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Effects of sulfonylurea agents on platelet arachidonic acid metabolism; study on platelet homogenates

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Abstract—Effects of three sulfonylurea agents on arachidonic acid metabolism of platelet homogenates were evaluated using HPLC. Gliclazide had no significant inhibitory effects on arachidonic acid metabolism. Glibenclamide and glimepiride both inhibited the production of cyclooxygenase-related metabolites, thromboxane B_2 (TXB_2) and 12-hydroxy 5,8,10-heptadecatrienoic acid (HHT), whereas 12-hydroxy 5,8,10,14-eicosatetraenoic acid (12-HETE), a 12-lipoxygenase-related product, was unaffected. These findings confirmed part of our previous report using intact platelets, except that we found in the present study that glibenclamide had no inhibitory effect on 12-lipoxygenase.

Key words: platelet; arachidonic acid metabolites; HPLC; sulfonylurea; cyclooxygenase; 12-lipoxygenase

We previously reported the effects of three sulfonylurea agents, gliclazide, glimepiride and glibenclamide on platelet function [1]. We measured platelet aggregation, $[Ca^{2+}]_i$ elevation, and arachidonic acid metabolism in platelets stimulated by thrombin, a Ca^{2+}-ionophore, and arachidonic acid. Based on these findings, we made a hypothesis on the action site of each sulfonylurea agent on arachidonic acid metabolism; glimepiride inhibits the cyclooxygenase pathway, and glibenclamide affects both the cyclooxygenase and 12-lipoxygenase pathways, while gliclazide has no effect on arachidonic acid metabolism. While an intact cell system provides a useful tool in assessing the site of action of a particular agent, ranging from the ligand–receptor interaction to $[Ca^{2+}]_i$ elevation and aggregation, it has a disadvantage of being affected by a number of factors. In particular, there is a positive feedback in the production of TXA_2* which is produced upon platelet stimulation by various agonists. TXA_2 activates phospholipase A_2, which leads to enhanced production of TXA_2. With this pathway in action, it is possible that even a minor change that affects a particular step(s) may result in a great change in the overall production of TXA_2. Thus, there is a risk of overestimating the effect of certain agents or of misjudging the site of action. In this study, we measured the production of arachidonic acid metabolites, TXB_2, HHT and 12-HETE, in homogenized platelet suspensions to clarify further the site of action for each sulfonylurea agent.

Materials and Methods

Agents. The sulfonylurea agents used in the present study include gliclazide (1-(4-methylbenzensulfonyl)-3-[3-azabicyclo(3,3,0)octyl]urea), glibenclamide (1-[4-[2-(chloro-2-methoxybenzamide)-ethyl]-phenyl-sulfonyl]-3-cyclohexyl-urea), and glimepiride (1-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-carboxamide)-ethyl]-phenyl-sulphonyl]3-(4-methylcyclohexyl)-urea). They were dissolved in dimethylsulfoxide at a concentration of 50 mM and stored at −60° until use.

Platelet homogenate preparation. Venous blood was obtained from healthy human donors who had not taken any drug for a minimum of 14 days preceding the experiments. Platelets were separated from blood and resuspended in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 7.2), and sonicated three times on ice for 5 sec.

Measurement of arachidonic acid metabolites by HPLC. Sonicated platelets in the volume of 360 μL were preincubated with various concentrations of gliclazide, glibenclamide or glimepiride at 37° for 5 min. [^{14}C]-arachidonic acid (10 μM) was added to the sonicate preparation, and the mixture was kept at 37° for 10 min with constant shaking. The lipids were extracted and then subjected to reversed-phase HPLC using TSK-Gel ODS-80Tm (Tosoh, Tokyo, Japan). HPLC analysis was performed as described for Fig. 1.

Results and Discussion

In this study, we evaluated the effects of sulfonylurea agents on arachidonic acid metabolism in cell homogenates by the analysis of radioactive arachidonic acid metabolites. Reversed-phase HPLC sensitively identified several arachidonic acid metabolites including HHT, 12-HETE and TXB_2 (Fig. 1). We used radioactive arachidonic acid in order to simultaneously measure TXB_2 along with other metabolites, since TXB_2 which lacks ultraviolet absorbance was undetectable in the system used in our previous report on the effects of sulfonylurea agents on arachidonic acid metabolism. Figure 2 summarizes the results of the present study. The changes in the production of TXB_2 correlated well with those of HHT, supporting our previous assumption that a change in HHT production represents the overall arachidonic acid metabolites that are catalysed by the cyclooxygenase pathway.

Gliclazide up to 100 μM had no significant inhibitory effects on arachidonic acid metabolism of exogenous arachidonic acid. Glimepiride as well as glibenclamide inhibited the production of TXB_2 and HHT in a dose-dependent manner, but not that of 12-HETE even at maximum concentrations. These findings are in accord with our previous findings using intact platelets [1] except that we previously assumed that glibenclamide had an inhibitory effect on the production of 12-HETE.

In the previous report using intact platelets, we used three agonists with different modes of platelet activation to assess the sites of action of sulfonylurea agents. When glibenclamide inhibited both the production of HHT and that of 12-HETE, albeit to a lower extent, we assumed that glibenclamide inhibited 12-lipoxygenase as well as cyclooxygenase. However, the present findings that glibenclamide has no inhibitory effect on 12-lipoxygenase suggests that we underestimated the effect of the TXA_2 positive feedback pathway; in intact cells, TXA_2 produced from exogenous arachidonic acid must have activated phospholipase A_2, mobilizing endogenous arachidonic acid

* Abbreviations: HHT, 12-hydroxy 5,8,10-heptadecatrienoic acid; 12-HETE, 12-hydroxy 5,8,10,14-eicosatetraenoic acid; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

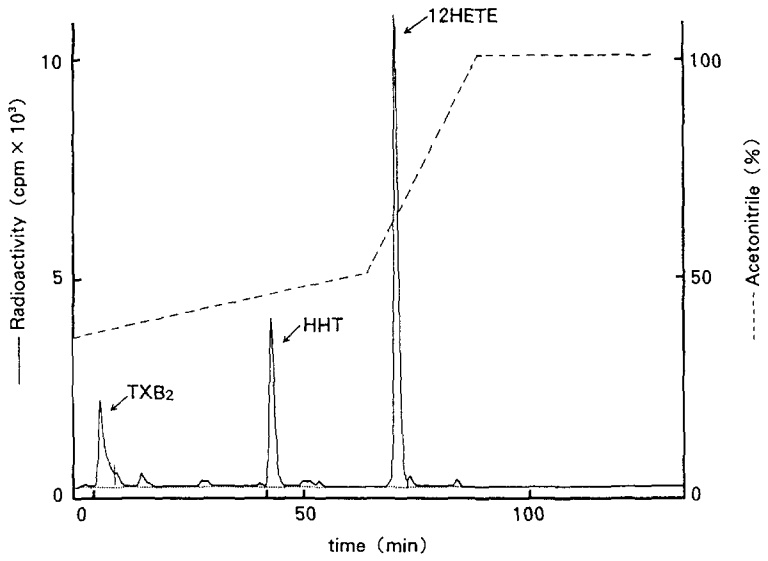

Fig. 1. Typical HPLC chromatogram showing resolution of [^{3}H]arachidonic acid metabolites. A platelet homogenate suspension was first incubated with various concentrations of sulfonylurea agents for 5 min. Arachidonic acid at a concentration of 10 μM was then added to the platelet homogenate, and the mixture was incubated for another 10 min. Lipids were extracted, and analysed with HPLC for production of TXB_2, HHT and 12-HETE. The mobile phase consisted of 0.017 M H_3PO_4:CH_3CN (65:35, v/v, pH 3.5) and 100% CH_3CN at a flow rate of 1 mL/min. The elution time was 6 min for TXB_2, 44 min for HHT, and 71 min for 12-HETE.

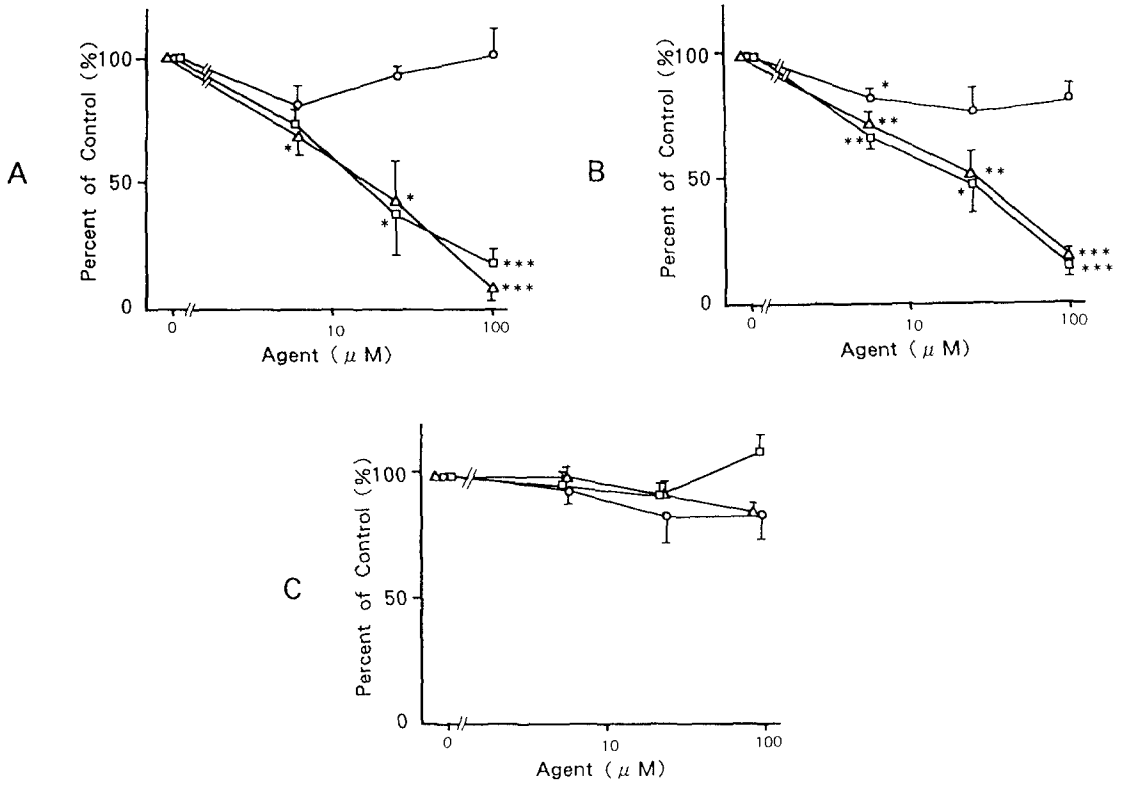

Fig. 2. Effects of three sulfonylurea agents on arachidonic acid metabolites [(A) TXB_2; (B) HHT; (C) 12-HETE]. Each agent was tested at the concentrations of 6.25, 25 and 100 μM. The data (percentage to the control) are presented as the means ± SD of six independent experiments. (○) Gliclazide, (□) glimepiride, (△) glibenclamide. The P values for significant differences are expressed as *0.02, **0.01, ***0.001.

with resultant increase both in TXA_2 and 12-HETE production. With this pathway in action, a decrease in the TXA_2 production induced by an inhibitor of phospholipase A_2 should result both in the reduced production of the cyclooxygenase products and that of 12-HETE.

As for gliclazide and glimepiride, we essentially confirmed the previous findings. Gliclazide had no inhibitory effect on cyclooxygenase or on 12-lipoxygenase in cell homogenates. In accord with this, we previously found that it inhibited neither the production of HHT nor that of 12-HETE in platelets activated by thrombin, a Ca^{2+}-ionophore, or arachidonic acid. Gliclazide-induced suppression of platelet function so far reported [1] should thus be related to effects other than those on arachidonic acid metabolism. Glimepiride suppressed both the production of TXB_2 and HHT, suggesting that its site of action is at cyclooxygenase but not at TXA_2 synthetase; if it had inhibited TXA_2 synthetase, the production of HHT should have increased in an inverse proportion to the decreased production of TXB_2.

In conclusion, we have confirmed part of our previous findings on the effects of sulfonylurea agents on arachidonic acid metabolism and furthermore found that glibenclamide had no effect on 12-lipoxygenase. Two hypoglycemic agents, glibenclamide and glimepiride are both potent inhibitors of cyclooxygenase. This implies that when administered clinically they are beneficial both for controlling the blood sugar level and for suppressing the production of TXA_2, which is a potent endogenous activator of platelets.

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REFERENCE

1. Ozaki Y, Yatomi Y and Kume S, Effects of oral hypoglycaemic agents on platelet functions. *Biochem Pharmacol* **44**: 687–691, 1992.

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