# Platelet activation: a new vascular activity of anandamide

Mauro Maccarrone, Alessandro Finazzi-Agrò*

*Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Via di Tor Vergata 135, I-00133 Rome, Italy*

Received 20 July 2000; revised 14 September 2000; accepted 15 September 2000

Edited by Matti Saraste

Endocannabinoids are amides and esters of long chain polyunsaturated fatty acids, which act as mediators in brain and peripheral tissues through the binding to cannabinoid receptors. Anandamide (*N*-arachidonylethanolamine, AEA) is a major endocannabinoid, showing cardiovascular activity [1] by induction of vasorelaxation [2]. Although AEA has been described as an endothelium-derived hyperpolarizing factor (EDHF), this hypothesis was debated and recent data suggest that EDHF is instead a cytochrome P450-metabolite [3]. Whether or not an EDHF, AEA is likely to play an important role in the control of vascular tone, as supported also by the observation that both rat endothelial cells and macrophages can release it [4].

We found and published in this journal [5] that AEA ($\leq$1.2 mM) activates human platelets by a cannabinoid receptor-independent mechanism, which involves a rise in intracellular calcium and does not depend on the arachidonate cascade. In the same paper we showed that human platelets have the biochemical machinery to degrade AEA, i.e. a high affinity transporter and a fatty acid amide hydrolase (FAAH). Afterwards, these data have been largely confirmed by Braud et al. [6], who found that AEA in rabbit platelets was active at physiological concentrations ($\leq$10 μM) when used in combination with $CaCl_2$ and fibrinogen. These authors attributed the aggregating effect of AEA to its cleavage into arachidonic acid by an AEA-degrading enzyme [6]. At any extent, these data suggest that AEA is an unlikely physiological agonist of platelets, but it can rather act in vivo as a co-agonist in combination with other 'classical' aggregating molecules such as arachidonic acid, fibrinogen or thrombin [5,6]. However, the role of endocannabinoids in the cross-talk between platelets and endothelium, which might be crucial in thrombosis, still awaits clarification.

Platelet activation by AEA appears of interest also because it can be released from endothelial cells and macrophages in rats [4]. This suggests an interplay between different blood cells in regulating the peripheral endocannabinoid system, hence the cardiovascular activities of these newly discovered lipid mediators [1–4]. In keeping with this concept, we showed that peripheral human cells such as lymphoma (U937) cells [7], lymphocytes [8], endothelial (HUVEC) cells [9] and mastocytes (HMC-1) [10] take up AEA and degrade it through FAAH. The apparent kinetic constants of FAAH in human blood cells (Table 1) suggest that a similar enzyme is expressed to different extents, showing similar $K_m$ but different $V_{max}$ values (Table 1). Finally, it should be stressed that platelet activation is paralleled by a decrease in nitric oxide, which stimulates AEA uptake by human cells [7–10]. Therefore, platelets can affect endocannabinoid degradation by the neighboring blood cells, contributing to the control of these compounds.

## References

[1] Wagner, J.A., Varga, K., Jàrai, Z. and Kunos, G. (1999) Hypertension 33, 429–434.
[2] Járai, Z., Wagner, J., Varga, K., Lake, K.D., Compton, D.R., Martin, B.R., Zimmer, A.M., Bonner, T.I., Buckley, N.E., Mezey, E., Razdan, R.K., Zimmer, A. and Kunos, G. (1999) Proc. Natl. Acad. Sci. USA 96, 14136–14141.
[3] Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D.R., Fleming, I. and Busse, R. (1999) Nature 401, 493–497.
[4] Varga, K., Wagner, J.A., Bridgen, D.T. and Kunos, G. (1998) FASEB J. 12, 1035–1044.
[5] Maccarrone, M., Bari, M., Menichelli, A., Del Principe, D. and Finazzi-Agrò, A. (1999) FEBS Lett. 447, 277–282.
[6] Braud, S., Bon, C., Touqui, L. and Mounier, C. (2000) FEBS Lett. 471, 12–16.
[7] Maccarrone, M., van der Stelt, M., Rossi, A., Veldink, G.A., Vliegenthart, J.F.G. and Finazzi-Agrò, A. (1998) J. Biol. Chem. 273, 32332–32339.
[8] Maccarrone, M., Valensise, H., Bari, M., Lazzarin, N., Romanini, C. and Finazzi-Agrò, A. (2000) Lancet 355, 1326–1329.
[9] Maccarrone, M., Bari, M., Lorenzon, T., Bisogno, T., Di Marzo, V. and Finazzi-Agrò, A. (2000) J. Biol. Chem. 275, 13484–13492.
[10] Maccarrone, M., Fiorucci, L., Erba, F., Bari, M., Finazzi-Agrò, A. and Ascoli, F. (2000) FEBS Lett. 468, 176–180.

Table 1
Kinetic parameters of FAAH activity in human peripheral cells

| Human cells | $K_m$ (μM) | $V_{max}$ (pmol min$^{-1}$ mg protein$^{-1}$) | Reference |
|---|---|---|---|
| Platelets | 10 ± 1.0 | 270 ± 30 | [5] |
| Lymphoma cells (U937) | 6.5 ± 0.6 | 520 ± 50 | [7] |
| Lymphocytes | 8.0 ± 1.0 | 187 ± 20 | [8] |
| Endothelial cells (HUVEC) | 7.0 ± 0.7 | 25 ± 3 | [9] |
| Mast cells (HMC-1) | 5.0 ± 0.5 | 160 ± 15 | [10] |

*Corresponding author. Fax: (39)-6-72596468.
E-mail: finazzi@uniroma2.it


PII: S0014-5793(00)02080-9