

# Compartment modeling of nitric-oxide mediated activation of the cAMP-dependent protein kinase in platelets

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Nitric oxide (NO) is known to be a physiological modulator of platelet aggregation, and is produced by epithelial cells in the blood vessels. Platelet aggregation is in general preceded by a change in their shape from a smooth, discoid to rough, outer surface with pseudopodia, through a reversible process involving reorganization of the cytoskeleton. In platelets, NO activates the soluble Guanylate cyclase (sGC), leading to elevated levels of cGMP, which has several downstream targets like the cGMP-dependent protein kinase (cGPK), cGMP-inhibited phosphodiesterase (PDE3A) and cGMP-stimulated phosphodiesterase (PDE2A). Interestingly, the inhibitory effect of NO on platelet aggregation and shape change can be mimicked by PDE3A-specific inhibitors. We have recently reported that the cAMP-dependent protein kinase (cAPK) plays a crucial part in the NO-mediated inhibition of platelet aggregation (Jensen et al. 2004). In particular, the cAPK mediated phosphorylation of the Vasodilator Stimulated Phosphoprotein (VASP) (Ser157) is highly correlated to platelet shape change.

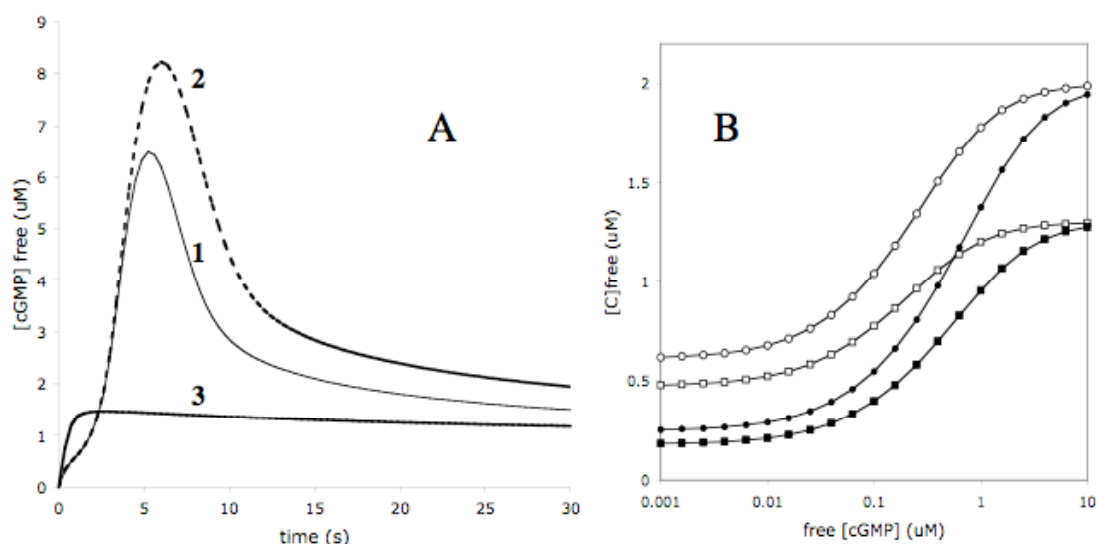
The model incorporates bulk cGMP turnover, through sGC and soluble PDE5 and PDE2A. In addition, we have included high concentrations of the cGPK (up to 7  $\mu\text{M}$ ), which is highly abundant in platelets (Eigenthaler et al. 1992). Binding of cGMP to the allosteric activation domain of PDE5 increases its  $V_{\max}$  10 fold. Similarly, PDE2A, which hydrolyses both cGMP and cAMP with similar efficiencies, is activated by cGMP, but its contribution to total cGMP hydrolysis is kept considerable lower than PDE5, as reported. A peak in cGMP concentration is observed in response to NO, in concordance with previous reports on the kinetics of NO-induced production of cGMP, (Fig. 1A). This property is dependent on the relatively slow activation of PDE5 relative to sGC, but it also renders the system susceptible to desensitisation. The physiological relevance of this property is not known, and thrombin treated platelets can be repeatedly inhibited through several sequential NO infusion/removal cycles (Jensen et al. 2004). Thus, the kinetics of PDE5 activation/deactivation largely governs the shape of the cGMP-response curve and the time needed for complete re-zeroing of the system. Due to high levels of cGPK, our model retains an elevated level of total cGMP (bound) as long as the system is exposed to NO.

Compartmentalization of cAMP signaling is well described in several cell systems and involves anchoring of the cAPK as well as localization of different phosphodiesterase isotypes. PDE3A, which is abundant in platelets, has low  $K_m$  value for cAMP (90 nM), and is therefore well suited to maintain a low basal level of this nucleotide. It is also inhibited at low levels of cGMP and can thereby facilitate cross-talk between these second messengers. Since stimulation with NO or inhibition of PDE3A only produces a small increase in the total cAMP level, it is suggested that

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NO-mediated activation of cAPK and its inhibition of platelet shape-change occurs in a compartment. Recently it was also reported that the cAPK holoenzyme binds cAMP with relatively low affinity ( $S_{0.5} = 2.9 \mu\text{M}$ ,  $H = 1.4$ ) and that the cAMP-bound holoenzyme is less dissociable than hitherto believed (Dao et al. 2006, Viste et al. 2005). This is taken into account for activation of cAPK at the high levels reported in platelets ( $>3 \mu\text{M}$ ). We find that the steady-state activation of cAPK at 3–6  $\mu\text{M}$  is most sensitive to cGMP concentrations between 0.05–1  $\mu\text{M}$ , and that the signaling capacity is dependent on the basal cAMP-level in the compartment (Fig. 1B).



**Fig. 1** (A) A peak in the kinetics of NO-mediated cGMP-response is observed due to relatively slow activation of PDE5 (5 nM NO, PDE5  $K_D$  cGMP 130 nM,  $k_d$   $1.3 \cdot 10^{-3} \text{ s}^{-1}$  (1),  $k_d$   $6.5 \cdot 10^{-4} \text{ s}^{-1}$  (2)) and on previous exposure to NO (10 nM NO, sGC inactive at initial conditions). (B) The steady state activation of cAPK at 6  $\mu\text{M}$  (○,●) and 3  $\mu\text{M}$  (□,■) in response to variable concentration of cGMP. Decreasing the basal influx of cAMP increases the total response capability of the localized cAPK (●,■).

#### References

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