Table 2. Effect of Rutaecarpine on Thromboxane Synthetase Activity

Treatment	Thromboxane B_2 (ng/mL)
PBS	1527.1 ± 14.0 (4)
DMSO (0.5%)	1535.1 ± 28.2 (4)
Imidazole (1 mM)	1329.4 ± 45.1* (4)
Rutaecarpine	
100 μM	1606.9 ± 39.1 (4)
200 μM	1632.8 ± 60.7 (4)

Volumes at 0.1 mL of aspirin-treated platelet microsomes were aliquoted into tubes, followed by the addition of DMSO (0.5%), imidazole (1 mM), or rutaecarpine (100 or 200 μM) at 25 °C for 3 min. Then, 2 μL of PGH_2 solution was added, vortexed, and incubated for 3 min at 25 °C. Finally, 10 μL of a $FeCl_2$ solution was added followed by centrifugation (3000 g, at 4 °C for 10 min). The supernatant thromboxane B_2 level was assayed by using an EIA kit. Data are presented as means ± S.E.M. (n = 4) *: $P < 0.001$ as compared with the PBS group. See Ref. 10.

boxane synthetase or phospholipase A_2 (PLA_2), Sheu et al.[10] found that rutaecapine (100 and 200 μM) did not significantly affect thromboxane synthetase activity in aspirin-treated platelet microsomes, indicating that inhibition of TxB_2 formation by rutaecarpine, at least in part, is not due to the inhibition of thromboxane synthetase in platelets (Table 2). Furthermore, rutaecarpine (100 and 200 μM) did not significantly affect PLA_2 activity in [^{3}H] arachidonic acid-labeled resting platelets.[10] These results indicate that rutaecarpine inhibition of TxA_2 formation in activated-platelets may be through other intracellular secondary pathways rather than by directly affecting PLA_2 activity on platelet membranes.

On the other hand, rutaecarpine (50-100 μM) dose-dependently inhibited both the increase in the $[Ca^{2+}]i$ level of Fura 2-loaded platelets (Fig. 3) and phosphoinositide breakdown stimulated by collagen (10 μg/mL) in [^{3}H] myoinositol-loaded platelets at different incubation times.[10] Collagen (10 μg/mL) induced a time-related increase in inositol monophosphate (IP) formation, which caused about a 1.3-fold rise in IP formation to occur during the initial 1 min, reaching a maximal IP formation approximately 2 min after collagen addition. In the presence of rutaecarpine (50, 100, and 200 μM), IP formation in collagen-stimulated platelets was markedly and dose-dependently decreased at different incubation times, respectively.[10] The IC_{50} value of rutaecarpine was estimated to be about 142 μM in this reaction. This IC_{50} value of rutaecarpine for inhibiting collagen-induced inositol phosphate formation is close to the IC_{50} value (166 μM) for inhibiting collagen-induced platelet aggregation.[31] Thus the antiplatelet activity of rutaecarpine may possibly be due to the inhibition of phospholipase C activity, leading to reduced phosphoinositide breakdown, followed by the inhibition of TxA_2 formation, and then inhibition of $[Ca^{2+}]i$ mobilization of platelet aggregation stimulated by agonists.

Effect on Uterotonia

Rutaecarpine was evaluated for *in vitro* uterotonic activity using isolated rat uteri. Proestrus (determined by vaginal smear) rats were pretreated with 100 μg of estradiol (in peanut oil by intramuscular injection) 24 h prior to the experiment.[32] The middle third of an isolated uterine horn was used for the study.[32] In the *in vitro* situation, it was found that the effect of rutaecarpine on isolated rat uterus contraction was not blocked by atropine at a concentration of 30 nM, but was blocked by

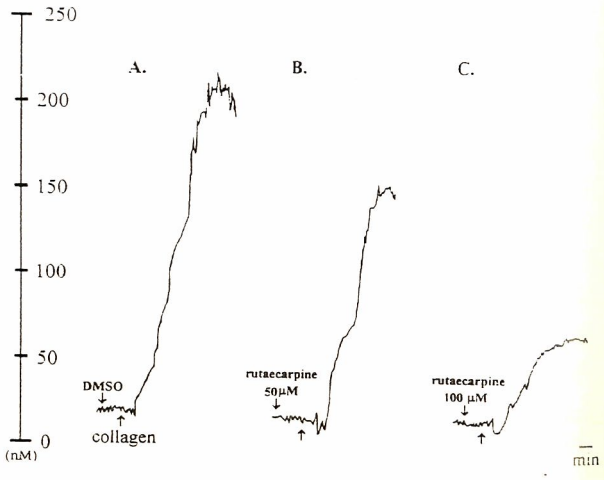

Fig. 3. Effect of rutaecarpine on collagen-induced intracellular Ca^{2+} mobilization of Fura 2-AM-loaded human platelets. Platelet suspensions were incubated with Fura 2-AM (5 μM) at 37 °C for 30 min, followed by the addition of collagen (10 μg/mL) in the presence of (A) DMSO (0.5%), control; (B) rutaecarpine (50 μM) or (C) (100 μM) which was added 2 min prior to the addition of collagen (10 μg/mL). For the detailed experimental procedure, see Ref. 10.