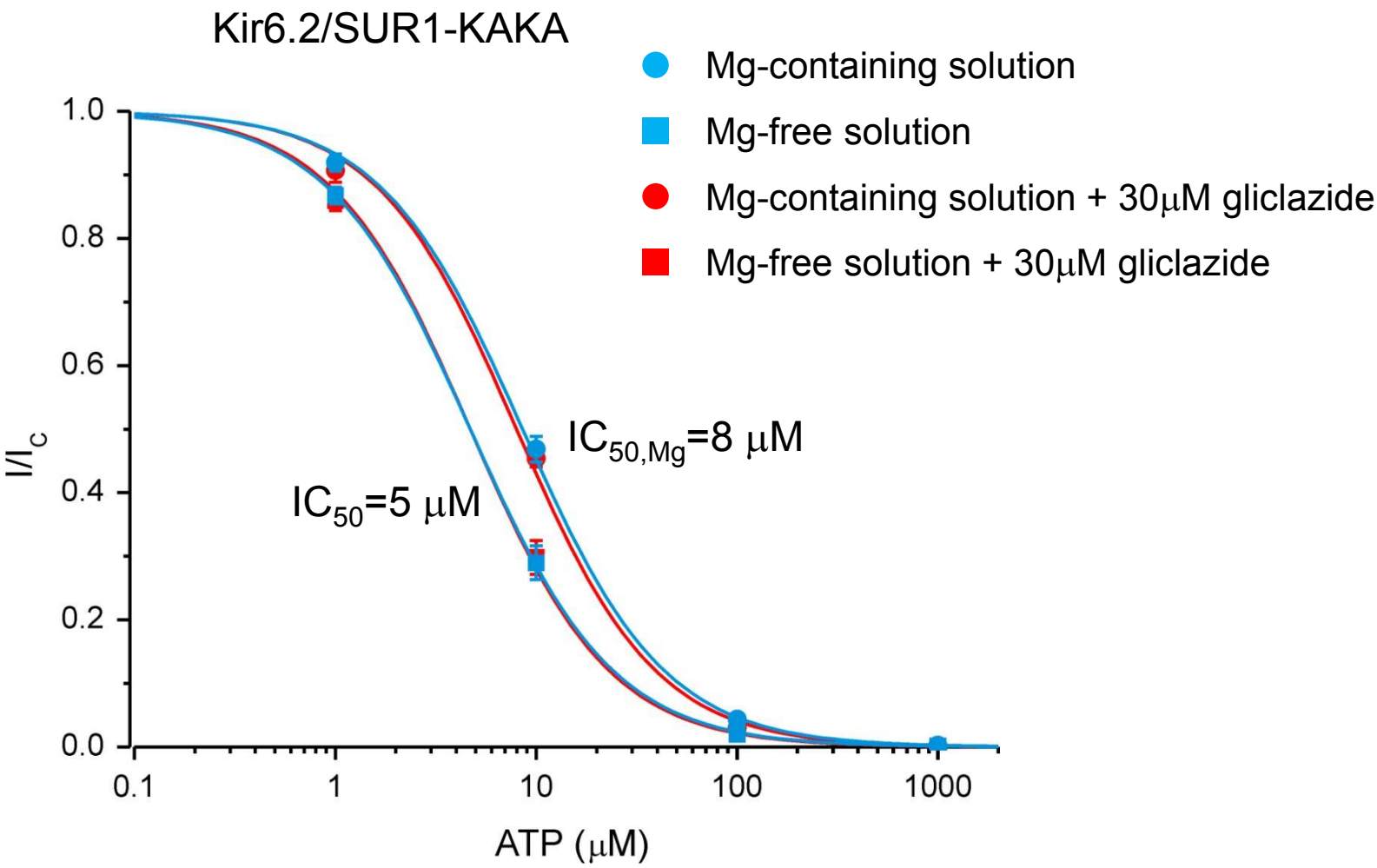


Gliclazide is without effect on ATP inhibition of Kir6.2/SUR1-KAKA channels



Enhanced inhibitory effect of ATP induced by gliclazide is likely to involve NBD1:

- ATP binding affinity (K_i=4μM) for NBD1 is much higher than that for NBD2 (K_i=60μM) so that it accounts more easily for gliclazide effects (*Matsuo et al., 2000 J Biol Chem* ***275****:28757*).

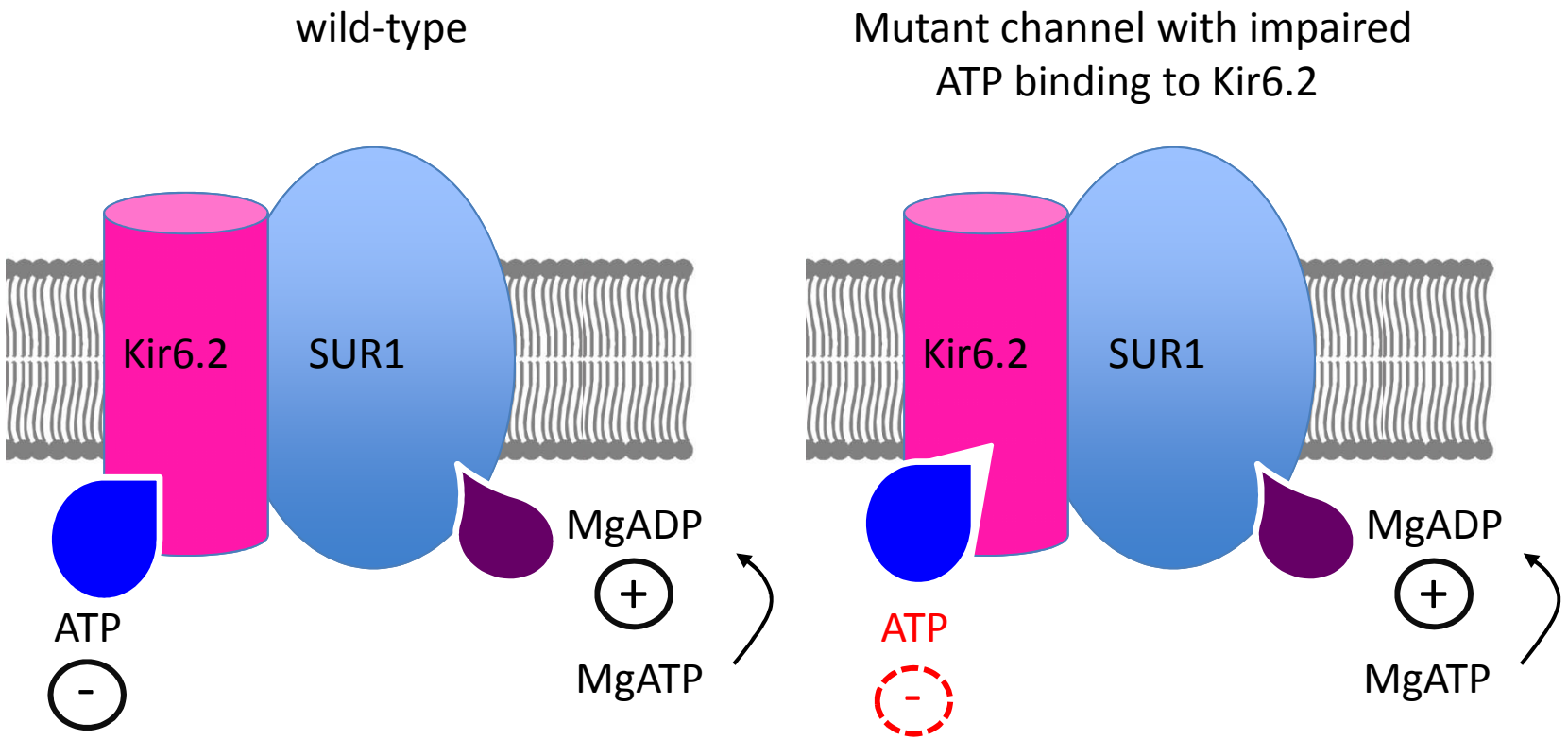
- Gliclazide effects are present in Mg-free solution Unlike for NBD2, binding of ATP to NBD1 does not require Mg^{2+} (*Matsuo et al., J Biol Chem 1999* ***274****:37479*).

CONCLUSIONS (1):

- Gliclazide suppresses binding of MgADP to NBD2
- Gliclazide impairs transduction mechanism by which MgADP binding to NBD2 promotes channel opening
- Gliclazide fully abolishes activation of wild-type K_{ATP} channels by MgADP
- Gliclazide enhances the inhibitory effect of ATP on wild-type K_{ATP} channels both in the absence and presence of Mg^{2+}; this effect is likely to involve NBD1

3. Effect of ATP on the efficacy of the high affinity gliclazide block of mutant K_{ATP} channels with impaired nucleotide inhibition

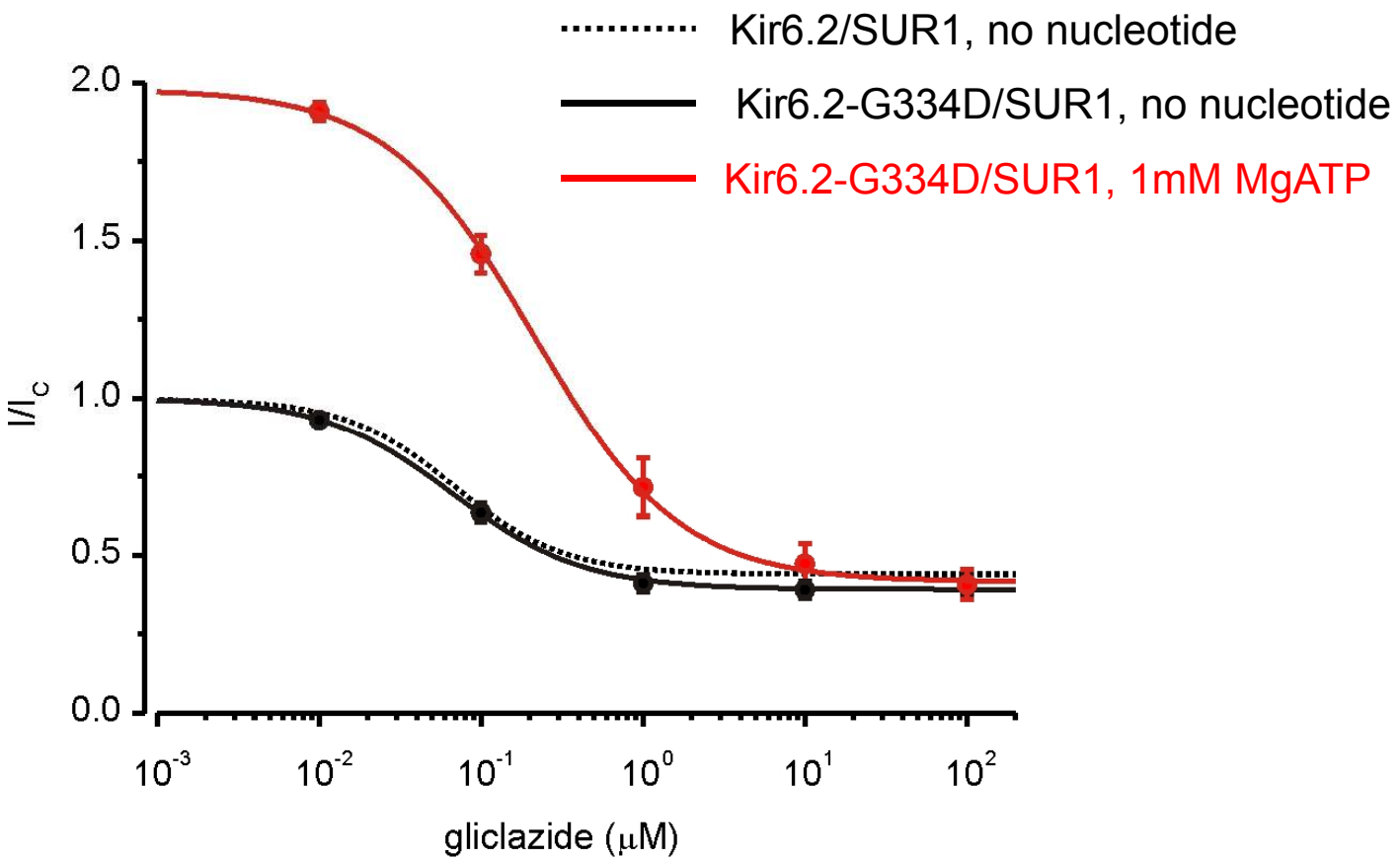
(1) Mutations in the putative inhibitory ATP binding site on Kir6.2 subunit



Kir6.2-G334D/SUR1

DEND syndrome

(neonatal diabetes with developmental delay and neurological features)

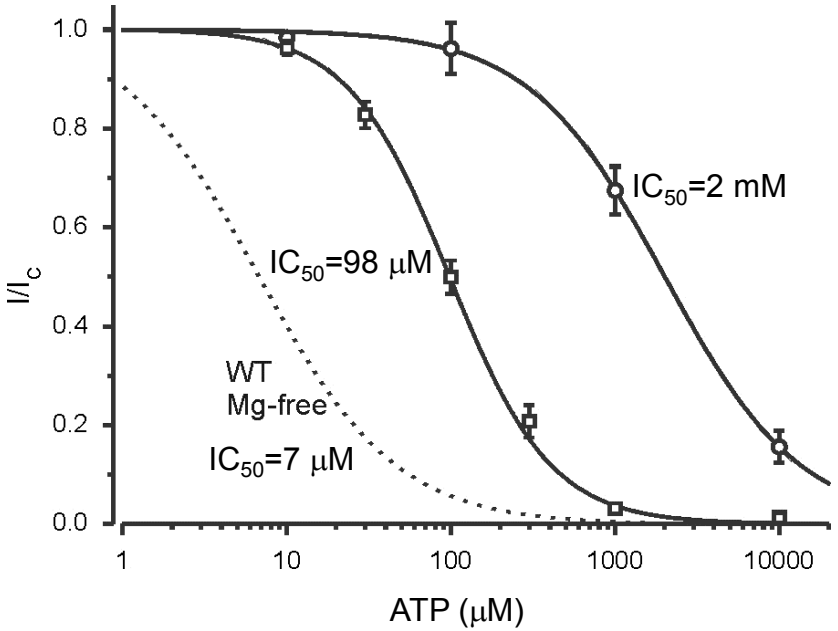


Kir6.2-R201C/SUR1

Permanent neonatal diabetes

Mg-containing solution

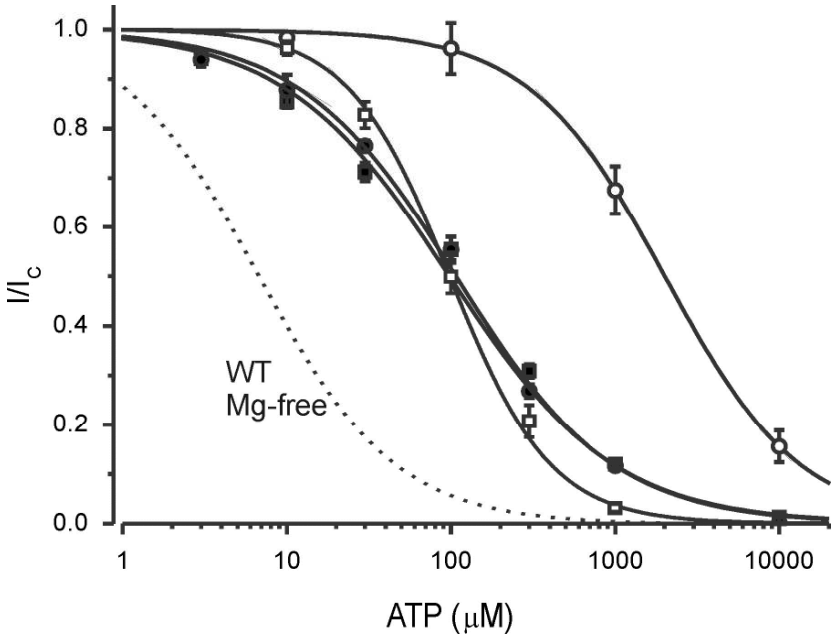
Mg-free solution



Kir6.2-R201C/SUR1

Gliclazide suppresses MgATP activation without enhancing ATP inhibition

- Mg-containing solution
- Mg-free solution
- Mg-containing solution + 30μM gliclazide
- Mg-free solution + 30μM gliclazide



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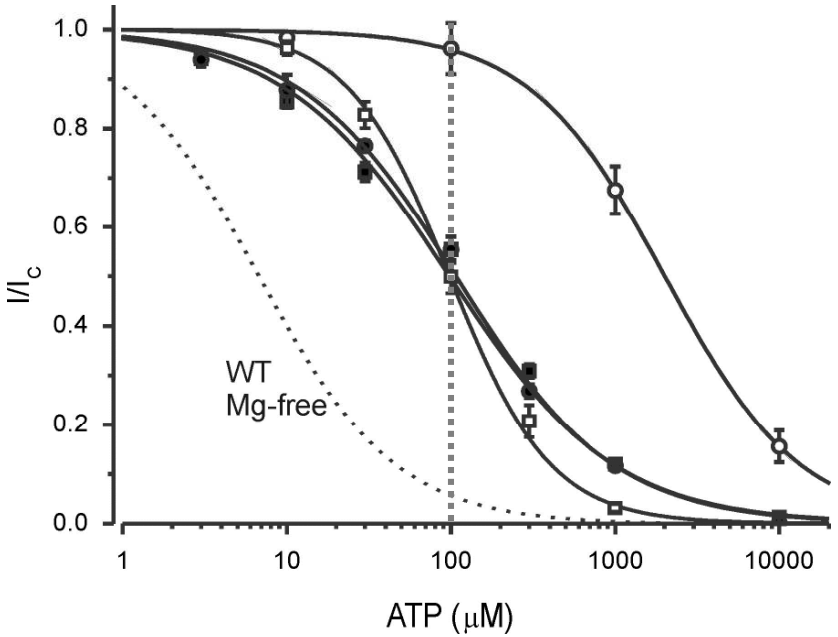
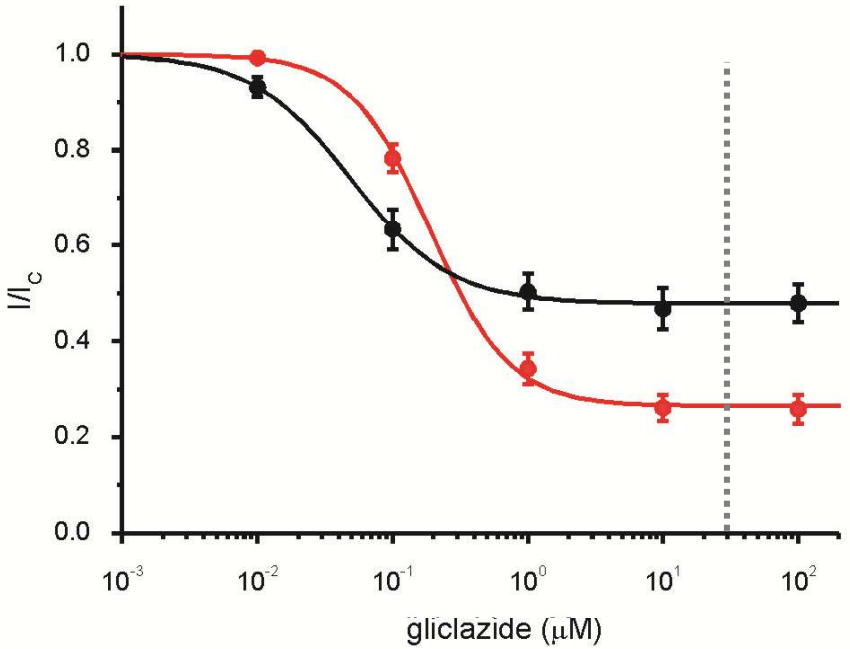
- Mg-free solution + 30μM gliclazide

- no ATP
- [MgATP]= 100 μM

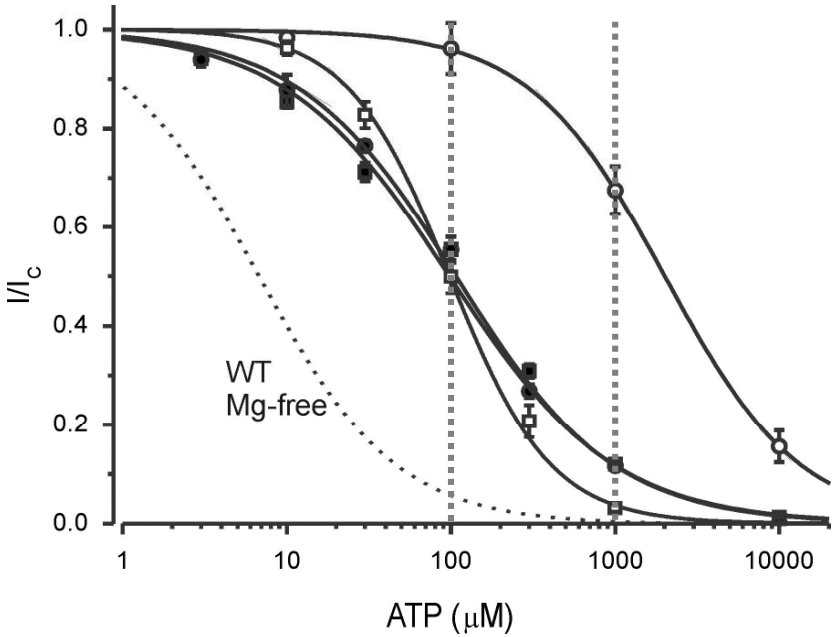




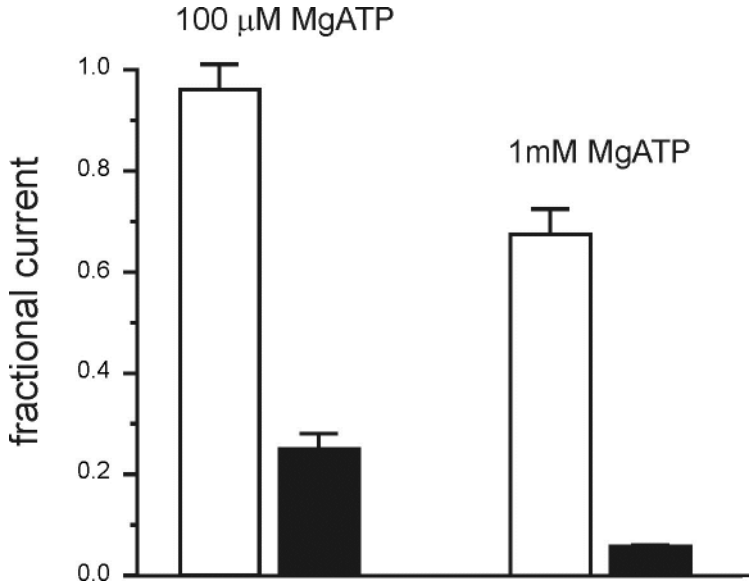
Kir6.2-R201C/SUR1

Gliclazide effectively inhibits Kir6.2R201C/SUR1channels in the physiological range of ATP concentrations

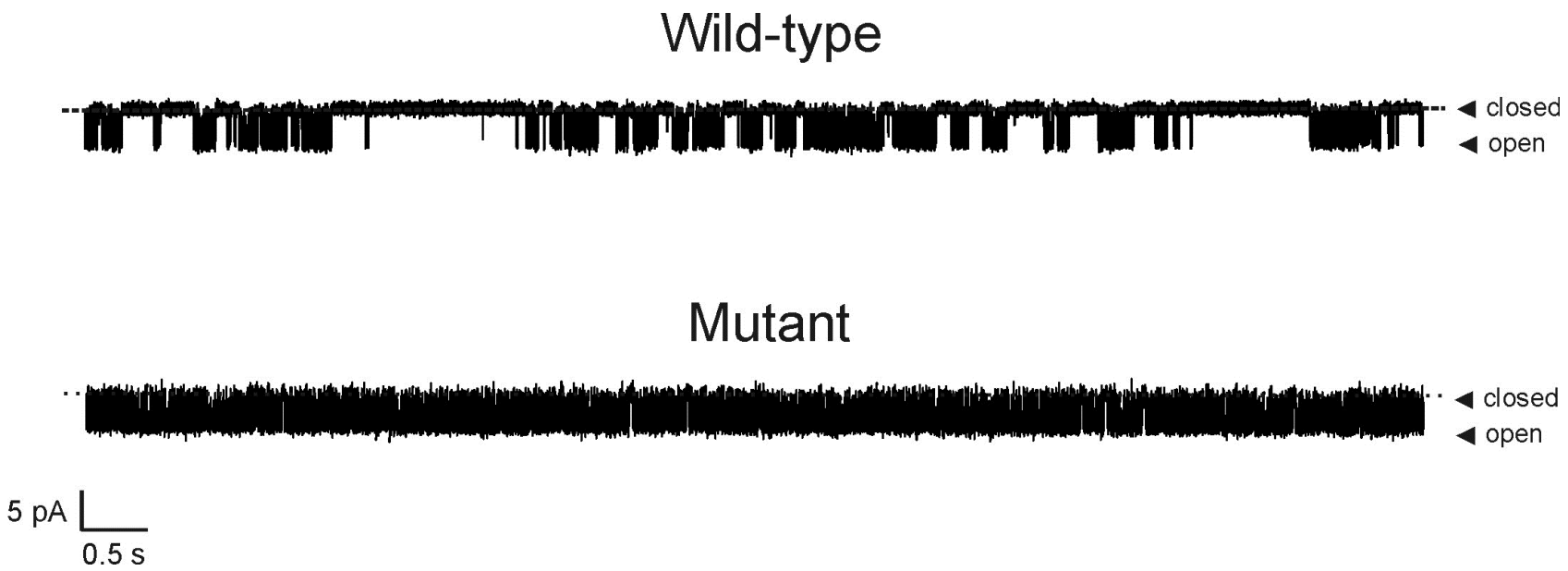
- Mg-containing solution
- Mg-free solution
- Mg-containing solution + 30μM gliclazide
- Mg-free solution + 30μM gliclazide



- [gliclazide]=0
- [gliclazide]=30 μM



(2) Mutations that impair channel gating

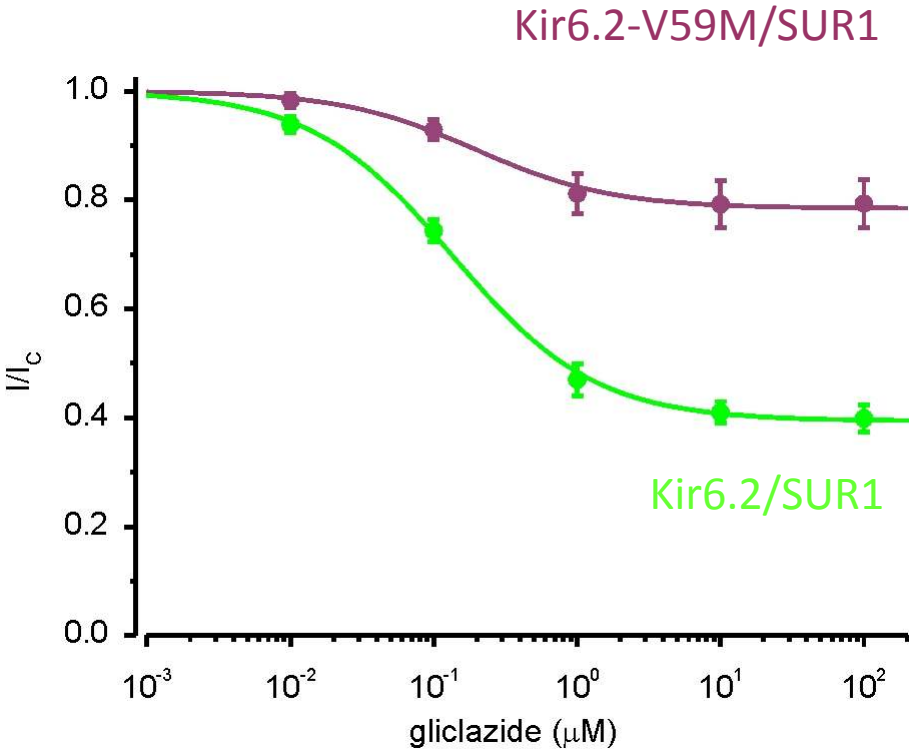


Kir6.2-V59M/SUR1

i-DEND syndrome

(neonatal diabetes, developmental delay and muscle weakness)

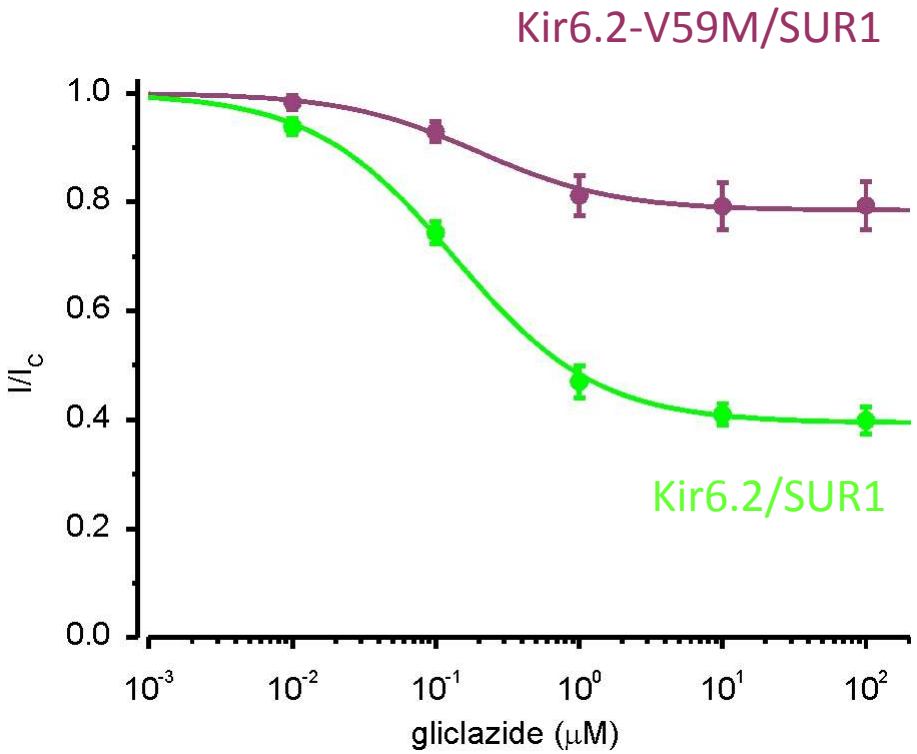
Excised patch, nucleotide-free solution

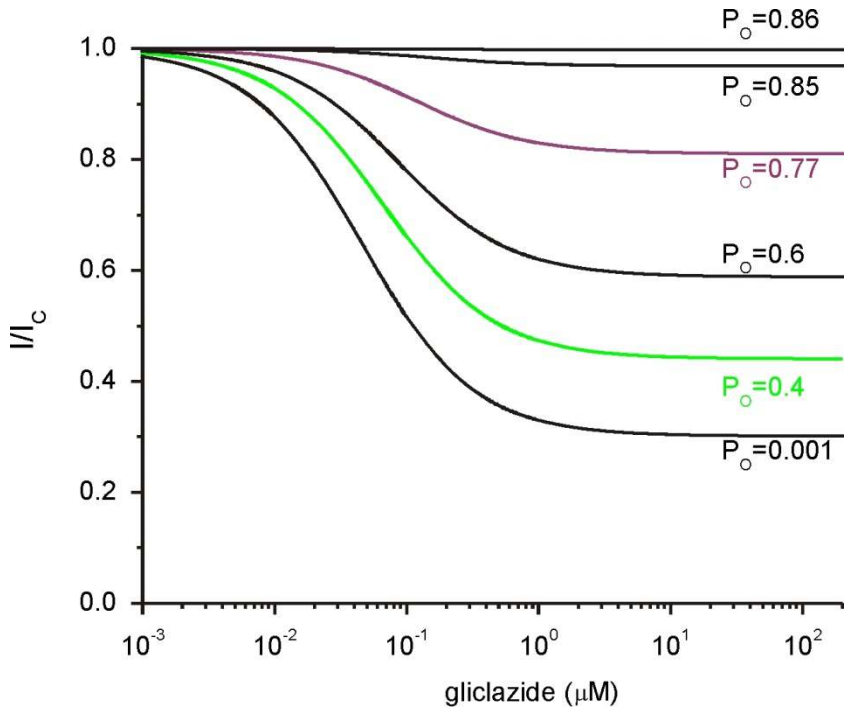


Kir6.2-V59M/SUR1

Simulation of the direct block by gliclazide for channels with different values of open probability

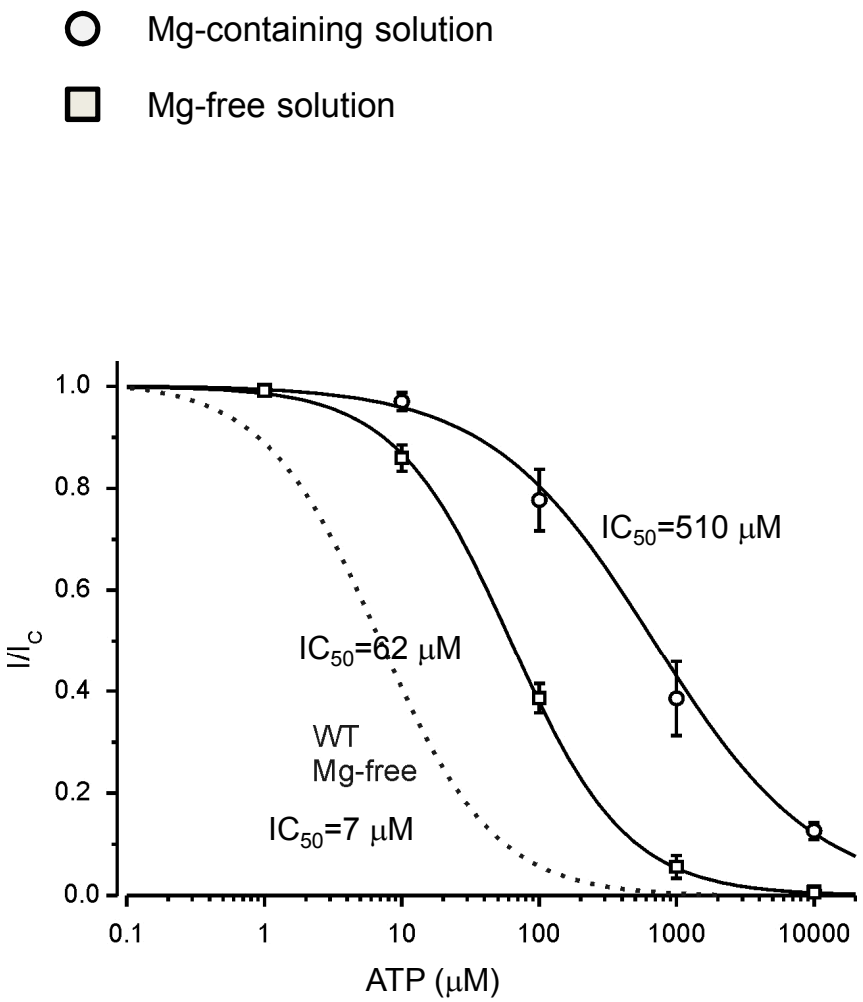
Excised patch, nucleotide-free solution





Kir6.2-V59M/SUR1

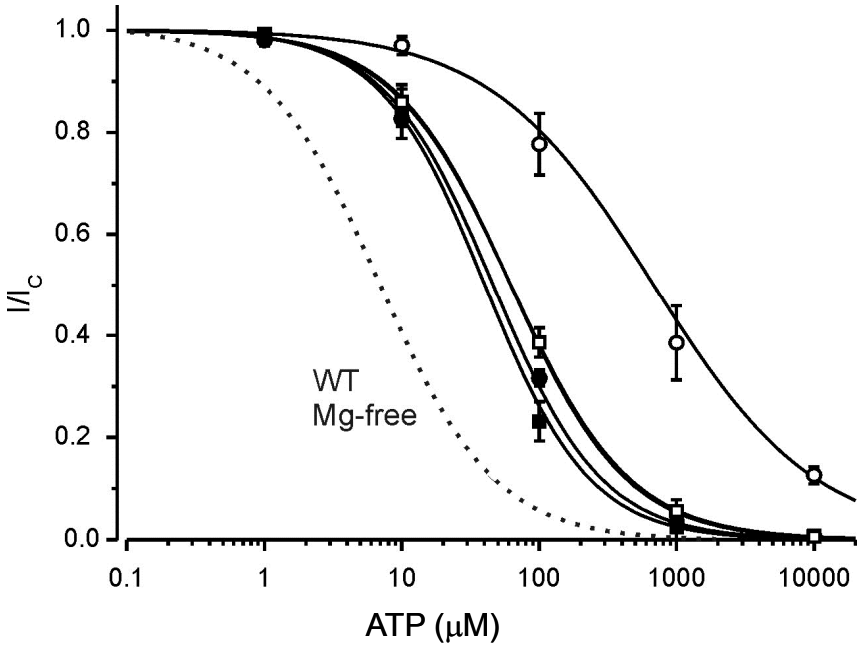
V59M mutation impairs ATP sensitivity of the K_{ATP} channel



Kir6.2-V59M/SUR1

Gliclazide abolishes MgATP activation and slightly enhances ATP inhibition

- Mg-containing solution
- Mg-free solution
- Mg-containing solution + 30μM gliclazide
- Mg-free solution + 30μM gliclazide



Kir6.2-V59M/SUR1

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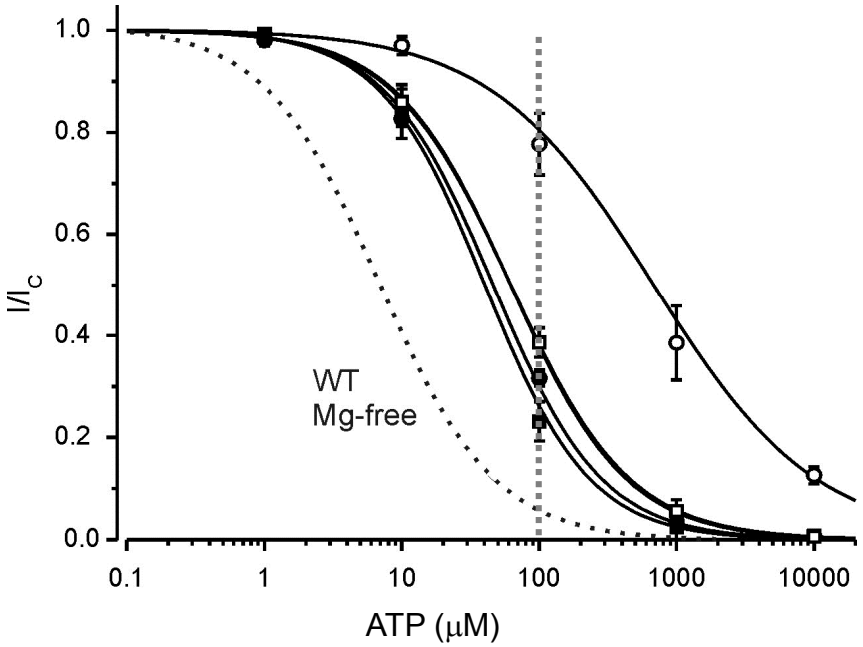
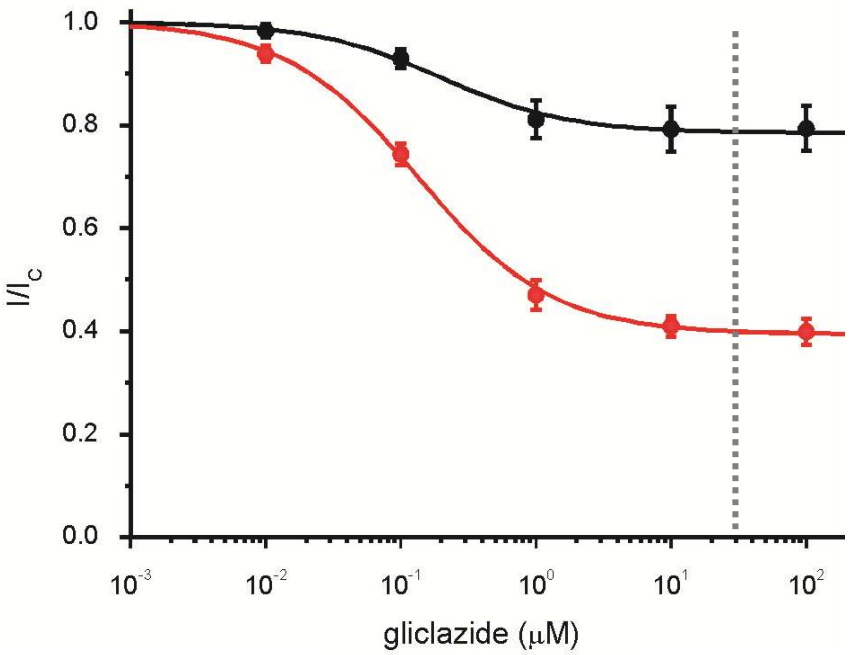
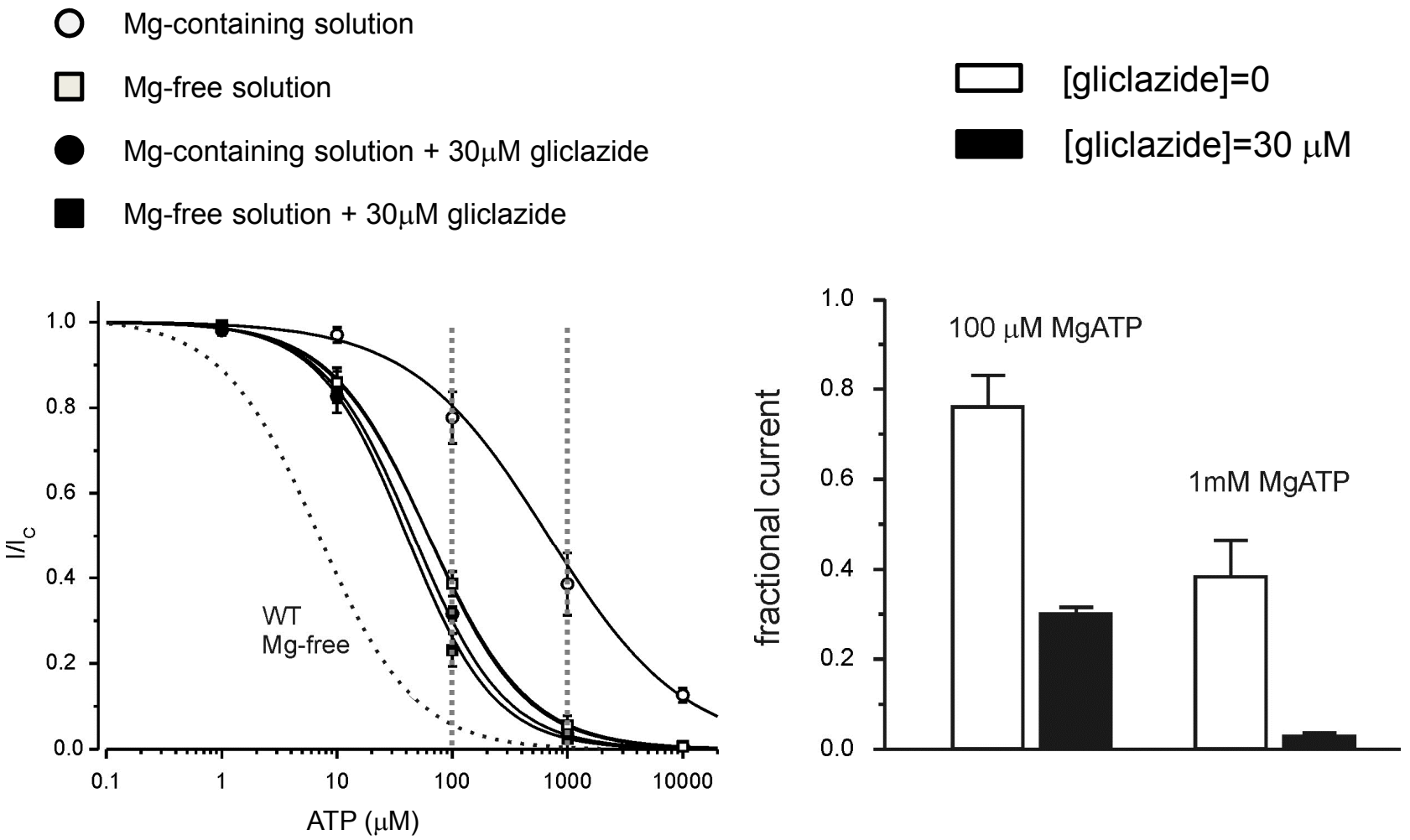




Kir6.2-V59M/SUR1

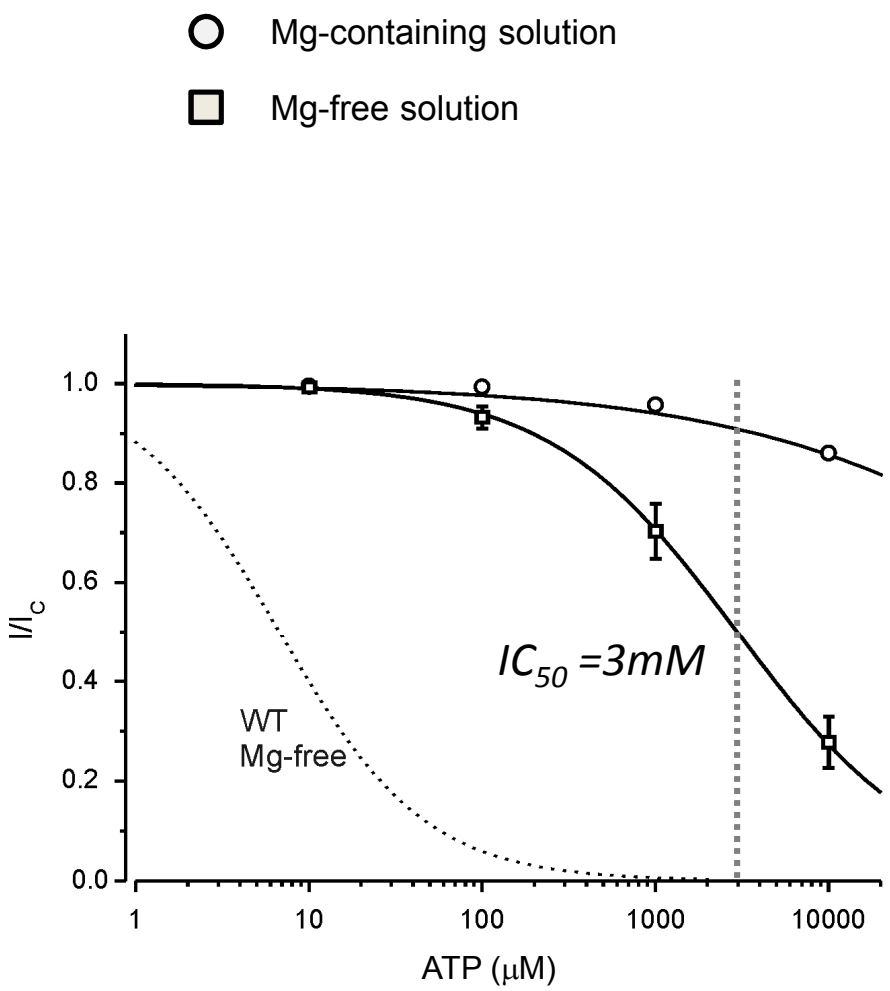
Gliclazide effectively inhibits Kir6.2-R201C/SUR1 channels in the physiological range of ATP concentrations



Kir6.2-I296L/SUR1

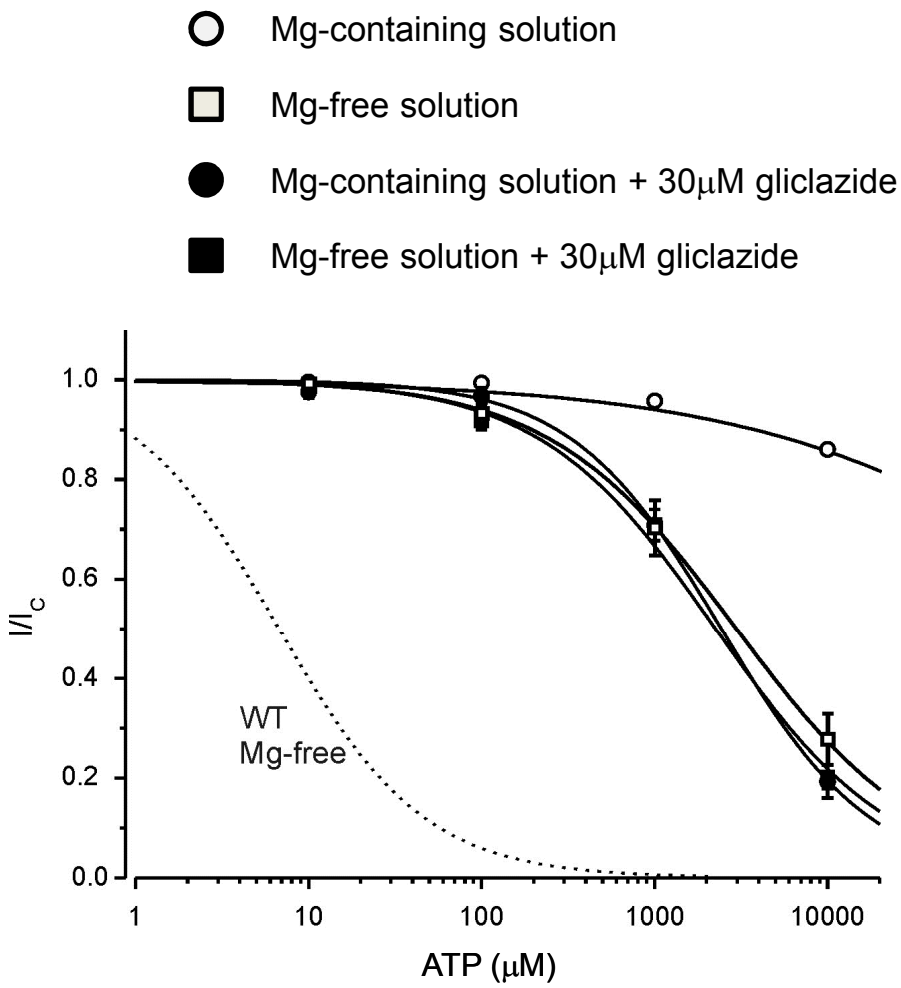
DEND syndrome

(neonatal diabetes with developmental delay and neurological features)



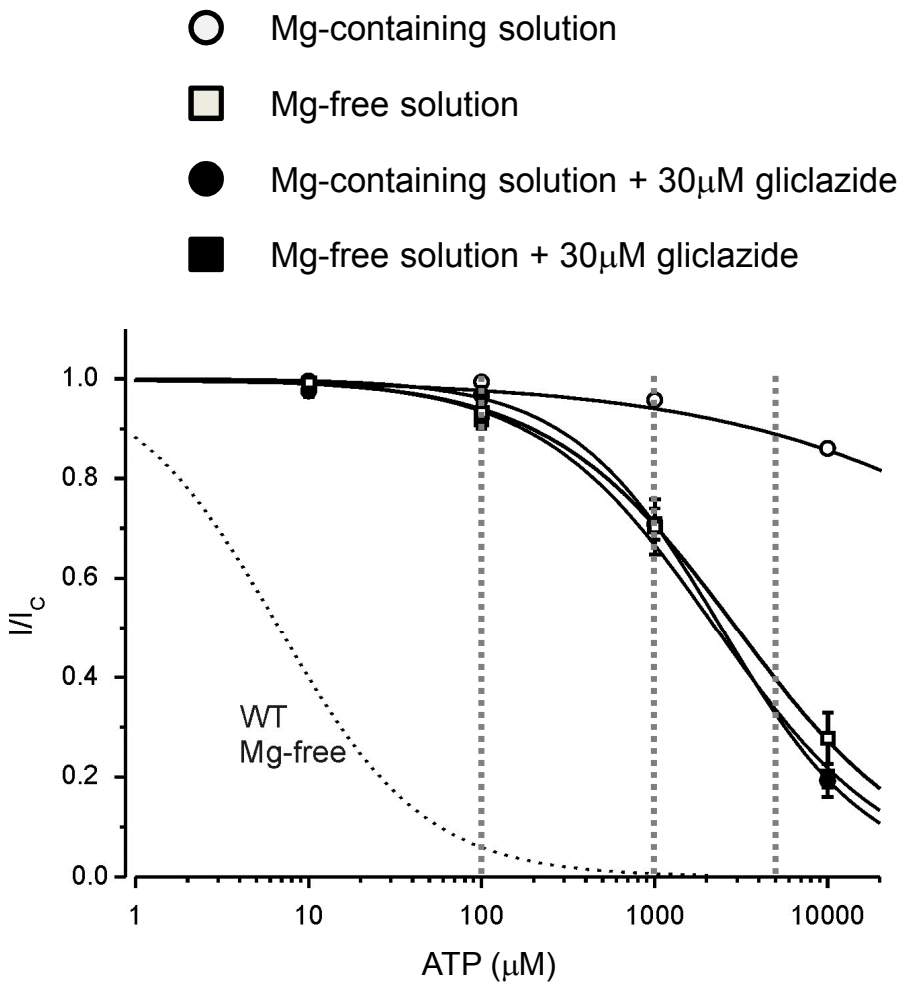
Kir6.2-I296L/SUR1

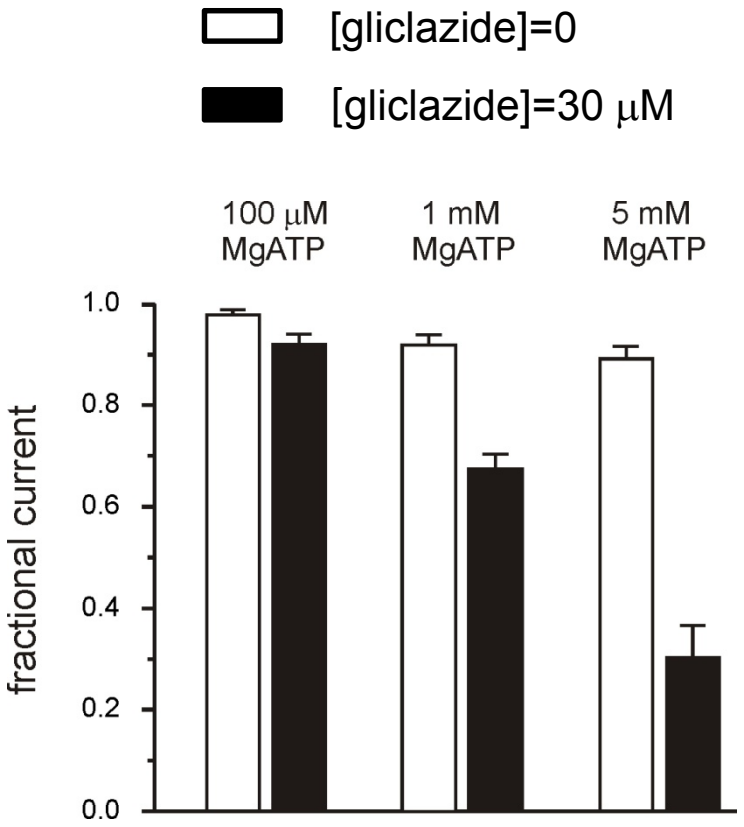
Gliclazide suppresses MgATP activation without substantially affecting ATP inhibition



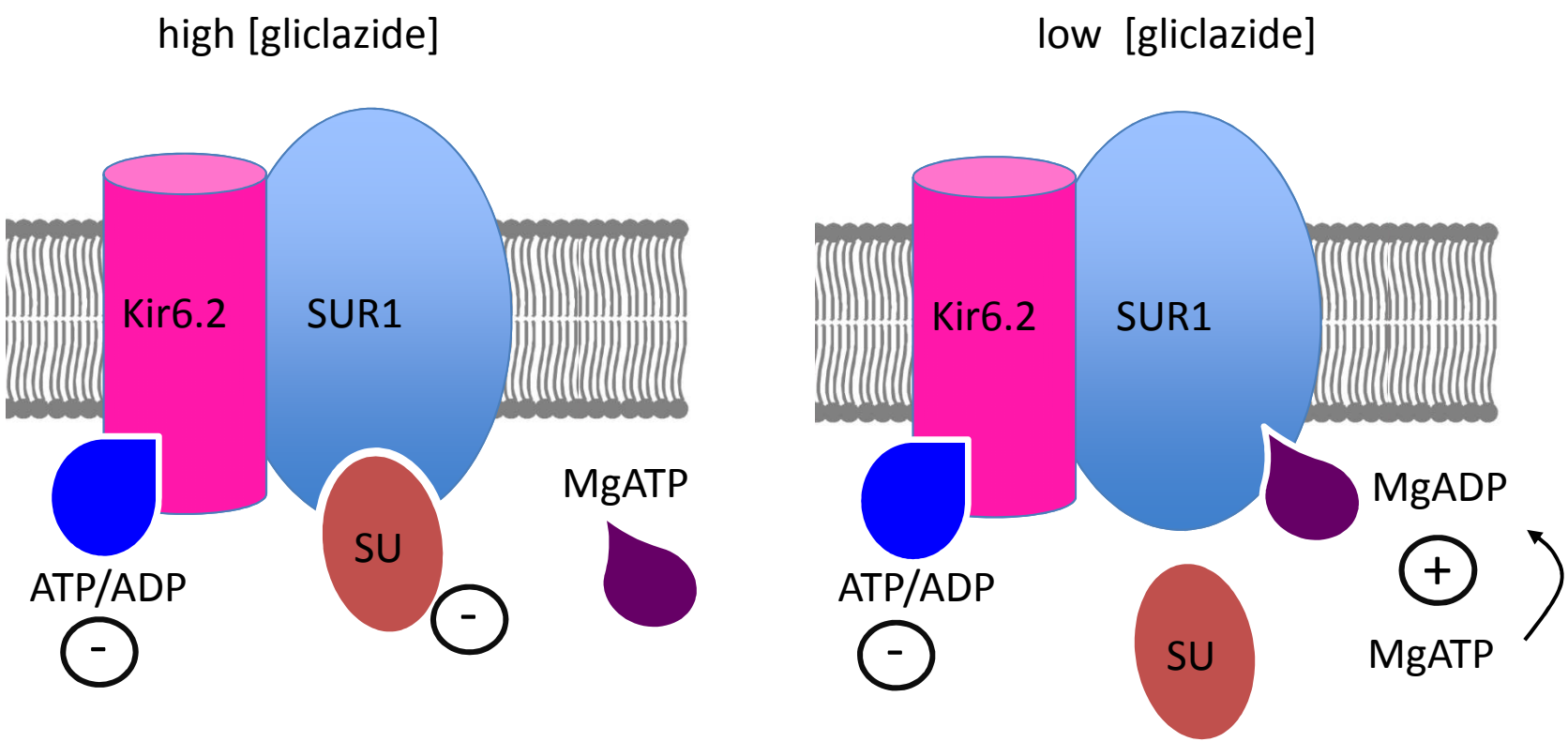
Kir6.2-I296L/SUR1

Gliclazide poorly inhibits Kir6.2-I296L/SUR1 channels





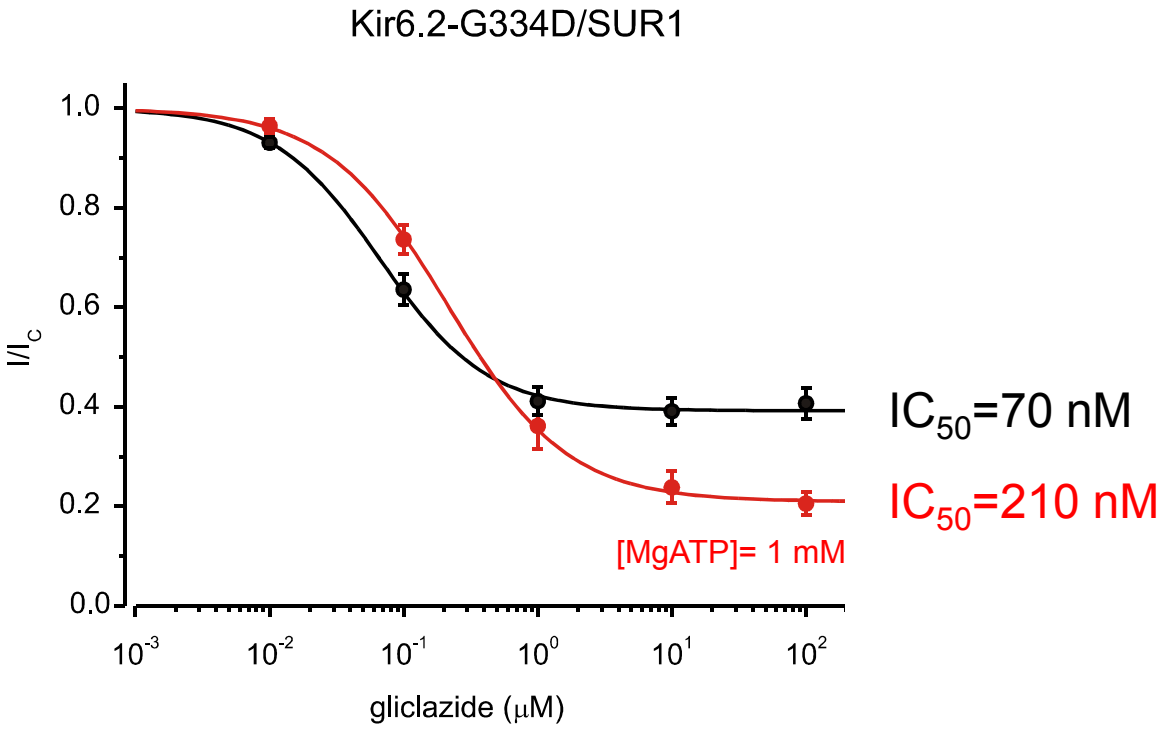
4. Reduction of gliclazide binding to SUR1 by adenosine nucleotides



MgATP increases the value of IC_{50} of high affinity block of gliclazide in excised patches

Excised patch, no nucleotide

Excised patch, MgATP



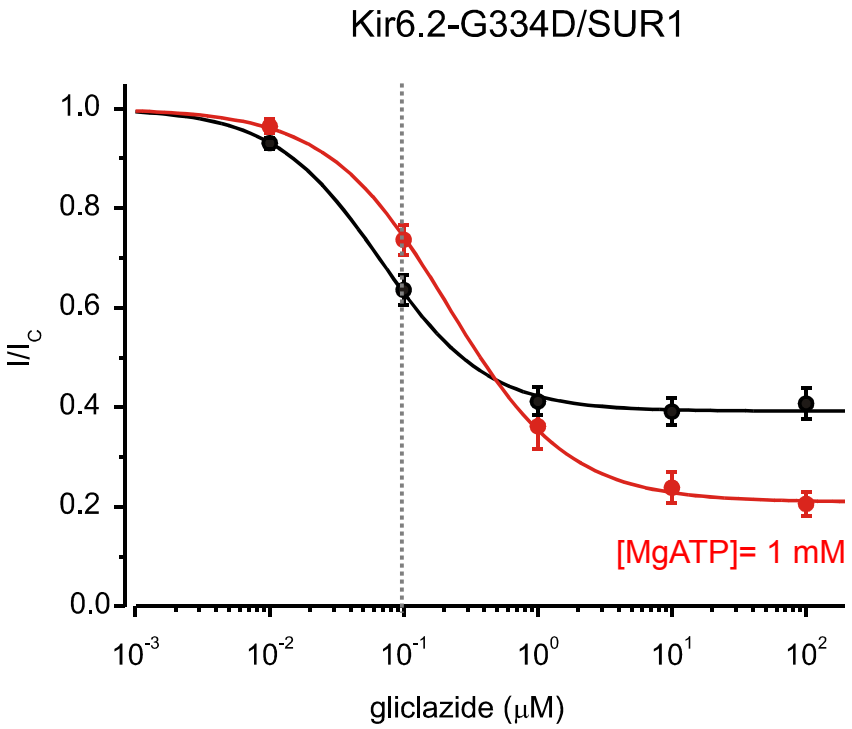
IC_{50}=70 nM

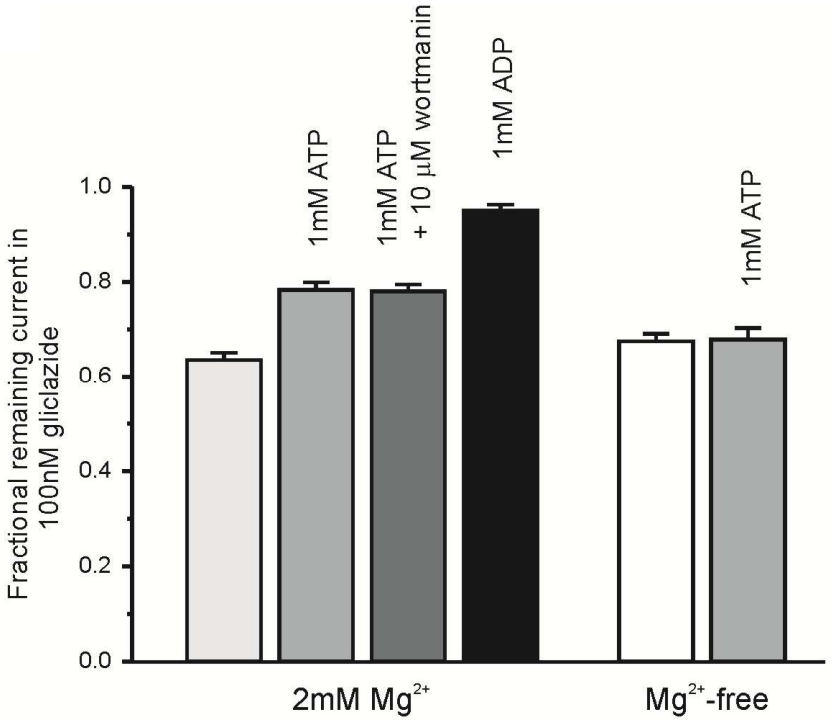
IC_{50}=210 nM

ATP reduces inhibitory effect of gliclazide
The mechanism involves nucleotide binding to NBD2

Excised patch, no nucleotide

Excised patch, MgATP



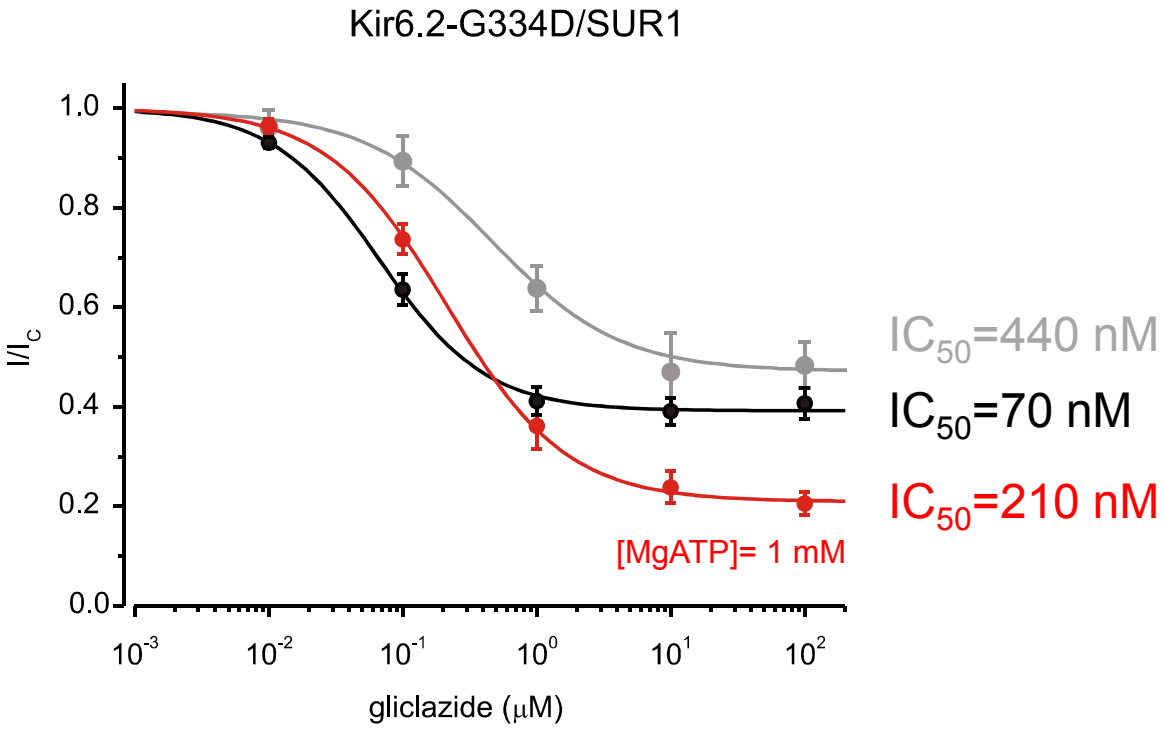


MgATP increases the value of IC_{50} of high affinity block of gliclazide in excised patches

Excised patch, no nucleotide

Excised patch, MgATP

Intact cell

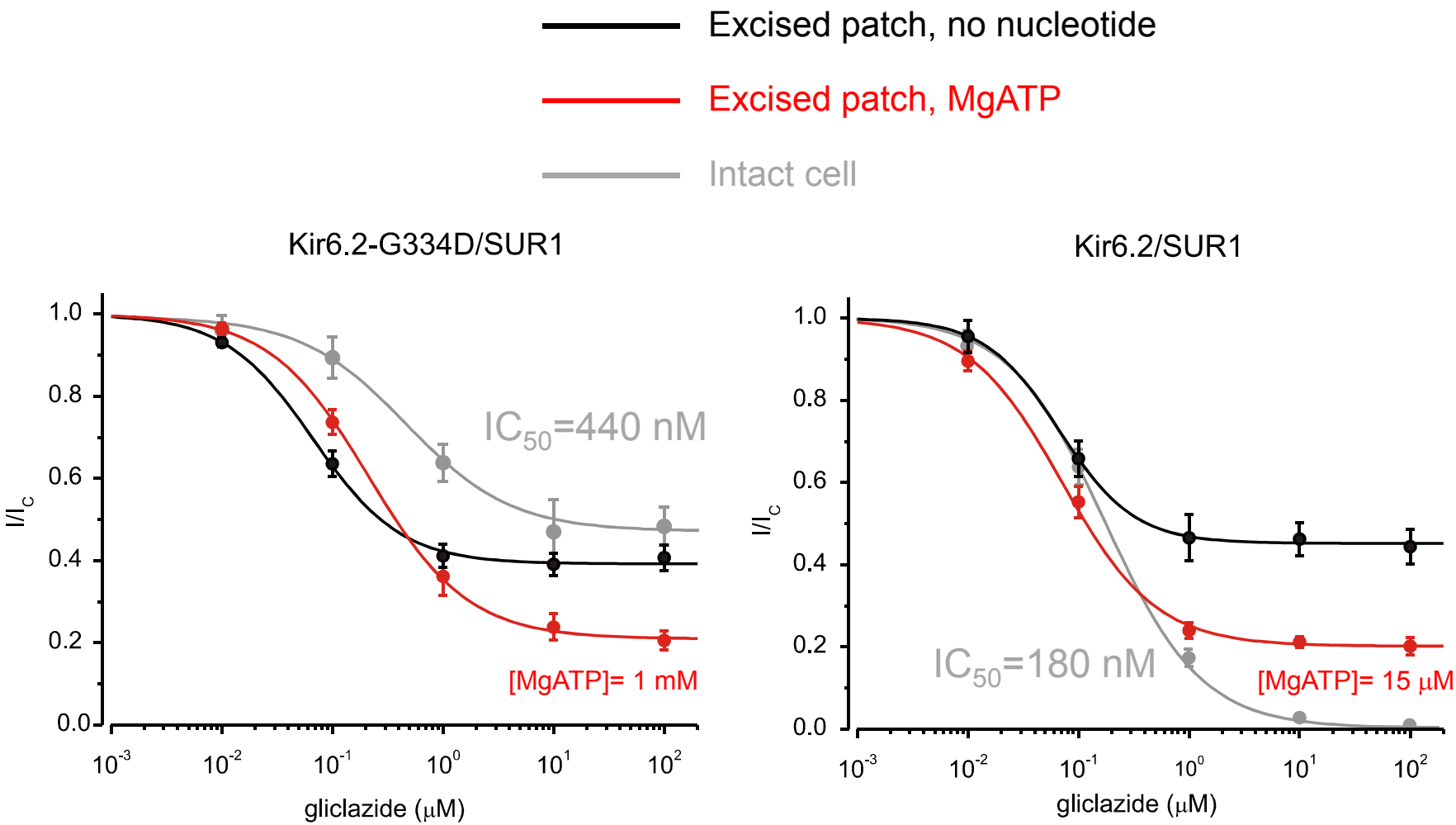


IC_{50}=440 nM

IC_{50}=70 nM

IC_{50}=210 nM

In the intact cell, the IC_{50} for high affinity gliclazide block is higher for mutant Kir6.2-G334D/SUR1 channels than for Kir6.2/SUR1 channels



CONCLUSIONS (2):

- K_{ATP} channels with mutations that cause mild impairment of ATP inhibition can be efficiently closed by gliclazide; channels with mutations that cause severe impairment of ATP inhibition can't.

- Gliclazide effectively abolishes MgATP activation in K_{ATP} channels with ND mutations

- Gliclazide-induced enhancement of the inhibitory effect of ATP is largely suppressed by ND mutations; this suppression may contribute to increased doses of sulphonylureas used in the treatment of this disease

- Mg-nucleotide binding to NBD2 impairs gliclazide binding to the high affinity binding site on SUR1

Acknowledgments:

Frances Ashcroft

Heidi De Wet

Mathilde Lafond

wellcome trust

I have yet to see any problem, however complicated, which, when looked at in the right way did not become still more complicated.

Poul Anderson (1926 –2001)
American science-fiction writer

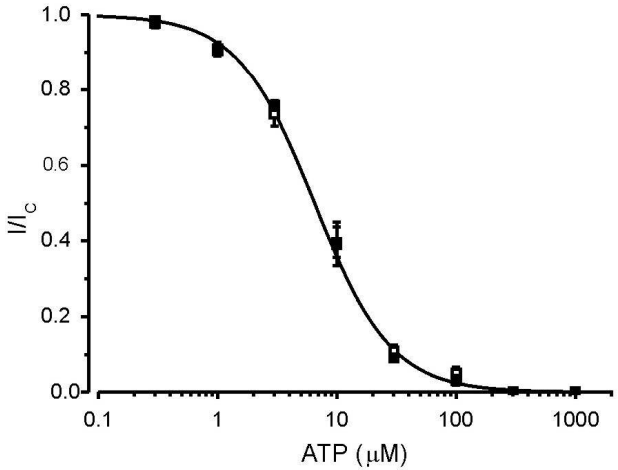
Mg^{2+}-free

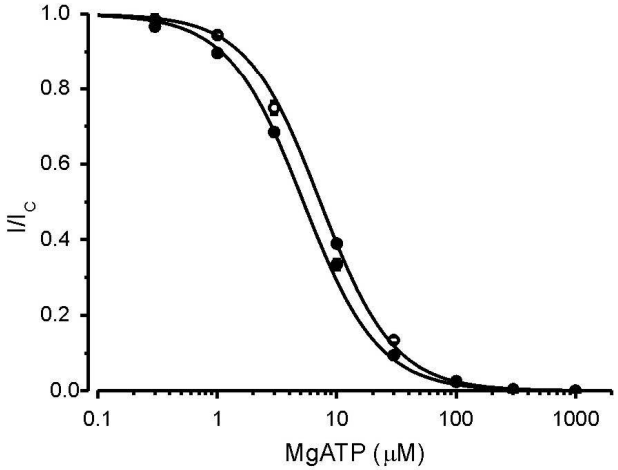
with Mg^{2+}

Kir6.2/SUR1-KA1

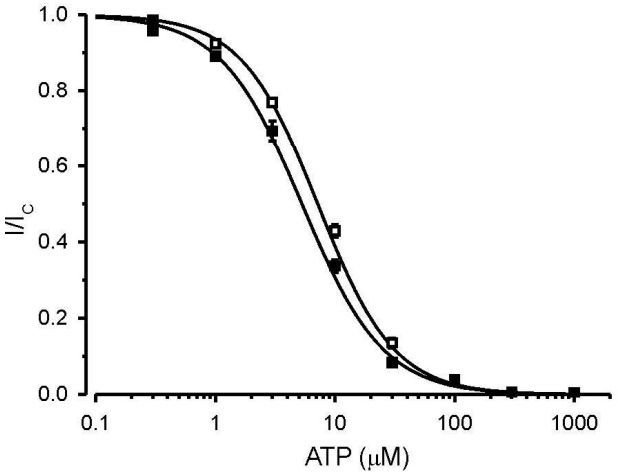
open symbols: [gliclazide]=0

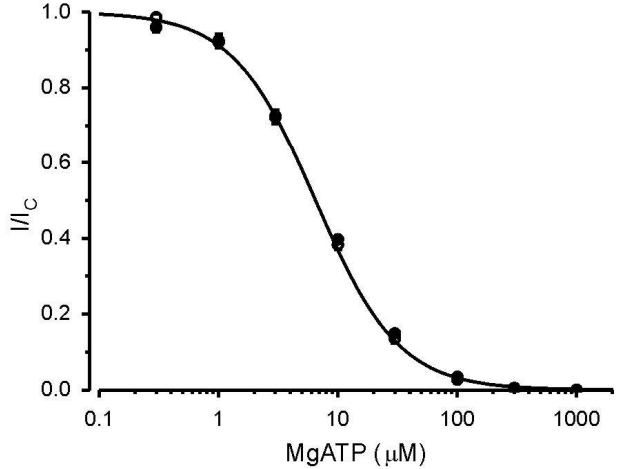
filled symbols: [gliclazide]=30μM





Kir6.2/SUR1-KA2



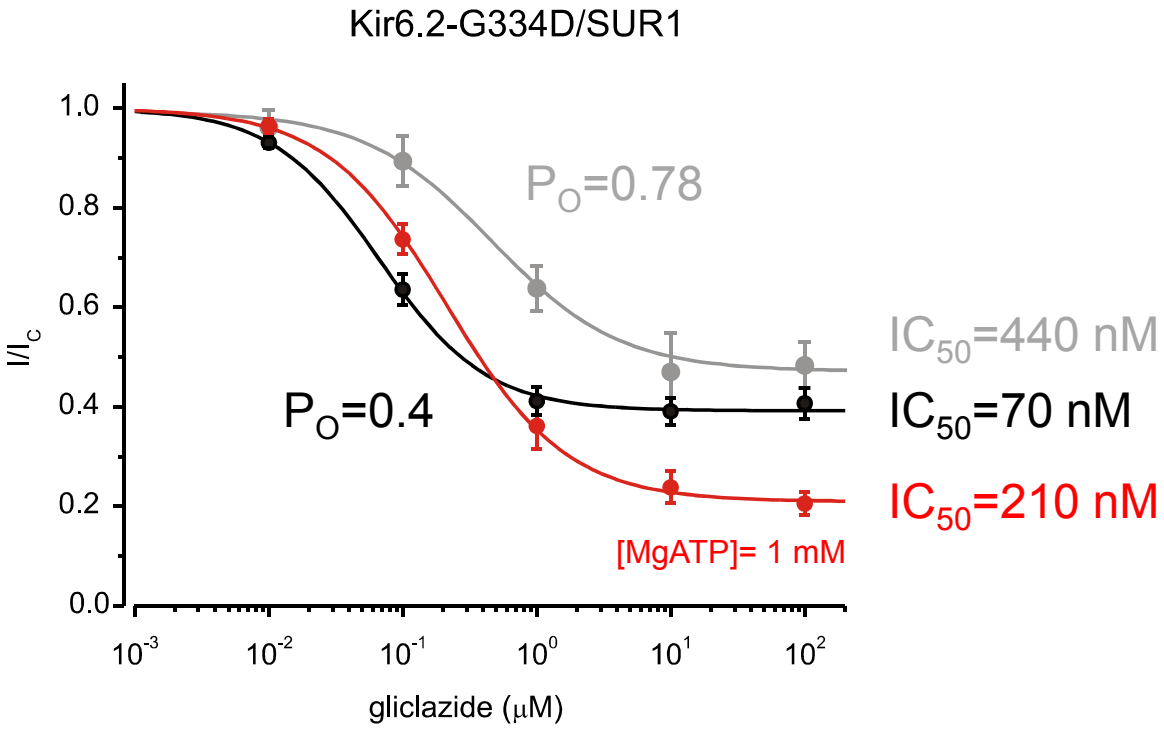


IC_{50} of high affinity block of gliclazide is further increased in the intact cell

Excised patch, no nucleotide

Excised patch, MgATP

Intact cell



Kir6.2-I296L/SUR1

Mg-containing solution

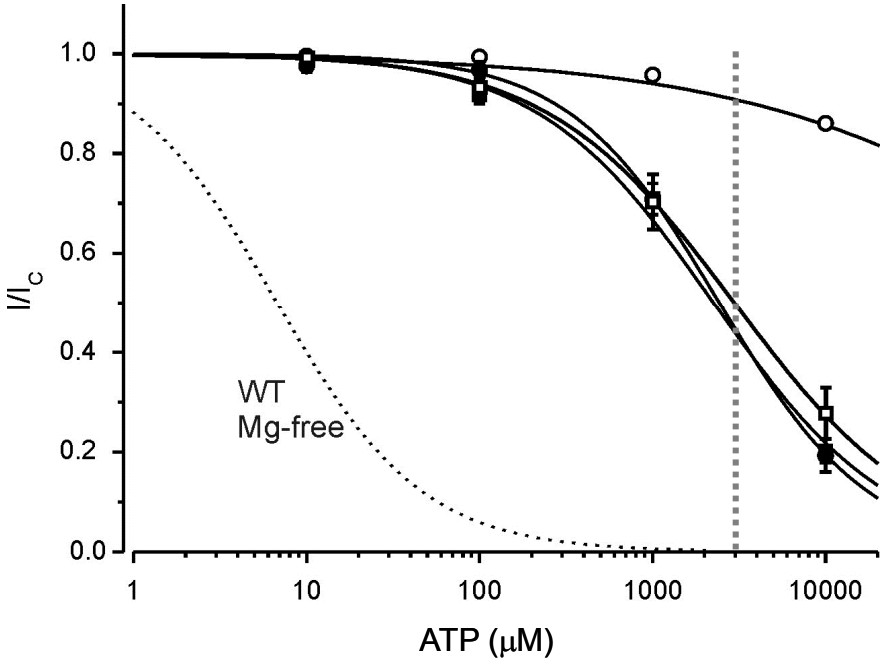
Mg-free solution

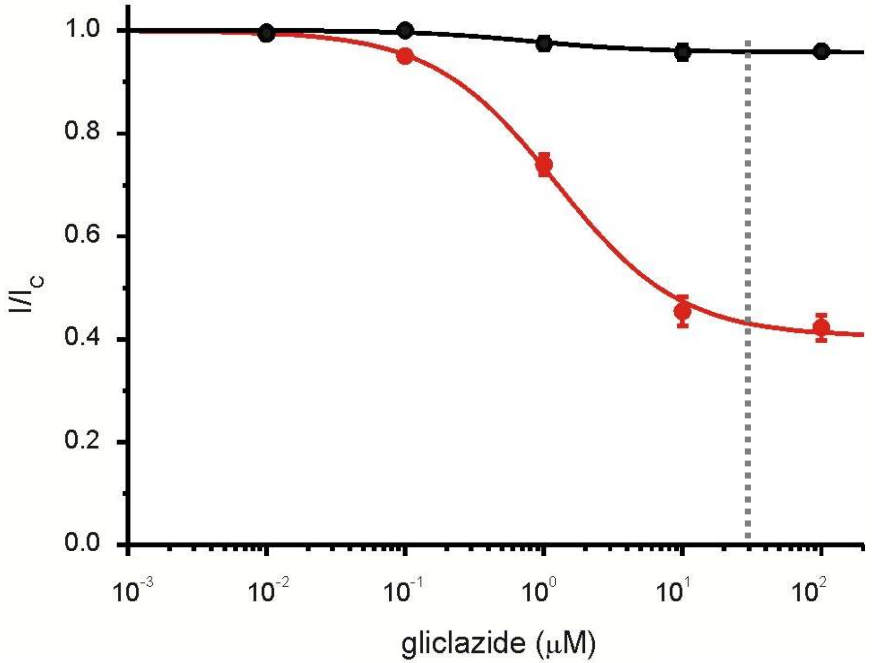
Mg-containing solution + 30μM gliclazide

Mg-free solution + 30μM gliclazide

no ATP

[MgATP]= 3 mM





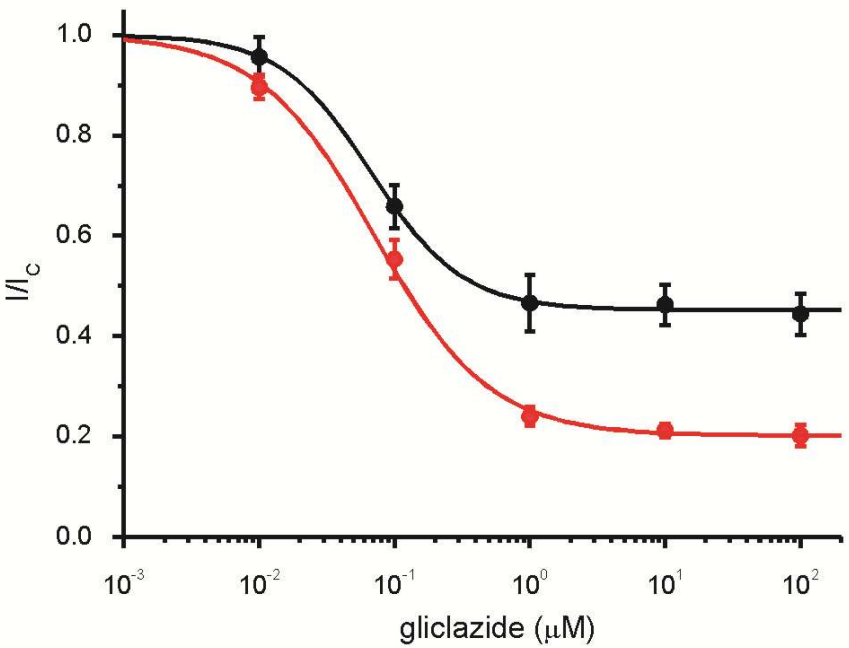
Kir6.2/SUR1

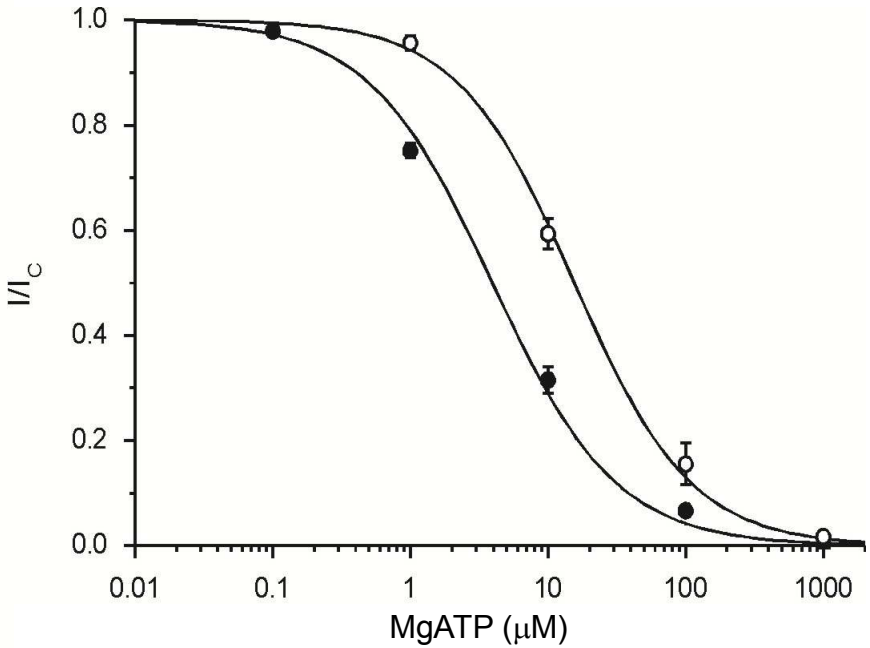
● no ATP

● [MgATP]= 15μM

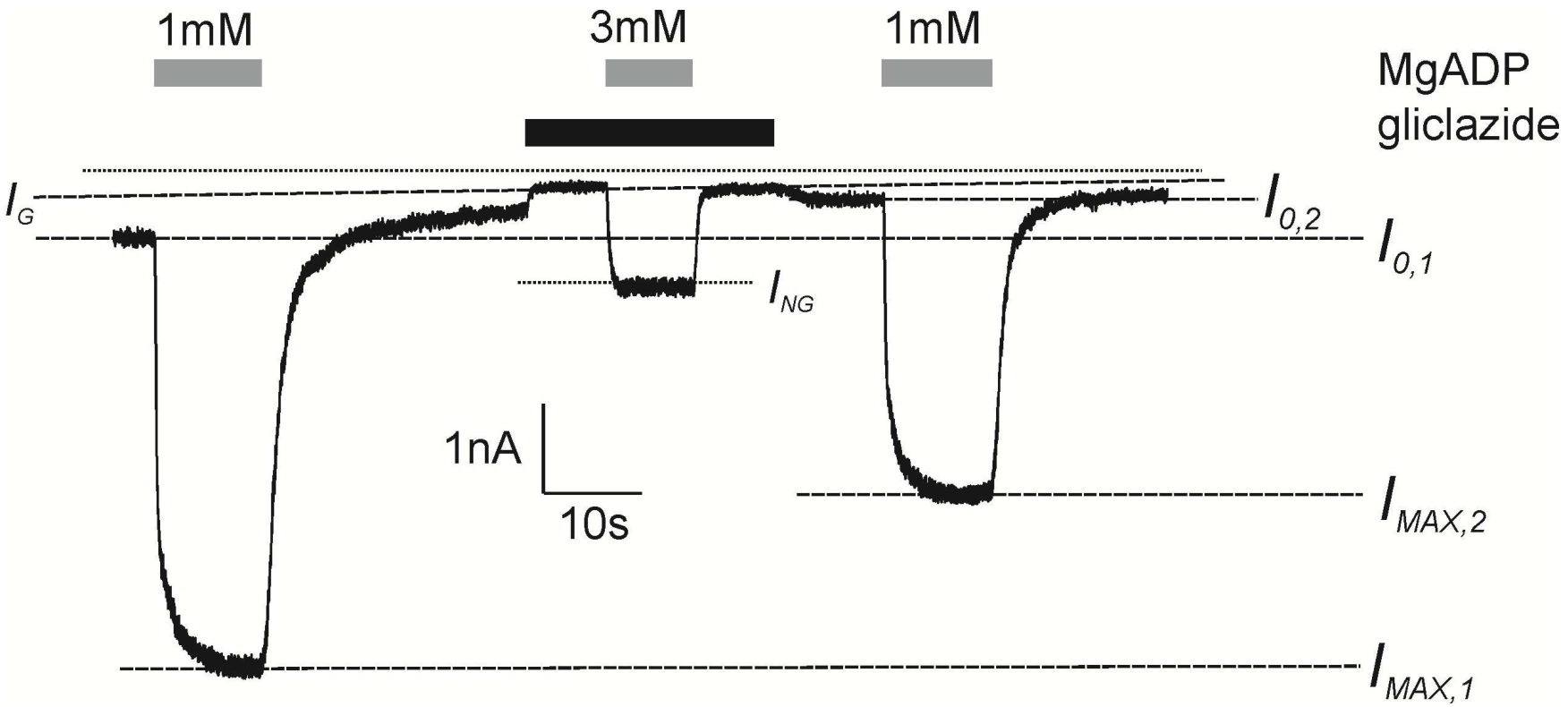
○ [gliclazide]=0

● [gliclazide]=30μM

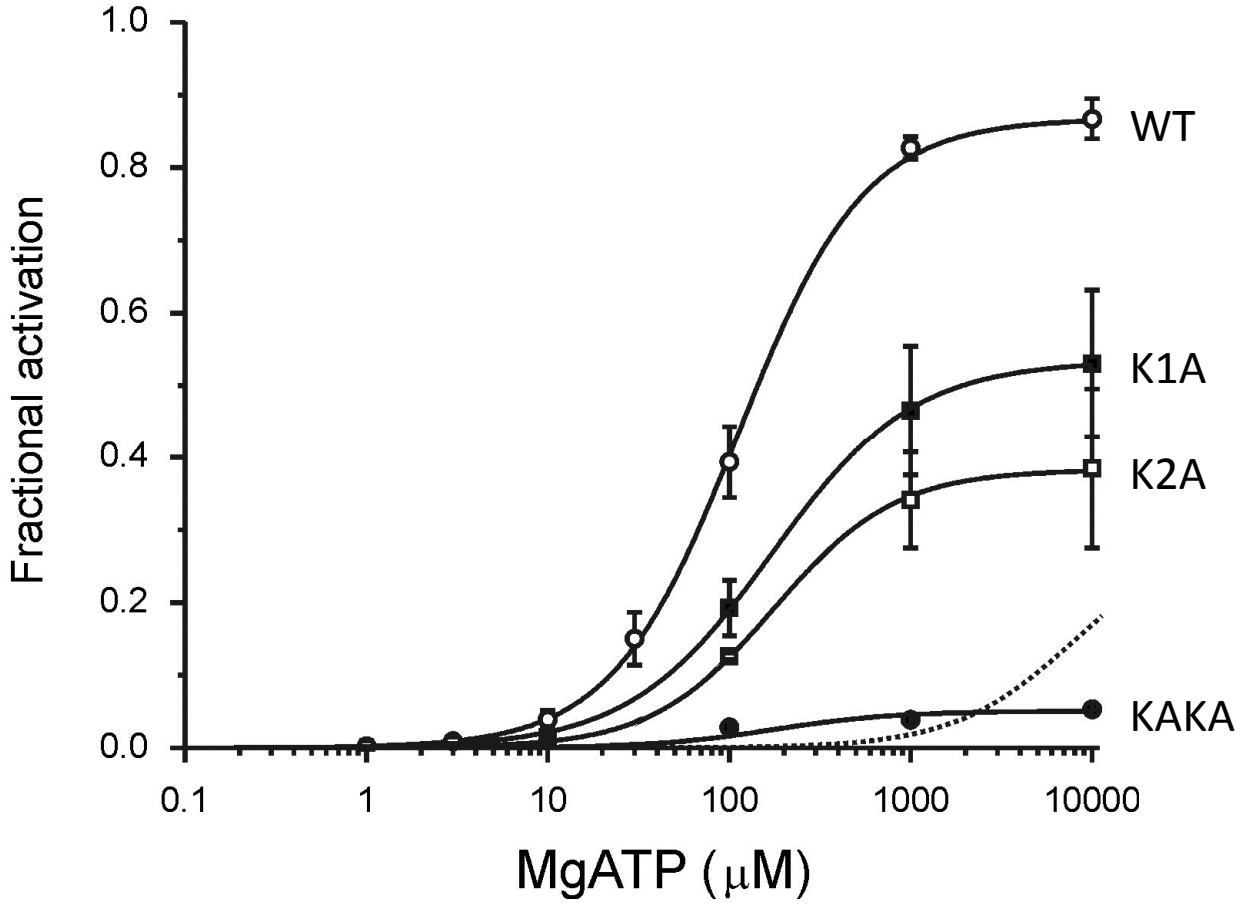




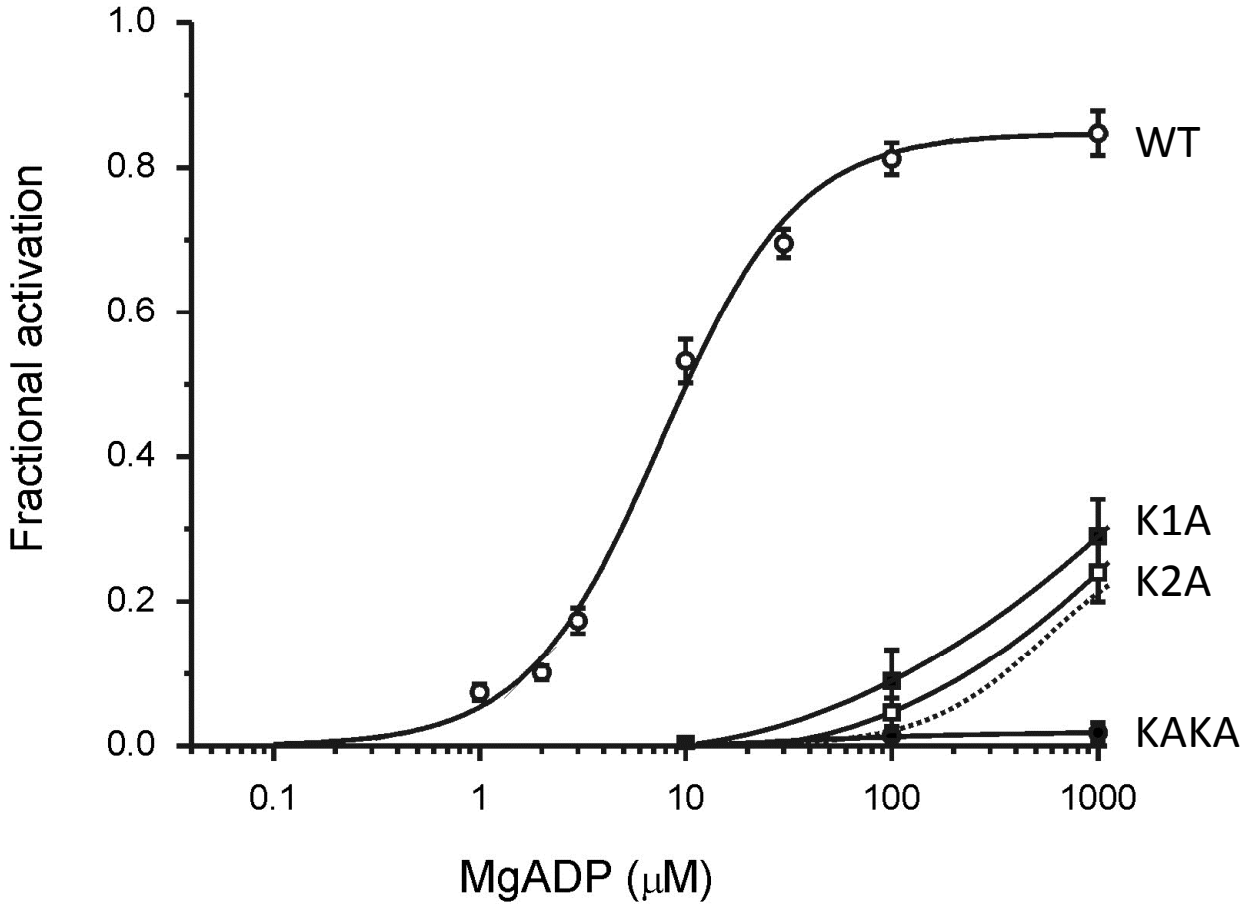
DAMN: Decline of Activation by Magnesium Nucleotides



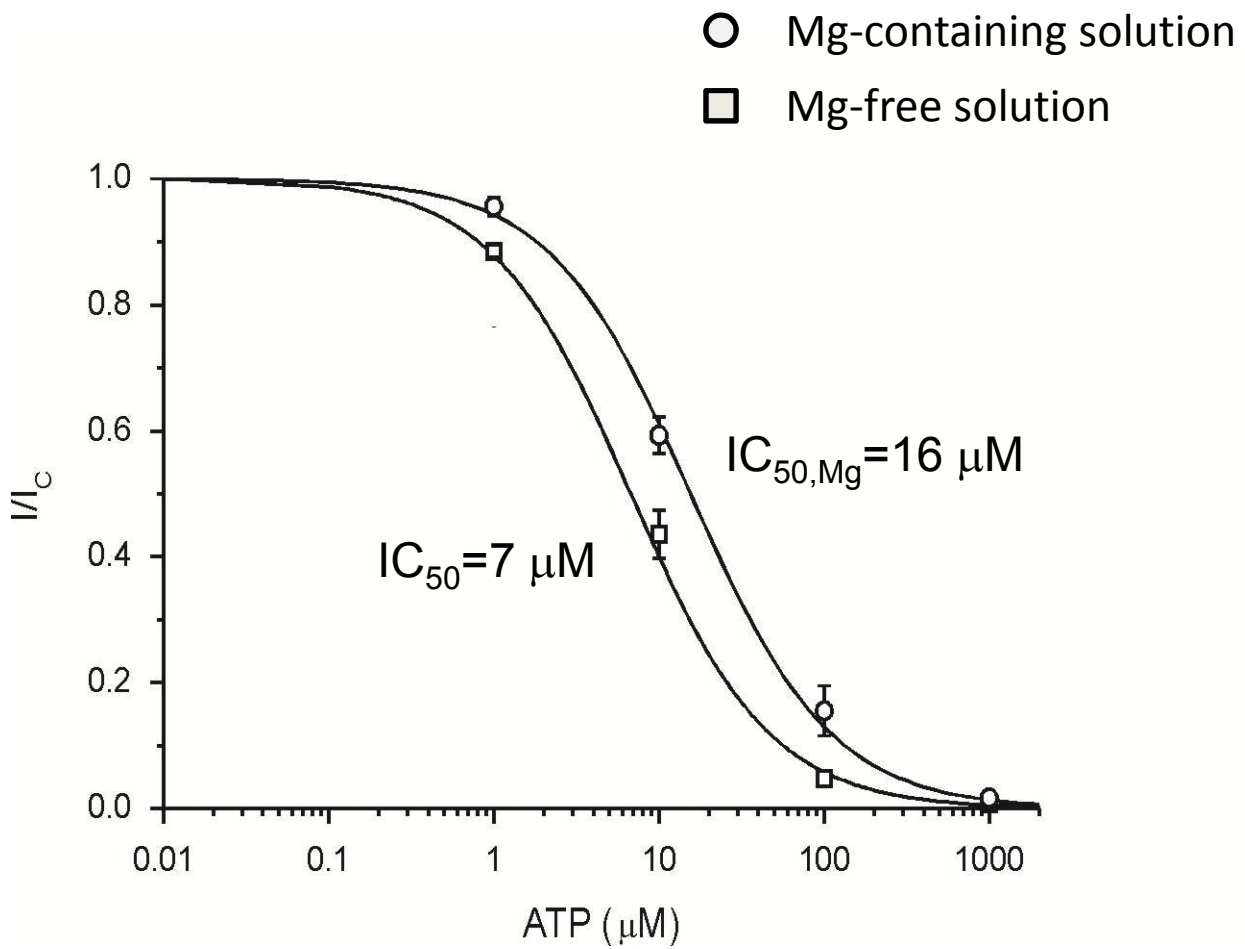
Comparison of the effects of gliclazide and the double KAKA mutation on activation of Kir6.2-G334D/SUR1 channels by MgADP



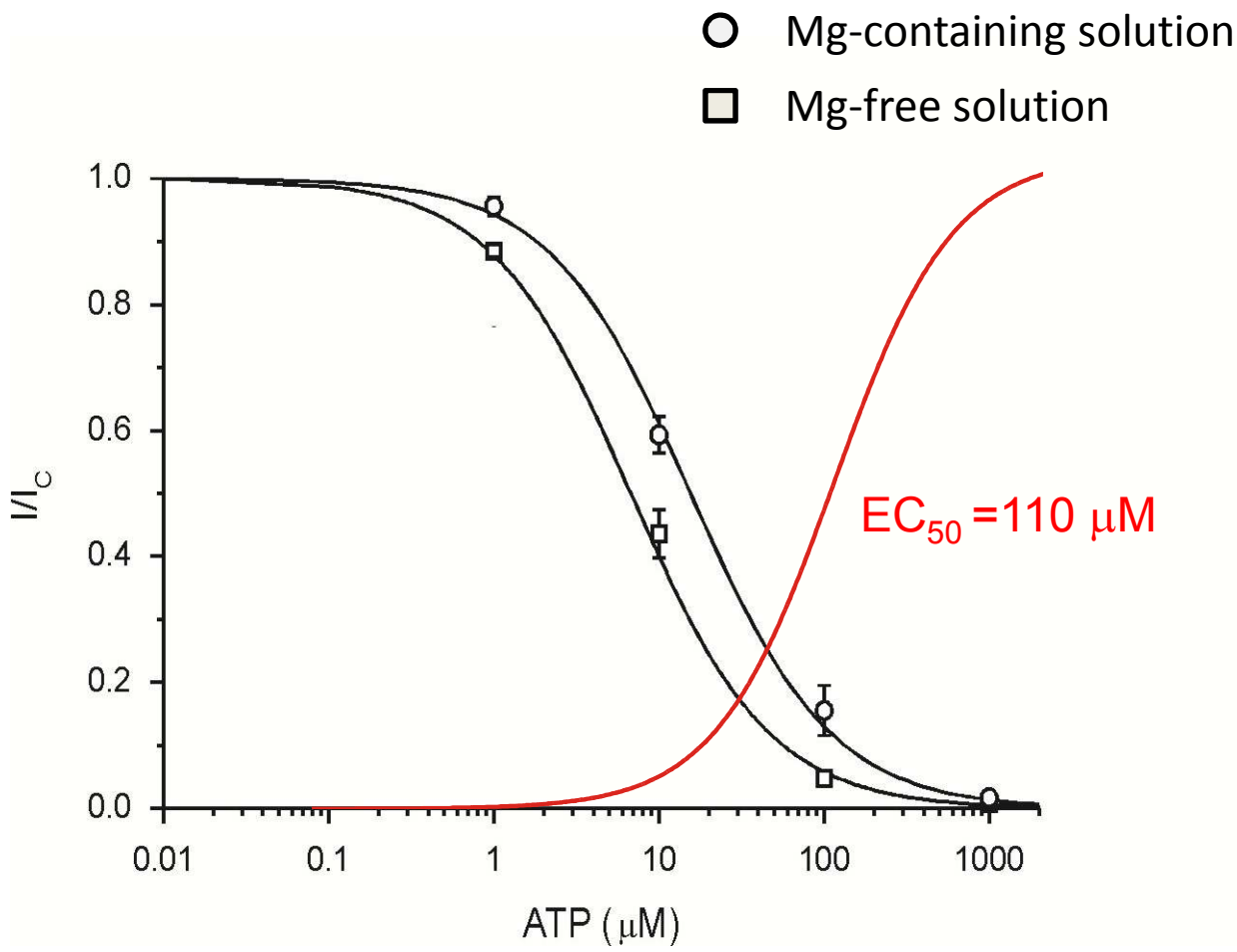
Comparison of the effects of gliclazide and the double KAKA mutation on activation of Kir6.2-G334D/SUR1 channels by MgATP

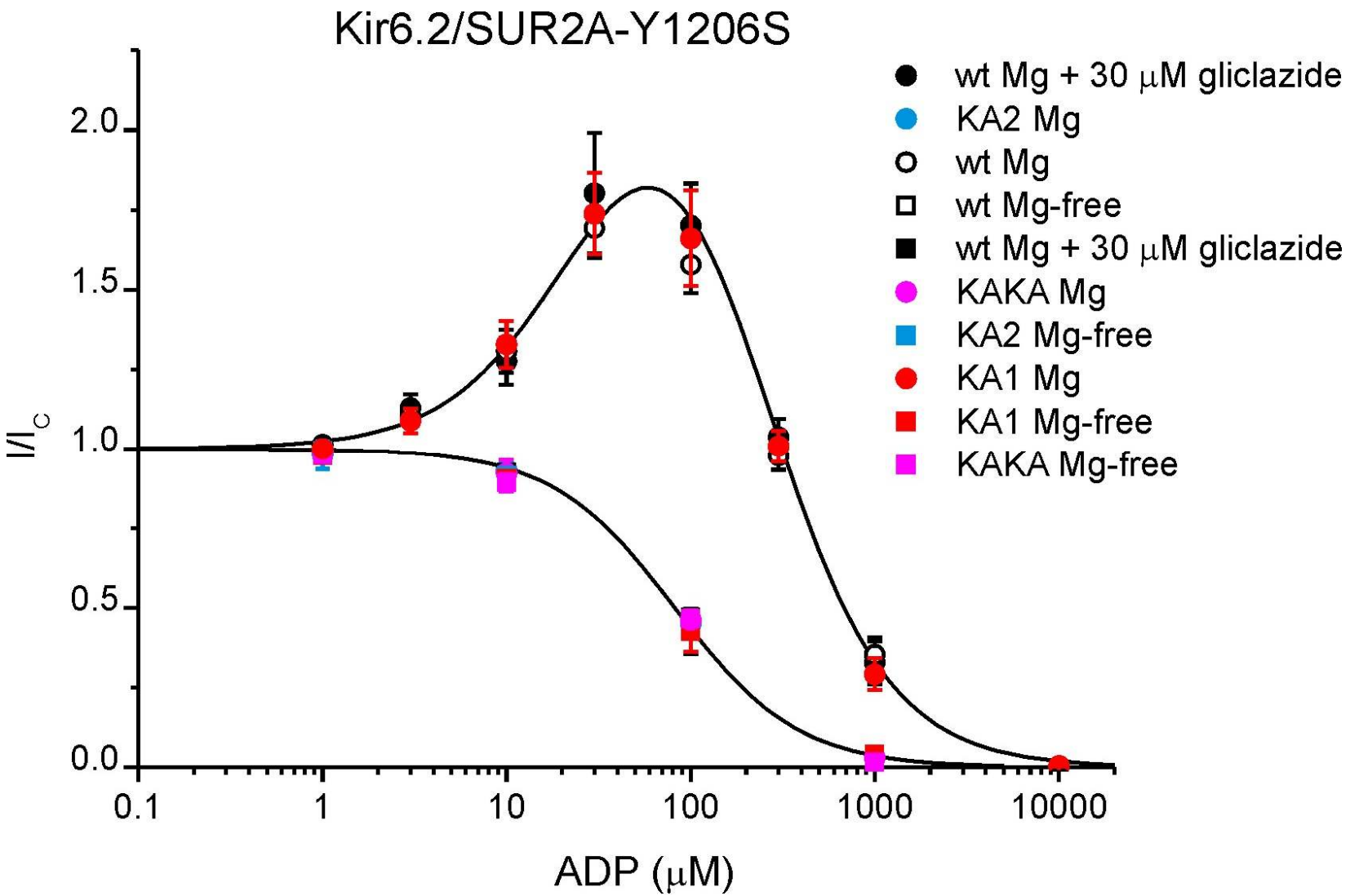


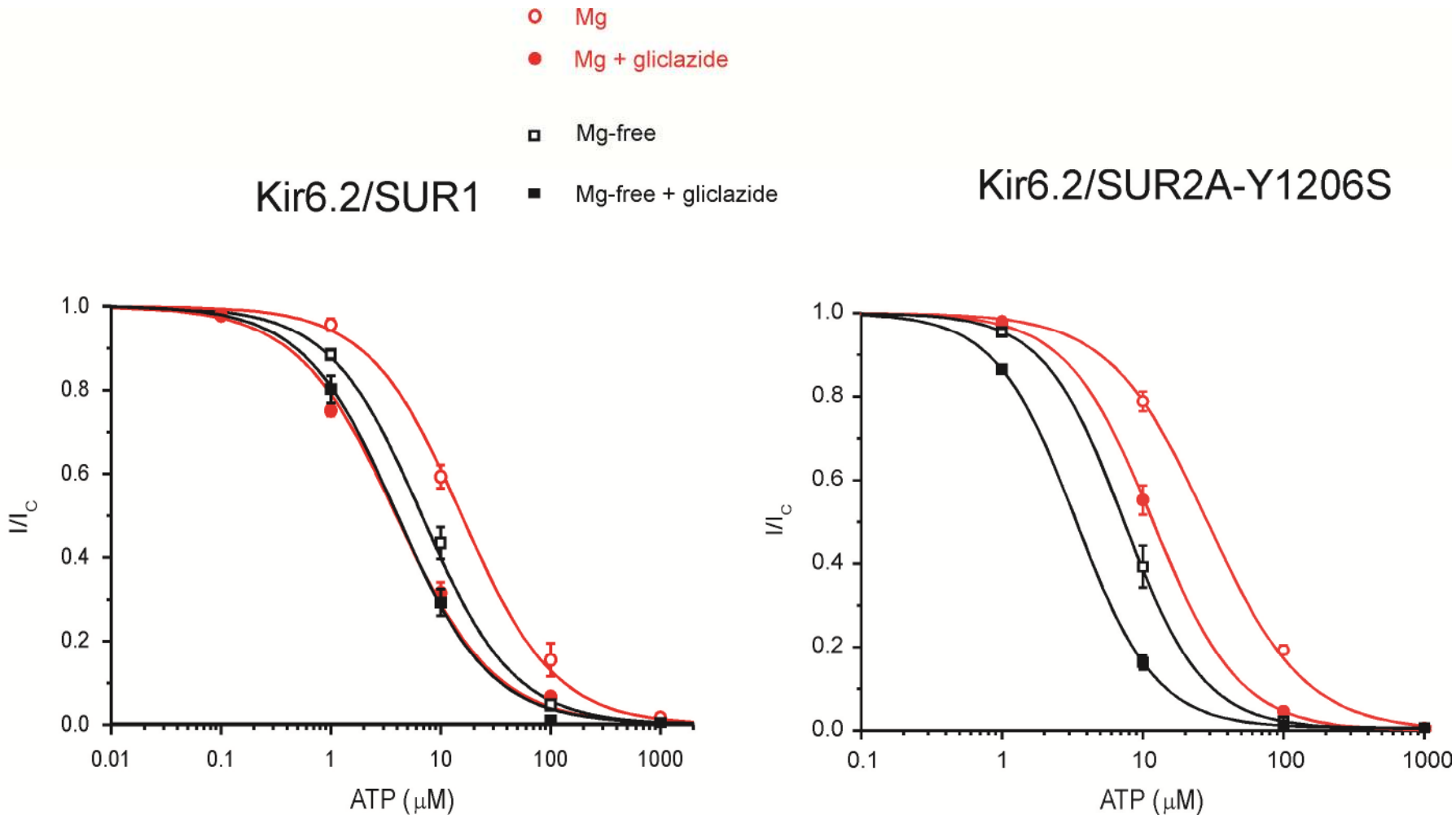
Concentration-response relationships for inhibition of wild-type K_{ATP} channels by ATP in the presence and absence of Mg^{2+}



Concentration-response relationships for inhibition of wild-type K_{ATP} channels by ATP in the presence and absence of Mg^{2+}

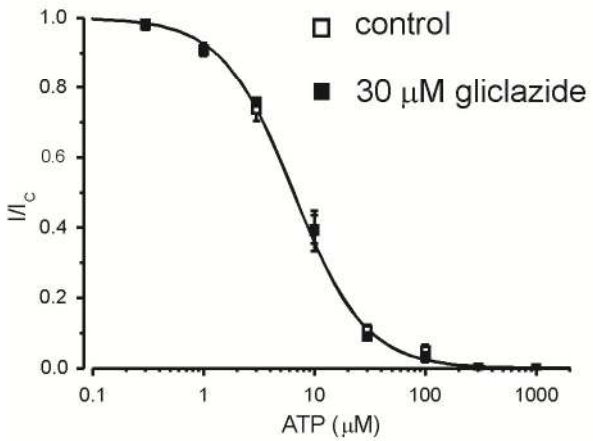



Kir6.2/SUR2A-Y1206S
wt Mg + 30 μM gliclazide
KA2 Mg
wt Mg
wt Mg-free
wt Mg + 30 μM gliclazide
KAKA Mg
KA2 Mg-free
KA1 Mg
KA1 Mg-free
KAKA Mg-free
I/I_C
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
10000
ADP (μM)

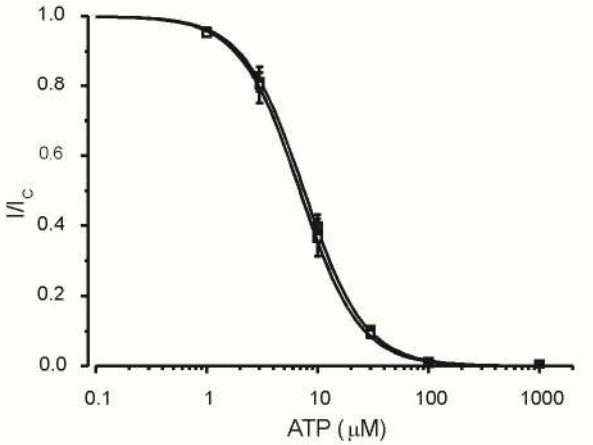

Mg
Mg + gliclazide
Mg-free
Mg-free + gliclazide
Kir6.2/SUR1
Kir6.2/SUR2A-Y1206S
I/I_C
ATP (μM)
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
1000

Mg^{2+}-free

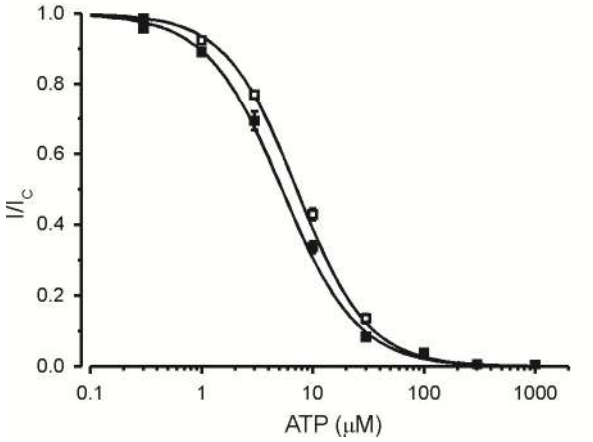
Kir6.2/SUR1-KA1



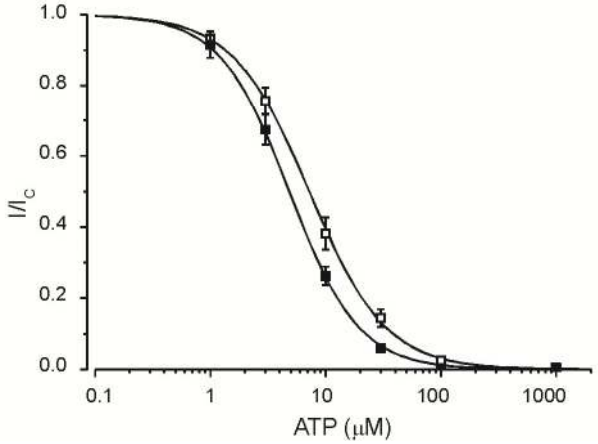
Kir6.2/SUR2A-Y1206S-KA1



Kir6.2/SUR1-KA2

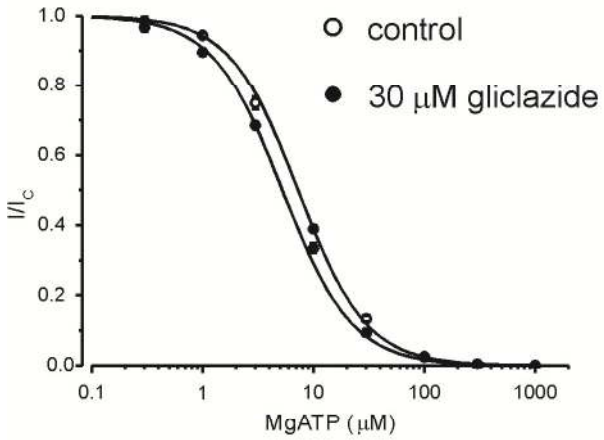


Kir6.2/SUR2A-Y1206S-KA2

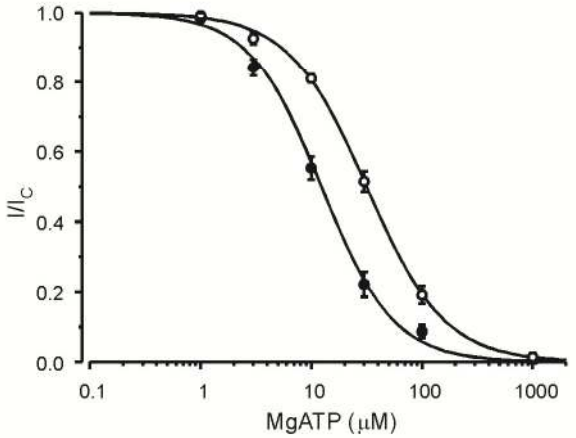


with Mg^{2+}

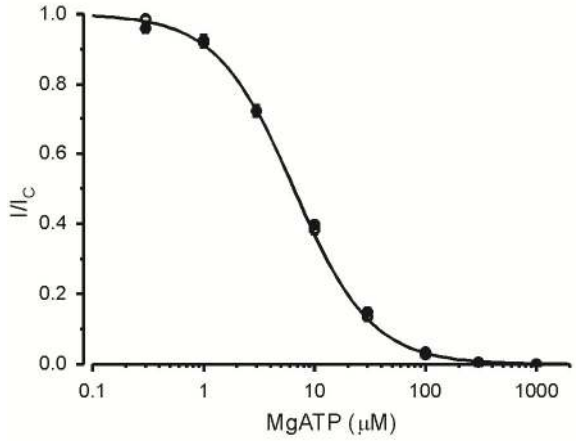
Kir6.2/SUR1-KA1



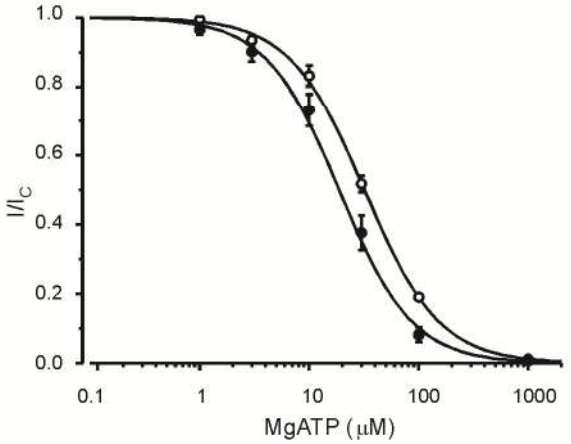
Kir6.2/SUR2A-Y1206S-KA1

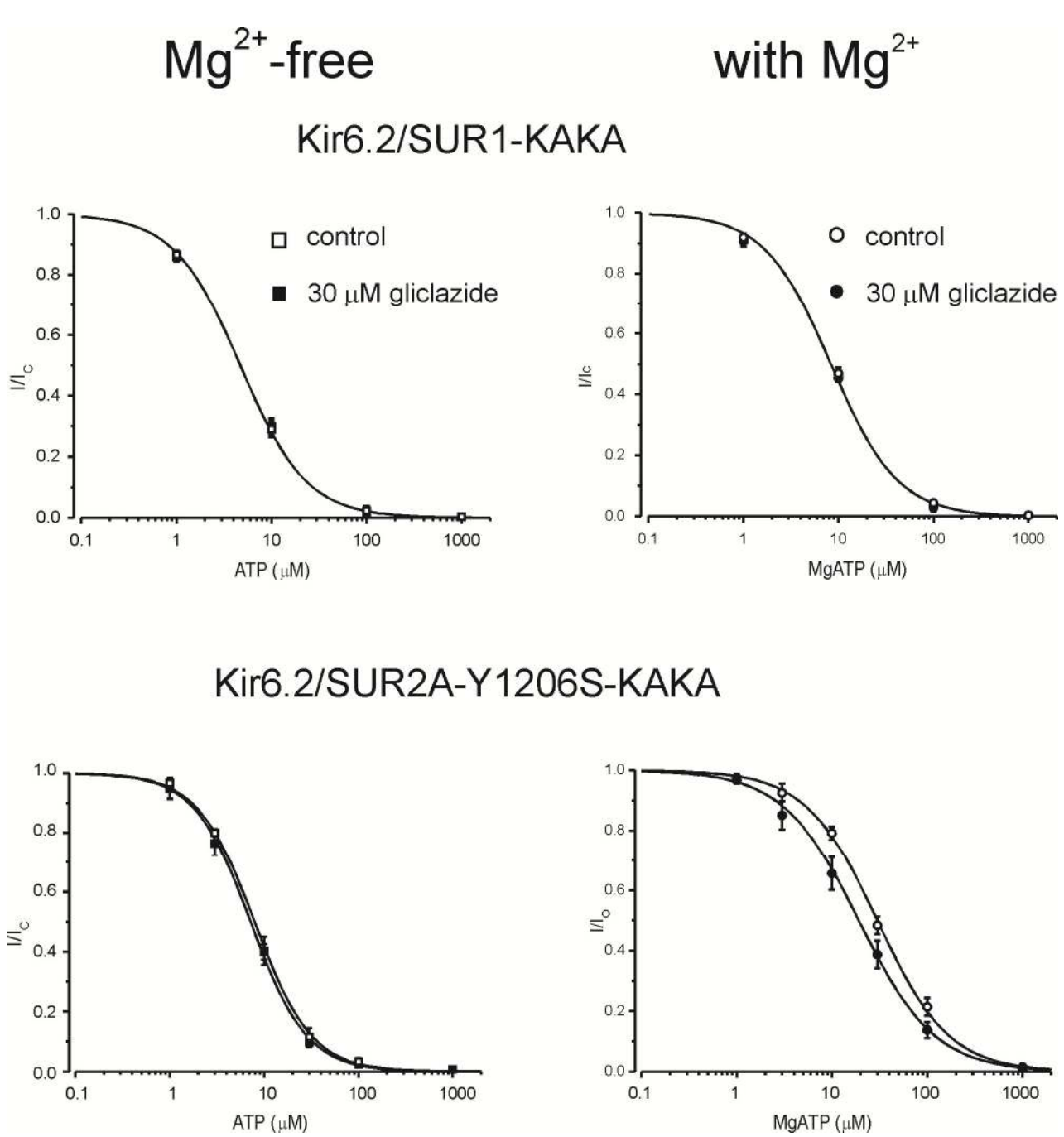


Kir6.2/SUR1-KA2



Kir6.2/SUR2A-Y1206S-KA2




Mg2+-free
with Mg2+
Kir6.2/SUR1-KAKA
control
30 μM gliclazide
I/IC
ATP (μM)
MgATP (μM)
Kir6.2/SUR2A-Y1206S-KAKA
I/IO