Platelet preparation

Freshly drawn whole blood from a normal healthy donor was collected in acid citrate dextrose in plastic vials and immediately fractionated by centrifugation at 25 °C to separate platelet-rich plasma (PRP) (Etablissement Français du Sang, France). The PRP fraction was stored at 22 °C under continuous and slow agitation until the beginning of the experiments. The isolated platelets were counted in 1% acetic acid solution using microscopy and contained no detectable erythrocytes as well as a very low amount of leukocytes. The platelets were then dispersed in Tyrode solution (pH = 7.5, NaCl 137 mM, KCl 2.7 mM, $NaHCO_3$ 1.2 mM NaH_2PO_4 0.36 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 2.0 mM, glucose 5.5 mM) containing 0.35% bovine serum albumin, fraction V.

Preparation of FITC-fibrinogen (Fg)

FITC-Fg was synthesized as previously described [20]. Briefly, thawed human Fg (20 mg/mL, Sigma-Aldrich, France) was incubated with celite-fluorescein isothiocyanate (FITC) (1 mg/mL) at room temperature for 15 minutes with intermittent vortexing. The FITC-Fg was separated from unreacted free FITC by passing through a Sephadex G25 column in phosphate-buffered saline.

Model of arachidonic acid-induced thrombosis

As previously described [17], the right common carotid artery of isoflurane-anesthetized animals was exposed. 4 to 6 µl of AA (Sigma Aldrich, St-Louis) at 200 mg/ml (or ethanol for sham surgery) was applied for 1 minute to the carotid isolated from surrounding tissues by a piece of parafilm. The diameter of the carotid upstream was slightly reduced by a loose permanent ligation. Blood flow was never interrupted.