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Haem Modulation of Arterial Large-Conductance Ca^{2+}- Activated K^+ (BK) channels.

Vasospasm is often associated with pathologies like haemorrhagic stroke or malaria but the mechanism responsible for the vasospasm is still poorly understood. During these diseases, severe haemolysis, bursting of red blood cells, promotes the release of haem from haemoglobin. Haem is normally degraded in cells by haem oxygenase (HO) enzymes to carbon monoxide (CO), biliverdin IX and iron (Fe^{2+}). However, haem build-up occurs during haemolysis because the HO system becomes saturated.

Arterial smooth muscle cells (SMCs) express a variety of K^+ channels including the large-conductance Ca^{2+}-activated K^+ (BK) channels which open in response to a rise in intracellular Ca^{2+} levels or depolarisation. The release of Ca^{2+} from intracellular stores (Ca^{2+} sparks) produces a local increase in intracellular Ca^{2+} concentration which triggers the opening of BK channels in close proximity to the release site. This results in the efflux of K^+ ions which causes hyperpolarisation and can be recorded as spontaneous transient outward currents (STOCs). BK channels regulate vascular tone by providing negative feedback to counteract vasoconstriction induced by voltage-gated Ca^{2+} entry. Earlier studies have shown that haem can interact with BK channels to decrease their activity (1). Conversely, CO, a breakdown product of haem, has been reported to enhance BK channel activity (2). Therefore, a balance between the interaction of haem and CO with BK channels may be involved in the vasospasm during these diseases. However, the mechanism of action of haem or CO with K^+ channels remains unclear. The aim of this study is to investigate the mechanism of interaction of haem and CO with BK channels and examine the influence of the intracellular environment on such interaction.

BK channel activity was recorded from smooth muscle cells isolated from the mesenteric artery of adult male Wistar rats. Single-channel currents and STOCs were recorded using inside-out and perforated patch techniques respectively. CO was supplied using CO-releasing molecule 3 (CORM-3) which was freshly prepared before each use. Data are presented as mean ± SEM, statistical analysis was performed using paired and unpaired Student's T-test. Cytoplasmic application of CORM-3 (30 µM) to inside-out patches in the presence of low Ca^{2+} (300 nM) increased single BK channel open probability (Popen) by 5.7 ± 0.4 ($n = 8$, $p< 0.05$) whereas haem (100 nM) reduced activity to $4.7 \times 10^{-3} \pm 3.4 \times 10^{-3}$ times that of control ($n = 4$, $p< 0.05$). Following haem application, the onset of channel inhibition was slower at pH 6.7 ($t_{1/2}= 83 \pm 4$ s) compared with pH 7.2 (($t_{1/2}= 15 \pm 2$ s) ($p< 0.0001$). In whole-cell recordings, extracellular application of haem (5 µM) at membrane potentials of - 30 and - 50 mV significantly increased the mean STOC charge by 1.2 ± 0.1 ($n= 7$, $p< 0.05$) and 1.5 ± 0.1 ($n= 4$, $p< 0.05$) respectively.

In conclusion, cytoplasmic haem inhibits BK channel activity in inside-out patches and our data shows that decreased intracellular pH slows the rate of inhibition. Furthermore, the positive effect of haem on the mean STOC charge may result from the action of CO generated from haem degradation within the cells. The contractile tone of arterial SMCs following haemolysis could be regulated by a balance between the effects of haem and CO on BK channels expressed in these cells.

1) Tang *et al*. (2003). *Nature* **425**: 531-535.

2) Jaggar *et al.* (2005). *Circ Res* **97**: 805-812.