



# ACTIVATION OF SULPHONYLUREA-SENSITIVE CHANNELS AND THE NO-cGMP PATHWAY DECREASES THE HEART RATE RESPONSE TO SYMPATHETIC NERVE STIMULATION

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## INTRODUCTION

Depolarization of the presynaptic sympathetic nerve terminal leads to an influx of calcium and exocytotic release of norepinephrine (NE).

Recently, activated neuronal ATP-sensitive potassium channels ( $K_{ATP}$ ) have been implicated as presynaptic inhibitors of stimulation-evoked NE release in the guinea pig atria. Activation of  $K_{ATP}$  channels either by  $K_{ATP}$  channel openers or by depletion of ATP has been proposed as the mechanism by which hyperpolarization of the nerve terminal and shortening of action potential duration reduces calcium influx and exocytotic release of NE.

Similarly, nitric oxide (NO), like  $K_{ATP}$  activation, inhibits peripheral sympathetic activity in the heart. Inhibition of endogenous NO production with non-isoform specific and neuronal NOS (nNOS) inhibitors increases NE release during cardiac sympathetic nerve stimulation (SNS).

Whether  $K_{ATP}$  channels play a functionally significant role in the sympathetic control of cardiac excitability and whether they are modulated by the NO-cGMP pathway is not known.

### Aims:

- To investigate whether modulators of sulphonylurea-sensitive channels ( $K_{ATP}$  channels) alters the peripheral sympathetic control of heart rate in the isolated guinea-pig atria.
- To determine whether an interaction between NO and  $K_{ATP}$  channels affects the HR response to peripheral sympathetic activation.

## METHODS

The atria and right stellate ganglion were dissected free and sutures placed on the lateral edges of both atria. The atria were vertically mounted with the suture in the left atrium connected to a stainless steel hook, and the suture in the right atrium attached to an isometric force transducer. Heart Rate (bpm) was triggered from contraction.

The right stellate ganglion was placed through a pair of platinum ring electrodes connected to a stimulator. Prior to each pharmacological intervention, the ganglion was stimulated at 1, 3 and 5 Hz (10 V, 1ms).

## PROTOCOLS

### Effect of $K_{ATP}$ modulators on the heart rate response to SNS and bath applied NE (Aim 1)

$K_{ATP}$  activation: Diazoxide (5, 10, 50 and 100 $\mu$ M)  
in-vitro bout of hypoxia (0%  $O_2$  / 5%  $CO_2$ )

$K_{ATP}$  inhibition: Glibenclamide (5, 10 and 30 $\mu$ M)

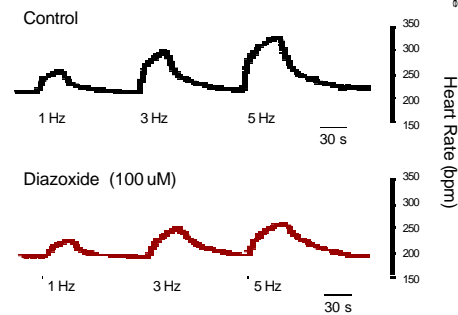
### Interaction between $K_{ATP}$ modulators and NO-cGMP pathway (Aim 2)

$K_{ATP}$  activation + NOS activation: Diazoxide (100 $\mu$ M) + 8-Br-cGMP (0.5mM)

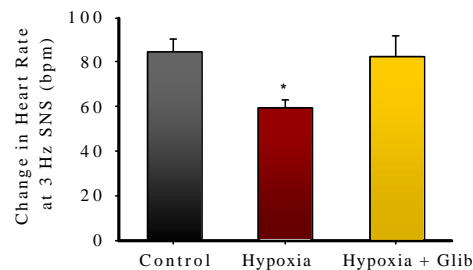
$K_{ATP}$  inhibition + NOS activation: Glibenclamide (30 $\mu$ M) + 8-Br-cGMP 0.5mM

## RESULTS

### AIM 1: Effect of $K_{ATP}$ modulators on the heart rate response to SNS and bath applied NE

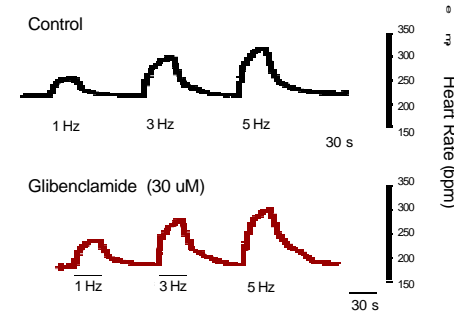


**Figure 1.** Raw data traces showing the effects of cardiac SNS (1, 3 and 5 Hz, 10V, 1ms pulse width) on heart rate (bpm) under control conditions (top trace) and in the presence of the  $K_{ATP}$  opener, diazoxide (100 $\mu$ M; lower trace). Diazoxide significantly attenuated the HR response to SNS ( $p \leq 0.05$ ; ANOVA).



**Figure 2.** The effect in-vitro hypoxia (0%  $O_2$ /5%  $CO_2$ ) and its reversal with glibenclamide (30 $\mu$ M) on the increase in heart rate with right stellate ganglion stimulation (10V, 1ms pulse width, 30 s duration) at 3 Hz (n=6). Hypoxia significantly decreased the magnitude of the positive chronotropic response ( $*p < 0.05$ ; ANOVA) to sympathetic activation and this effect was significantly enhanced with glibenclamide

### AIM 2: Interaction between $K_{ATP}$ modulators and NO-cGMP pathway

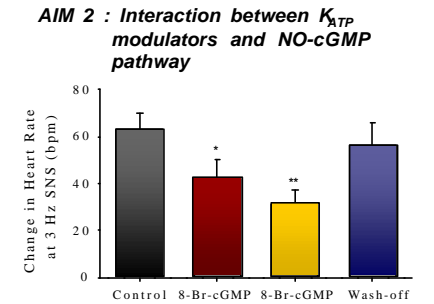


**Figure 3.** Raw data traces showing the effects of cardiac SNS (1, 3 and 5 Hz, 10V, 1ms pulse width) on heart rate (bpm) under control conditions (top trace) and in the presence of the  $K_{ATP}$  inhibitor, glibenclamide (30 $\mu$ M; lower trace). Glibenclamide (n=6) enhanced the HR response to SNS in a concentration dependent manner, reaching a maximal response at 30 $\mu$ M.

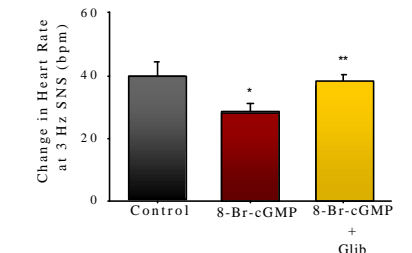
K-ATP Opener	Increase in heart rate with Applied NE			
	0.1	0.3	0.5	0.7 $\mu$ M
<b>Diazoxide (<math>\mu</math>M), n=6</b>				
control	27 ± 7	56 ± 8	68 ± 7	80 ± 6
5	25 ± 5	55 ± 8	69 ± 6	77 ± 5
10	30 ± 5	61 ± 6	72 ± 4	81 ± 5
50	28 ± 6	61 ± 6	70 ± 5	79 ± 5
100	31 ± 7	60 ± 8	76 ± 7	83 ± 8
100 + 30 Glib	37 ± 6	72 ± 5	86 ± 5	89 ± 5
<b>K-ATP Inhibitors</b>				
<b>Glibenclamide (<math>\mu</math>M), n=6</b>				
control	24 ± 4	55 ± 5	74 ± 4	77 ± 3
5	33 ± 5	68 ± 6*	84 ± 5	88 ± 5
10	34 ± 8	75 ± 9*	89 ± 7	89 ± 4
30	43 ± 2*	80 ± 10*	95 ± 8*	88 ± 7

values are expressed mean ± SEM

\*  $p < 0.05$  control vs intervention, ANOVA



**Figure 4.** Effect 8-Br-cGMP (0.5mM) and Diazoxide (100 $\mu$ M) on the increase in heart rate with right stellate ganglion stimulation at 3 Hz (n=7). 8-Br-cGMP significantly reduced the HR response to SNS ( $*p < 0.05$ ; ANOVA) and this effect was then further significantly reduced ( $**p < 0.05$ ) in the presence of diazoxide.



**Figure 5.** Effect of glibenclamide (30 $\mu$ M) in the presence of the cGMP analogue, 8-Br-cGMP (0.5mM; n=12) on the increase in heart rate with SNS. Glibenclamide could still increase the HR response to SNS ( $**p < 0.05$ ; ANOVA) when the response had been significantly reduced ( $*p < 0.05$ ) at 3 Hz nerve stimulation with 8-Br-cGMP.

## CONCLUSIONS

The new findings in this study are that:

- Sulphonylurea-sensitive channels ( $K_{ATP}$  channels) and the NO-cGMP pathway can significantly regulate the HR response to peripheral cardiac sympathetic nerve stimulation.
- Activation of both the NO-cGMP pathway and  $K_{ATP}$  channels reduced the HR response to SNS further than activation of the NO-cGMP pathway alone, indicating that both pathways can act independently.

## ACKNOWLEDGEMENTS

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