— **Rapid Communication** —

Prazosin Stimulates the Release of Hepatic Triacylglycerole Lipase Caused from Primary-culture Rat Hepatocytes

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Prazosin is an alpha 1 adrenoceptor antagonist, and it is used as an antihypertensive agent. The effects of prazosin on the activity of hepatic triacylglycerole lipase (HTGL) are not fully understood. In this study, we demonstrated that prazosin stimulates the release of HTGL activity from primary cultures of rat hepatocytes in a time- and dose-dependent manner. U-73122, a phopholipase C (PLC) inhibitor, suppresses prazosin's stimulation of the release of HTGL activity. Moreover, prazosin stimulated the increase of PLC activity in the hepatocytes in a time- and dose-dependent manner. In addition, the prazosin-stimulated release of HTGL activity was reduced by Quin2/AM (an intracellular Ca^{2+}-chelator), W-7 (a Calmodulin inhibitor), and KN-93 [an inhibitor of Ca^{2+}/Calmodulin dependent protein kinase (CaMK)-II]. These results suggest that the prazosin-stimulated release of HTGL activity is partly due to the activation of CaMK-II that is associated with the elevation of PLC activity in the hepatocytes.

Key words —— hepatic lipase, prazosin, hepatocyte, phospholipase C

INTRODUCTION

Prazosin ([4-(4-amino-6,7-dimethoxy-quinazolin-2-yl)piperazin-1-yl]-(2-furyl)methanone), which is a well-known depressor, competes with alpha 1 adrenoceptor.[1,2] Previous reports show that administration of prazosin, *in vivo*, causes increases in lipoprotein lipase (LPL) activity and decreases in 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity.[3–6] Prazosin also affects clinical indicators of lipid metabolism; for example, it results in the rise of the cholesterol ratio [high density lipoprotein (HDL)/very low density lipoprotein (VLDL) + low density lipoprotein (LDL)].[7–9] However, the various effects of prazosin on lipid metabolism are not understood in detail.

Hepatic lipase (hepatic triacylglycerole lipase; HTGL, EC 3.1.1.3) plays an important role in lipid metabolism.[10–12] It is synthesized primarily by hepatocytes and released on the luminal endothelial cell surface where it hydrolyzes the triacylglycerole in high and intermediate density lipoproteins. HTGL deficiency causes hyperlipemia and arteriosclerosis.[12] In addition, administration of doxazosin, another type of alpha 1 adrenoceptor antagonist, was shown to increase HTGL activity in the liver,[13] but the mechanism of this process is currently not known.

In this study, we examined the effects of prazosin on the release of HTGL activity from primary cultures of rat hepatocytes.

MATERIALS AND METHODS

Materials —— Glycerol tri[1-^{14}C]oleate (2.0 GBq/mmol) was obtained from Amersham (Tokyo, Japan), Phosphatidyl[2-^{3}H]-inositol 4,5-bisphosphate (740 GBq/mmol) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, U.S.A.) Prazosin, doxazosin, U-73122, U-73343, W-7 and collagenase were purchased from Wako Pure Chemical Industries (Osaka, Japan). Terazosin and 5-methylurapidil were obtained from Sigma (St. Louis, MO, U.S.A.). Quin2/AM and KN-93

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were purchased from Calbiochem (La Jolla, CA, U.S.A.). Williams' medium E was from Gibco (Grand Island, NY, U.S.A.). All other chemicals used were of analytical grade.

Preparation and Incubation of Hepatocytes —— Male Wistar rats, weighing 200–250 g, were fed on a commercial laboratory chow *ad libitum* and fasted for 24 hr before the experiments. Hepatocytes were isolated by *in vitro* collagenase perfusion and low speed centrifugation with modifications.[14, 15] Contamination by Kupffer cells of the hepatocyte preparation was confirmed to be less than 2% by peroxidase staining.[16] Cell viability was determined by trypan blue exclusion and ranged from 85 to 95%. The hepatocytes were cultured for 24 hr in monolayers in a plastic dish (1×10^5 cell/cm^2) in Williams' medium E (containing 10% fetal calf serum, 10 nM insulin, 10 nM dexamethasone, and 5 kIU/ml aprotinin) in a 5% CO_2 atmosphere. After removal of the medium by aspiration, monolayers of hepatocytes in the dish were further incubated for 0–105 min in Williams' medium E containing 2% bovine serum albumin with various concentration (0–200 μM) of alpha 1 adrenoceptor antagonists (prazosin, terazosin, doxazosin, and 5-methylurapidil). The hepatocytes were harvested and centrifuged at $50 \times g$ for 5 min to remove cellular debris. The supernatant obtained served as the preparation for assaying the release of HTGL activity.

Determination of HTGL Activity —— HTGL activity was determined by a method using glycerol tri[1-^{14}C]-oleate (1.2 μM; 2.5 kBq/ml) as a substrate.[17, 18] The HTGL activity was expressed as pmol of free fatty acids (FFA) produced/min par 10^6 cells.

Determination of Phospholipase C (PLC) Activity —— PLC activity was determined by the method of Higashi *et al.*[19] Briefly, the hepatocytes incubated with prazosin 0–100 μM over 30-min period, were homogenized in 20 mM Hepes-K buffer (pH 7.0) containing 1 mM dithiothreitol, 0.25 M sucrose, 1 mM EDTA and 0.7% cholate-Na by Physcotron (a microhomogenizer; NS-310E model, Niti-on Co., Tokyo, Japan), and were centrifuged at $105000 \times g$ for 60 min at 4°C. The supernatant contained the cytosolic and membrane fractions. The supernatant was used as the PLC enzyme preparation to hydrolyze phosphatidyl[2-^{3}H]-inositol 4,5-bisphosphate as a substrate.[20] The free inositolphosphates released were extracted and quantified. The PLC activity was expressed as fmol/min par 10^6 cells.

Data Analysis —— In each experiment, results are the mean ± S.E. of three or four observations from separate experiments using different hepatocyte preparations.

RESULTS

Figure 1(a) shows the changes in the release of HTGL activity from the hepatocytes incubated for 60 min with different adrenoceptor antagonists: prazosin, terazosin, doxazosin, or 5-methylurapidil. Only prazosin stimulated the release of HTGL activity from hepatocytes into the medium. Next, the hepatocytes incubated (100 μM prazosin) over a 105-min period [Fig. 1(b)]. A time-dependent release of HTGL activity from the hepatocytes was observed. Moreover, the effect of prazosin on the release of HTGL activity increased in a dose-dependent manner up to 100 μM. When prazosin stimulated the release of HTGL from the hepatocytes into the medium, the enzyme activity in the hepatocytes decreased (data not shown).

Whether the stimulatory release of HTGL activity by prazosin is involved in PLC activity was examined. The hepatocytes were incubated with prazosin in the presence of U-73122, an inhibitor of PLC. The prazosin-stimulated release of HTGL activity was markedly suppressed by U-73122 (Fig. 2), but it was not suppressed by U-

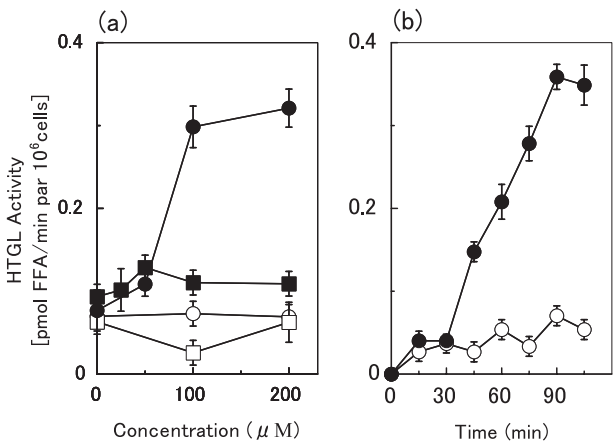

Fig. 1. Stimulatory Effects of Prazosin on the Release of HTGL Activity from Cultured Hepatocytes

(a) The hepatocytes were incubated for 60 min with various concentrations (0–200 μM) of alpha 1 adrenoceptor antagonists; prazosin (●), terazosin (○), doxazosin (■) and 5-methylurapidil (□). (b) The hepatocytes were incubated with (●) 100 μM prazosin or without (○) over a 105-min period. The activity of HTGL released into the medium was measured as described in Materials and Methods.

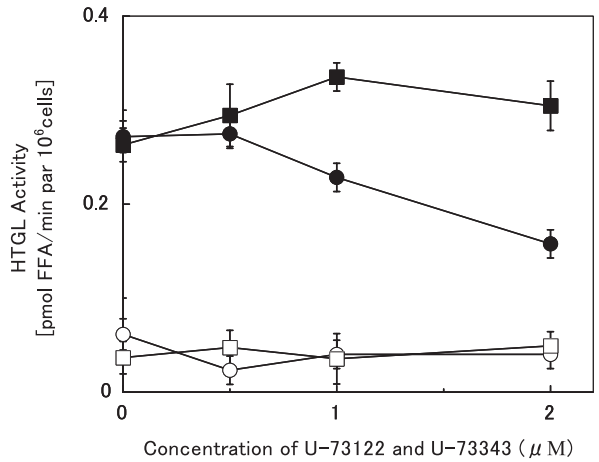

Fig. 2. Effects of U-73122 and U-73343 on the Prazosin-stimulated Release of HTGL Activity

The hepatocytes were incubated for 60 min with 100 μM prazosin (●, ■) or without (○, □) in the presence of U-73122 (●, ○) and U-73343 (■, □).

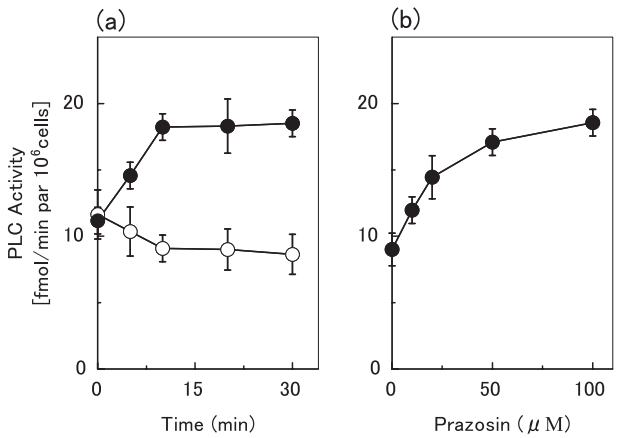

Fig. 3. Effects of Prazosin on PLC Activity in Hepatocytes

(a) The hepatocytes were incubated for 0–30 min with (●) or without (○) 100 μM prazosin addition. (b) The hepatocytes were incubated for 10 min with various concentrations (0–100 μM) of prazosin.

73343, which was an inactive analog of U-73122[21] and used as a negative control.

This increase in PLC activity in the hepatocytes by prazosin was examined. When the hepatocytes were incubated with prazosin at a concentration of 100 μM for up to 30 min, the PLC activity in the preparation from the incubated hepatocytes as described in Materials and Methods was increased in a time-dependent manner over 10 min [Fig. 3(a)]. Next, the hepatocytes incubated with different concentrations of prazosin (0–100 μM) for 10 min, PLC activity increased in a dose-dependent manner [Fig. 3(b)].

In addition, we investigated the effects of various modulators on the release of HTGL activity by prazosin (Fig. 4). The prazosin-stimulated release of HTGL activity was suppressed by Quin2/AM[22] (an intracellar Ca^{2+}-chelator), W-7[23] (a Ca^{2+}/calmodulin inhibitor), and KN-93[24] [an inhibitor of Ca^{2+}/calmodulin-dependent protein kinase (CaMK)-II] (Fig. 4).

DISCUSSION

Changes in *in vivo* lipid metabolism following administration of prazosin have been reported previously.[7–9] However, the contribution of HTGL to these changes in lipid metabolism was not shown. In this report, we demonstrate that prazosin stimulates the release of HTGL from rat hepatocytes [Fig. 1(a) and 1(b)]. In contrast, the stimulation of HTGL release was hardly evident with any other alpha 1 adrenoceptor blocker at the concentrations

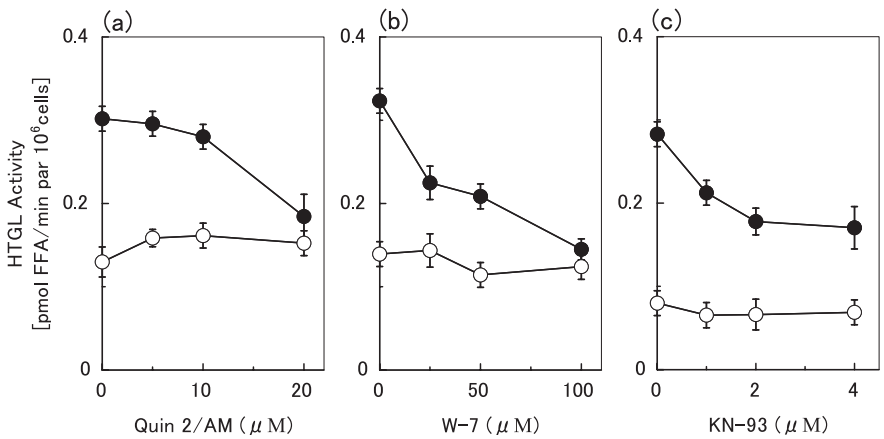

Fig. 4. Effects of Various Ca^{2+}-Modulators on the Prazosin-stimulated Release of HTGL Activity

(a) The hepatocytes were incubated with prazosin (100 μM, ● or without ○) in the presence of Quin2/AM (0–20 μM). (b) The hepatocytes were incubated with prazosin (100 μM, ● or without ○) in the presence of W-7 (0–100 μM). (c) The hepatocytes were incubated with prazosin (100 μM, ● or without ○) in the presence of KN-93 (0–4 μM).

examined [Fig. 1(a)]. Then, the stimulatory effect of prazosin was preserved in the presence of alpha 1 adrenoceptor agonists (data not shown). In this study, it is possible that the action of prazosin was caused by the uptake of prazosin into the hepatocytes, not by the interactions between the alpha 1 adrenoceptor and prazosin. Our data also suggested that the prazosin-stimulated release of HTGL activity is due to an activation of PLC activity in the hepatocytes (Figs. 2 and 3). It is well-known that an activation of PLC results in an increased intracellular Ca^{2+} concentration.[20] In our experiments, the stimulatory release of HTGL activity by prazosin was suppressed by an intracellular Ca^{2+}-chelator (Fig. 4). According to the report,[25] the maturation and the secretion of HTGL is inhibited by adrenaline though changes in Ca^{2+} homeostasis.[25] The intracellular Ca^{2+} contents may be important for the stimulatory release of HTGL activity by prazosin, an alpha 1 adrenoceptor antagonist. The prazosin-stimulated release of HTGL activity is also suppressed by inhibitors of Ca^{2+}/calmodulin inhibitor and CaMK-II (Fig. 4). Activation of CaMK-II induced by heparin was also reported to cause the stimulatory release of HTGL activity.[26] However, prazosin-stimulated release of HTGL activity was not suppressed by protein kinase C inhibitors (data not shown).

In conclusion, our results show that prazosin stimulated the release of HTGL activity from cultured rat hepatocytes, and the stimulatory action by prazosin may be due to the pathway involving an increase in the cytosolic Ca^{2+} concentration through an activation of PLC activity, following activation of CaMK-II activity.

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