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LETTER TO THE EDITOR

Activation of platelets by endocannabinoids: Distinct agonists or arachidonate reservoirs?

Mauro Maccarrone^{1,2}

¹Center of Integrated Research, Campus Bio-Medico University of Rome, Rome, Italy and ²European Center for Brain Research (CERC)/Santa Lucia Foundation, Rome, Italy

To the editor,

Endocannabinoids (eCBs) are signaling lipids with manifold actions in the central nervous system and in the periphery, therefore their dysregulation has a significant impact on human health and disease. Research efforts have clearly documented that these compounds play a relevant role within the cardiovascular system, where their binding to type-1 (CB₁) and type-2 (CB₂) cannabinoid receptors can promote or protect from atherosclerosis, respectively. In particular, the prominent eCBs 2-arachidonoylglycerol (2-AG) and N-arachidonylethanolamine (anandamide, AEA) have been shown to act as an agonist or a weak co-agonist of human platelets, respectively. To date, independent investigations into the mechanism of platelet activation by eCBs have provided conflicting results, that span from evidence for a CB₁/CB₂-dependent mechanism, to a non-CB₁/CB₂-dependent signal transduction or even to a classical arachidonic acid (AA)-dependent pathway. Here, I would like to supplement relevant information, that leads to the conclusion that the complexity of eCBs signaling and the plasticity of platelets, both highly reactive systems that strongly depend on environmental conditions, make it rather unlikely that a drug can hold the answer to the molecular details of their interactions. Therefore, the effects of inhibitors of metabolic enzymes and/or of receptor agonists/antagonists (especially when used at non-optimal doses) should be interpreted with caution.

Endocannabinoids (eCBs) are signaling lipids with manifold actions in the central nervous system and in the periphery, therefore their dysregulation has a significant impact on human health and disease. Research efforts have clearly documented that these compounds play a relevant role within the cardiovascular system, where their binding to type-1 (CB₁) and type-2 (CB₂) cannabinoid receptors can promote or protect from atherosclerosis, respectively [1]. In particular, the prominent eCBs 2-arachidonoylglycerol (2-AG) [2] and N-arachidonylethanolamine (anandamide, AEA) [3] have been shown to act as an agonist or a weak co-agonist of human platelets, respectively. The latter cell fragments are pivotal players in acute atherothrombosis as well as in the pathogenesis and progression of atherosclerosis [4]. To date, independent investigations into the mechanism of platelet activation by eCBs have provided conflicting results, that span from evidence for a CB₁/CB₂-dependent mechanism, to a non-CB₁/CB₂-dependent signal

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transduction or even to a classical arachidonic acid (AA)-dependent pathway. Indeed, AA can be released from eCBs by specific enzymes, namely monoacylglycerol lipase (MAGL) for 2-AG, and fatty acid amide hydrolase (FAAH) for AEA [5]. A recent article published in *Platelets* by Brantl et al. [6] has presented a balanced view of the state of art of platelet activation by eCBs, discussing how differences in platelet preparation (washed platelets vs. platelet-rich plasma), their source (rabbit vs. human), and composition of the aggregation medium (from albumin-free buffers to whole blood) can clearly affect activation of these extremely reactive cell fragments. In particular, in their investigation Brantl et al. [6] show that 2-AG induces human platelet aggregation in platelet-rich plasma and whole blood, corroborating previous data from my group and others; yet, they suggest that this effect of 2-AG occurs through its hydrolysis by MAGL, subsequent release of AA and cyclooxygenase-dependent conversion of the latter fatty acid to thromboxane A₂ [6]. As in previous related reports, the conclusions of Brantl and colleagues were essentially based on the ability of admittedly high doses of acetylsalicylic acid (5.4 mM), a well-known cyclooxygenase inhibitor [7], and of JZL184 (20 μM), a selective inhibitor of MAGL at nanomolar concentrations (IC₅₀ ~ 8 nM) [8], to block the effect of 2-AG [6].

Here, I would like to supplement that an emerging issue concerning the biological activity of eCBs is the different localization of their target receptors and metabolic enzymes within the cell. CB₁ and CB₂ are G-protein-coupled receptors with an extracellular binding site, whereas MAGL and FAAH have distinct membrane-bound intracellular localizations [9]. Therefore, the question arises as to how lipid signals like eCBs can find the right target and reach it at the right time in an aqueous medium. This point highlights the relevance of intracellular trafficking of eCBs as a key determinant of their biological activity, including cardiovascular effects. It also suggests that intracellular transporters of eCBs should be considered as important players when trying to understand the regulation of eCBs signaling, eventually developing novel therapeutic strategies to combat eCBs-related human diseases. Some of these transporters have been identified [9, and references therein], and

remarkably they have been shown to drive indeed eCBs signaling in a specific manner [10]. Against this background, here I would like to make a warning that selective inhibitors like JZL184 (especially if used at quite high concentrations) might hit unexpected off-targets that significantly contribute to the biological activity of eCBs. Incidentally, besides potentially unknown off-targets JZL184 inhibits FAAH with an IC_{50} of $\sim 4 \mu M$, and can affect CB_1 -dependent signaling through down-regulation and desensitization of this receptor [11]. Another major point that I would like to make is that eCBs have been clearly demonstrated to be good substrates for the key-enzymes responsible for the classical arachidonate cascade (i.e., cyclooxygenases and lipoxygenases), leading to the generation of oxygenated derivatives endowed with distinct biological activities [12]. Therefore, any inhibitory effect of acetylsalicylic acid might be due to blockade of eCBs oxidation by cyclooxygenase activity, rather than to blockade of the conversion of eCBs-released AA into thromboxane A_2 [12]. On a final note, I would like to comment that it appears unlikely that lipid signals like eCBs, which are biosynthesized and degraded through strictly regulated pathways [5, 9], are used as a simple reservoir of AA. In this event, one should predict that also AEA activates platelets as efficiently as does 2-AG; indeed, platelets have an active FAAH, that promptly cleaves AEA and releases AA [3]. Instead, AEA has been consistently found to be much weaker than 2-AG (if active at all) as platelet agonist [2, 3, 6].

In conclusion, I believe that the complexity of eCBs signaling and the plasticity of platelets, both highly reactive systems that strongly depend on environmental conditions, make it rather unlikely that a drug (be it acetylsalicylic acid or JZL184) can hold the answer to the molecular details of their interactions. Therefore, the effects of inhibitors of metabolic enzymes and/or of receptor agonists/antagonists (especially when used at non-optimal doses) should be interpreted with caution. Overall, I believe that platelet activation by eCBs still remains an open and rather complex question.

Declaration of interest

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