Check for updates

To the editor:

Roles of cGMP/cGMP-dependent protein kinase in platelet activation

Within recent correspondence[1,2] concerning the roles of cyclic guanosine monophosphate (cGMP)/cGMP–dependent protein kinase (cGK or PKG), Du et al[1(p4371)] state that their data[3,4] and ours[5] "clearly show that PKG inhibitors attenuate platelet aggregation induced by ristocetin and thrombin, and inhibit thrombin-induced ERK phosphorylation." Unfortunately, this statement is used out of context, is completely misleading, and does not reflect the major message of our paper. Although PKG inhibitors did attenuate platelet aggregation, our further experiments led us to conclude that this was PKG *in*dependent, opposite to the conclusion that Du et al reached. A very important finding of our study was that both PKG activators and inhibitors had potent and rapid "unspecific" (ie, *un*related to PKG activity!) inhibitory effects on platelet activation in response to different agonists. The claim of Li et al[3,4] that PKG activation causes extracellular signal-related kinase (ERK) phosphorylation and subsequent platelet activation could not be reproduced by us[5] or Marshall et al[6] using a variety of conditions and PKG stimulators. Our study[5] focused only on human platelets, and used platelet isolation methods and chemicals exactly as reported by Li et al[3,4] in order to minimize possible methodological differences.

Furthermore, the strong disagreement of our study[5] with the claim of Li et al[3,4] that PKG stimulation contributes to platelet activation is not only at the level of "*interpretation and conclusions*" as stated by Du et al,[1(p4371)] but also in essential findings, ours being in fact similar to and complementary to those reported by Senis et al[2] and Marshall et al.[6]

We also disagree with the soundness of other protein kinase inhibitory data presented by Li et al.[3] Earlier critical warnings by Burkhardt et al[7] and others, concerning problems with the use of certain protein kinase inhibitors, were essentially ignored by Li et al.[3,4] For example, H89, even when used at much lower concentrations (5-10 μM) than those used by Li et al[3] (25-50 μM), is known to inhibit both PKG and protein kinase A (PKA).[7] KT5823 is an unreliable in vitro inhibitor of PKG, and in intact cells has unpredictable effects, including enhancing vasodilator-stimulated phosphoprotein (VASP) phosphorylation in platelets.[7] In contrast to Li et al,[3] we showed that VASP phosphorylation induced by 8-Br-PET-cGMP is inhibited by Rp-8-Br-PET-cGMPS, but not by Rp-8-Br-cAMPS, and that myristoylated PKI (a PKA inhibitor) even had stimulatory effects on platelets. Thus, none of our data supports the Du et al thesis that cGMP inhibitory effects on platelets are mediated by PKA rather than PKG.

We would like to emphasize again that the main consensus of our results and conclusions differs from that published by Li et al.[3,4] Importantly, both our study[5] and that of Marshall et al[6] found no evidence supporting Du et al's hypothesis that the cGMP/PKG pathway is involved in platelet activation, but rather (re)establish the important inhibitory roles of both cyclic adenosine monophosphate (cAMP)/PKA and cGMP/PKG in human platelets.

Ulrich Walter and Stepan Gambaryan

Correspondence: Ulrich Walter, Institut für Klinische Biochemie & Pathobiochemie, Josef Schneider Str 2, 97080 Würzburg, Germany; e-mail: uwalter@klin-biochem.uni-wuerzburg.de

References

1. Du X, Marjanovic JA, Li Z. On the roles of cGMP and glycoprotein Ib in platelet activation [letter]. Blood. 2004;103:4371-4372.
2. Senis Y, Marshall S, Watson S. On the roles of cGMP and glycoprotein Ib in platelet activation: a response [letter]. Blood. 2004;103:4372-4373.
3. Li Z, Ajdic J, Eigenthaler M, Du X. A predominant role for cAMP-dependent protein kinase in the cGMP-induced phosphorylation of vasodilator-stimulated phosphoprotein and platelet inhibition in humans. Blood. 2003;101:4423-4429.
4. Li Z, Xi X, Gu M, et al. A stimulatory role for cGMP-dependent protein kinase in platelet activation. Cell. 2003;112:77-86.
5. Gambaryan S, Geiger J, Schwarz UR, et al. Potent inhibition of human platelets by cGMP analogs independent of cGMP-dependent protein kinase. Blood. 2004;103:2593-2600.
6. Marshall SJ, Senis YA, Auger JM, et al. GPIb-dependent platelet activation is dependent on Src kinases but not MAP kinase or cGMP-dependent kinase. Blood. 2004;103:2601-2609.
7. Burkhardt M, Glazova M, Gambaryan S, et al. KT5823 inhibits cGMP-dependent protein kinase activity in vitro but not in intact human platelets and rat mesangial cells. J Biol Chem. 2000;275:33536-33541.

Downloaded from http://ashpublications.org/blood/article-pdf/104/8/2609/1702777/zh802004002607c.pdf by guest on 13 August 2024