that NO and guanylyl cyclase were likely endothelial mediator and effector, respectively, responsible for the endothelium-dependent actions of rutaecarpine.[28]

For experiments designed to study the possible roles of Ca^{2+} in the actions of rutaecarpine, both removal of extracellular Ca^{2+} and treatment with the intracellular Ca^{+2} antagonist, 8-(N,N-diethylamino) octyl-3,4,5,-trimethoxybenzoate (TMB-8) (0.1 mM), suggested that influx of extracellular Ca^{2+} was the major factor contributing to the action of rutaecarpine.[28] The vasorelaxant effect of rutaecarpine appeared to be largely dependent on extracellular Ca^{+2}, as rutaecarpine failed to induce any relaxation in Ca^{2+}-free, EGTA-containing medium, indicating the possible involvement of transmembrane Ca^{2+} influx. Moreover, pertussis toxin (100 ng/mL) suppressed the relaxation potency of histamine but had no effects on the action of rutaecarpine.[28] Sodium fluoride (NaF; 1, 2, or 3 mM), a G protein activator,[29] attenuated the action of acetylcholine (ACh), but only minimally affected rutaecarpine.[28] 1-[6-{[17β-3-Methoxyestra-1,2,3(10)-trien-17-yl]amino}hexyl]-1H-pyrrole-2,5-dione (U73122) (1-10 μM), a phospholipase C inhibitor,[30] suppressed the actions of ACh but had little effect on rutaecarpine.[28]

Therefore, rutaecarpine induced an endothelium/nitric oxide-dependent vasodilatation in rat aorta precontracted by phenylephrine. These responses could be inhibited by the removal of extracelluar Ca^{2+} in the medium. This vasodilatation induced by rutaecarpine depended primarily on the influx of Ca^{2+} and not on the mobilization of intracellular Ca^{2+} ($[Ca^{+2}]i$). Because pertussis toxin, sodium fluoride, and U73122 did not affect rutaecarpine-induced endothelium-dependent vasodilatation, it is speculated that G_i proteins or G protein-phospholipase C coupling pathways are probably not involved in the action of rutaecarpine on vascular endothelial cells.[28]

Effect on Platelet Aggregation

In human platelet-rich plasma, rutaecarpine (40-200 μM) inhibited aggregation stimulated by a variety of agonists (i.e., collagen, ADP, epinephrine, and arachidonic acid) as shown in Fig. 2.[31] At 120 μM, rutaecarpine almost completely inhibited platelet aggregation induced by arachidonic acid (Fig. 2). Furthermore, rutaecarpine also dose-dependently inhibited collagen (10 μg/mL)- and ADP (20 μM)-induced platelet aggregation.[31] However, even at 200 μM, it did not completely inhibit platelet aggregation induced by collagen, ADP, or epinephrine (Fig. 2). The IC_{50} values for platelet aggregation induced by collagen, epinephrine, ADP, and arachidonic acid were estimated to be about (μM) 166.2, 64.8, 159.6, and 76.5, respectively.[31] The antiplatelet activity of rutaecarpine (120 μM) was not significantly attenuated by pretreatment with the nitric oxide synthase inhibitors, N^G-mono-methyl-L-arginine (L-NMMA) (100 μM) or N^G-nitro-*L*-arginine methylester (L-NAME) (200 μM), or with the guanylyl cyclase inhibitor, methylene blue (100 μM). In addition, rutaecarpine (40-200 μM) did not significantly affect cyclic AMP or cyclic GMP levels in washed human platelets, whereas it (40-200 μM) significantly inhibited thromboxane B_2 (TxB_2) formation stimulated by collagen (10 μg/mL) and thrombin (0.1 U/mL).[31] In a further characterization of whether or not the inhibition of TxB_2 formation was due to the inhibition of throm-

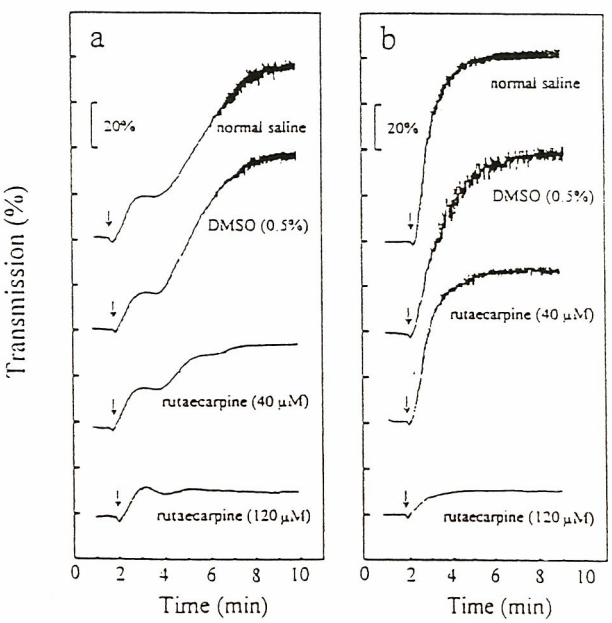

Fig. 2. Typical antiplatelet effect of rutaecarpine on adrenaline (10 μM)- and arachidonic acid (100 μM)-induced aggregation of human platelet-rich plasma. Human platelet-rich plasma was preincubated with normal saline (control), DMSO (0.5%), and rutaecarpine (40 and 120 μM) at 37 °C for 1 min. (a) Adrenaline (10 μM; ↓) or (b) arachidonic acid (100 μM; ↓) was then added to induce platelet aggregation. For the detailed experimental procedure, see Ref. 31.