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Different effect of hydrogen sulfide donors AP39 and AP123 in endothelial cells grown in hyperglycaemic environment

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Introduction: Diabetes represents a major disease associated to vascular complications, such as atherosclerosis and other inflammatory-based conditions, converging in hypertension (1-2). Mechanisms underlying these events involve endothelial nitric oxide synthase (eNOS). eNOS activity is suppressed in hyperglycaemic conditions. Resulting NO reduced bioavailability is coupled with reduced levels of eNOS and/or increased levels of caveolin-1 (Cav-1), a regulator of eNOS (3). Diabetic conditions are also linked to reduced circulating hydrogen sulfide (H_2S) levels (4), further undermining endogenous vasorelaxant response (5) and confirming that diabetes couples with H_2S deficiency (6). Here, we wanted to test whether novel H_2S donors, AP123 and AP39 (7), could affect changes to NO-signalling observed in hyperglicaemia.

Method: Bovine aortic endothelial cells (BAEC) were grown in high-glucose environment (HG,50mM,3h) as *in vitro* model for hyperglycaemia. AP123 or AP39 were added at time 0 (same as HG) or at time 1 (1h after HG induction). Cells were then challenged with calcium ionophore A23187 (1µM,30min), to stimulate eNOS activation, then cell pellets and supernatants were collected for western blot analysis and NO levels determination.

Results: Incubation of BAEC with AP39 or AP123 (3h,0.1nM-1µM) restored NO levels in a similar fashion, independently from administration time (370nM and 332nM respectively vs HG 210nM;P&lt0.05, n&gt5). However, the results obtained following concentration-response experiments at time 1, showed that AP39 and AP123 differently affected NOx levels. AP39 induced an "on/off-like" effect (100% at 1nM), while AP123 modulated NOx amount in a concentration-dependent manner (100% at 10nM;P&lt0.05). Western blot analysis showed that AP39 was unable to restore activation (peNOS) and expression of eNOS, impaired by HG conditions. Conversely, HG-dependent reduction in eNOS phosphorylation/expression were both positively modulated by AP123 (100% at 10nM;P&lt0.05).

Conclusions: Overall, these data highlight that AP39 and AP123 modulate NOx levels in a different fashion: AP123 is more efficient than AP39 in restoring eNOS/NO signalling in hyperglycaemic conditions. Although preliminary, these studies suggest that H_2S is a crucial vasculoprotective mediator in diabetic vasculature and that H_2S-releasing molecules may be useful in counteracting the detrimental effects of high glucose environment to the vasculature.

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