25) Two Diverse Platelet Reactions of Release and Aggregation in SHRSP and WKY: Different Responses to Cytosolic Free Calcium Concentrations, ATP Releasing Potencies, and Aggregation Activities. Hideaki Higashino, Yukinori Yamanishi, and Aritomo Suzuki. Department of Pharmacology, Kinki University School of Medicine, Osaka-Sayama 589.

It has been reported that two diverse platelet responses of release and aggregation is dependent on the cytosolic free calcium level, and SHRSP platelet is less potent in these physiological responses compared with WKY platelet. Using the platelets of these two strains, we have investigated the differences of strain-related platelet reactions, especially in response to cytosolic free calcium concentrations, ATP releasing potencies, and aggregation activities, which might be of some relevance to hypertensive state of SHRSP.

METHODS

Male SHRSP and WKY at ages of 9 months, each consisting of 20 rats, were used in this experiment. Platelets separated from PRP by mild centrifugation were resuspended in the Ca^{2+} free Hepes buffer solution at pH 7.4. In order to measure cytosolic free Ca^{2+} , platelets loaded with fura-2AM were suspended in the fluid divided into two portions; Ca^{2+} free Hepes buffer ((-)Ca sample) and buffer containing 1 mM CaCl_2 (+)Ca sample). Cytosolic free Ca^{2+} , $[\text{Ca}^{2+}]_i$, was measured by means of two wavelength excitation method (340/380 nm). ATP released in the solution was measured by means of photoluminescence method using luciferin-luciferase, and aggregation activity was observed by the method of Born. These three parameters were taken by a free Ca^{2+} analyzer (CAF-100, JASCO) with 400 μl volume of samples before and after addition of the reagents such as thrombin (0.3 U/ml), collagen (10 $\mu\text{g}/\text{ml}$), ADP (10 μM), ionomycin (1 μM) and TPA (10 nM). On measurement of $[\text{Ca}^{2+}]_i$ in (-)Ca samples, 0.1 mM MnCl_2 was added in the sample tubes 30 seconds before addition of reagents, which would prevent Ca^{2+} influx across the membrane.

RESULTS

In the absence of extracellular Ca^{2+} , no different $[\text{Ca}^{2+}]_i$ on platelet reaction was found in two strains, however, in the presence of extracellular Ca^{2+} receptor-mediated Ca^{2+} influx induced by thrombin and collagen was decreased and TPA induced Ca^{2+} influx was more enhanced in SHRSP platelets compared with WKY platelets. ATP releasing potencies from platelets of SHRSP were reduced by thrombin and collagen regardless of extracellular Ca^{2+} , but without Ca^{2+} , ionomycin potentiated SHRSP platelet reaction rather than WKY platelets. The ATP release inducing reagents were not affected by extracellular Ca^{2+} . TPA induced ATP release was never observed. No difference was found in the total ATP content in the platelets of SHRSP and WKY. Collagen and ADP induced aggregation which were closely connected with fibrinogen receptor showed lower activities in SHRSP than WKY, and thrombin and ionomycin induced ones were not different between these strains regardless of extracellular Ca^{2+} . In the presence of extracellular Ca^{2+} , TPA induced aggregation, in which C-kinase system may be involved, was larger in platelets of SHRSP than those of WKY.

CONCLUSIONS

1. In platelets, ATP releasing activity is largely dependent on the Ca^{2+} release from Ca^{2+} store rather than Ca^{2+} influx through plasma membrane. 2. Owing to seemingly higher C-kinase activity in SHRSP platelets, ATP release may be less potentiated. On the other hand, thrombin and TPA induced aggregation would be more stimulated compared with WKY platelets. 3. SHRSP platelets might have some impairment in fibrinogen induced aggregation pathway in which ADP is involved.