

NO-cGMP pathway increases the hyperpolarisation-activated current (I_f) and heart rate during adrenergic stimulation in the guinea pig *in-vitro*.

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Nitric oxide (NO) donors and cGMP analogues can increase heart rate by stimulating the hyperpolarisation activated current, I_f (Musialek *et al.* 1997), although others suggest that this current is inhibited by NO during adrenergic stimulation (Yoo *et al.* 1998). We tested the hypothesis that NO increases heart rate following adrenergic activation via cGMP dependent stimulation of I_f . In the presence of noradrenaline (NA, 1 μ M), the effects of the NO donor sodium nitroprusside (SNP) or the membrane permeable cGMP analogue 8Br-cGMP were evaluated in spontaneously beating sinoatrial node/atrial preparations and permeabilised patch clamped sinoatrial node cells (all at 37°C) from adult guinea pigs (Schedule 1 killing, Animal Scientific Procedures Act). The expression of phosphodiesterase (PDE) 2 and 3 levels was also evaluated by RT-PCR of RNA from the central sinoatrial node region. In the presence of NA, SNP (10 and 100 μ M, n=5) or 8Br-cGMP (n=6) transiently increased heart rate (See Figure 1A). The guanylyl cyclase inhibitor 1H-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ, 10 μ M, n=5) abolished (unpaired Students t-test, $p < 0.05$) the increase in heart rate to SNP (100 μ M) during adrenergic stimulation as did the I_f blockers caesium chloride (2mM, n=7) and 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino)-pyrimidinium chloride (ZD7288, 1 μ M, n=7). Addition of SNP (10 μ M) during adrenergic stimulation also caused a significant (ANOVA) transient increase in I_f (n=5, see Figure 1B). RT-PCR analysis of central sinoatrial node mRNA showed a high level of expression of cGMP inhibited PDE3 compared to PDE2. These results suggest that nitric oxide via a guanylyl cyclase, cGMP dependent pathway still has a stimulatory effect on I_f and heart rate in the presence of adrenergic stimulation. This may be related to the relatively low levels of PDE2 compared to PDE3 being expressed in guinea pig nodal tissue.

Figure 1: Sodium nitroprusside (SNP) increases heart rate (A) and the hyperpolarisation activated current I_f (B) during adrenergic stimulation with noradrenaline (NA)

References:

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