Adenosine 3′,5′-Cyclic Monophosphate Involvement in Hepatic Triacylglyceride Lipase Release from Prazosin-Stimulated Primary Cultured Rat Hepatocytes

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We recently found that hepatic triglyceride lipase (HTGL) was released from primary cultured rat hepatocytes after treatment with prazosin, an antagonist of alpha-1 adrenoceptors. However, the details of prazosin-induced HTGL release remain uncertain. Here we investigated whether changes in cAMP levels in hepatocytes were related to HTGL release from prazosin-stimulated hepatocytes. When hepatocytes were treated with prazosin, cAMP levels during stimulated release of HTGL increased in a time- and dose-dependent manner. Stimulated release of HTGL was suppressed by the adenylate cyclase inhibitors MDL-12,330A and 2′,5′-dideoxyadenosine. Further, cAMP-dependent protein kinase A (PKA) activity in prazosin-stimulated hepatocytes also increased in a time- and dose-dependent manner. Moreover, prazosin-stimulated HTGL release was suppressed by the PKA inhibitors H-89 and KT5720. These results suggest that prazosin-stimulated HTGL release from hepatocytes was due to cAMP production and partly due to subsequent PKA activation in hepatocytes.

Key words prazosin; hepatic lipase; cAMP; protein kinase A; hepatocyte

Materials and Methods

Materials [32P]ATP (1,111 TBq/mmol) was from PerkinElmer, Inc. Japan (Yokohama, Japan). Glycerol tri[14C]oleate (2.0 GBq/mmol) and a cAMP enzyme immunoassay system (RPN. 2251) were from GE Healthcare Japan (Tokyo, Japan). PKA assay kits (# 17–134) were from Millipore (Billerica, MA, U.S.A.). Prazosin, 2′,5′-dideoxyadenosine, H-89, KT5720, collagenase, and Williams’ medium E were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). MDL-12,330A was from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were of analytical grade.

Hepatocyte Preparation and Culture Male Wistar rats (weight: 200–300 g) were fed a commercial laboratory chow ad libitum and fasted for 24 h before the experiments. Hepatocytes were isolated by in vitro collagenase perfusion and low speed centrifugation, with modifications.19) Kupffer cell contamination in hepatocyte preparations was confirmed to be <2% by peroxidase staining. Cell viability was determined by trypan blue exclusion and ranged from 85 to 95%. Hepatocytes were cultured as monolayers for 24 h in a plastic dish (1×106 cells/cm2) in Williams’ medium E, containing 10% fetal calf serum, 10 ng/mL insulin, 10 ng/mL dexamethasone, and 5 KIU/mL aprotinin, in a 5% CO2 atmosphere. After removing the medium by aspiration, hepatocyte monolayers were incubated for an additional 0–60 min in Williams’ medium E containing 2% bovine serum albumin with prazosin and other reagents. Hepatocytes were harvested and centrifuged at 50 × g for 5 min to remove cellular debris. The supernatant was then used for HTGL activity assay.

HTGL Activity Determination HTGL activity was determined using glycerol tri[14C]oleate (3.7 MBq/mL) as the substrate,20) and it was expressed as pmol of free fatty acids...
(FFA) produced/min/10⁶ cells.

**cAMP Determination** Incubated hepatocytes (1×10⁵ cells/cm²) were homogenized in ice-cold 5% trichloroacetic acid (TCA) and centrifuged at 10000×g for 20 min. Supernatants were extracted with H₂O-saturated diethyl ether and then subjected to quantitative analysis for cAMP using a commercial cAMP enzyme immunoassay system (GE Healthcare), as previously described.²¹

**PKA Activity Determination** Hepatocytes incubated with prazosin were homogenized in 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.2) that contained 25 mM β-glycerophosphate, 5 mM ethylene glycol tetraacetic acid (EGTA), 1 mM sodium orthovanadate, and 1 mM dithiothreitol using a Vibra-cell ultrasonic processor (model VC-130PB, Sonics, Newtown, CT, U.S.A.), and then centrifuged at 10000×g at 4°C for 20 min. The supernatant was used as an enzyme preparation to determine PKA activity using a PKA assay kit. Radioactivity was measured and expressed as nmol phosphate/min/10⁶ cells.

**Data Analysis** Results are expressed as mean±standard error of the mean for three or four determinations from independent experiments using different hepatocyte preparations.

**RESULTS**

In a previous study, we found that prazosin stimulation increased HTGL release from primary cultured rat hepatocytes.²¹ In this study, we investigated whether this prazosin-stimulated HTGL release was due to a mechanism involving an increase in intracellular cAMP levels. We found that prazosin-stimulated HTGL release was markedly suppressed by the adenylate cyclase (AC) inhibitors 2'/uni²⁵,5'/uni²⁵-dideoxyadenosine (Fig. 1a) and MDL-12,330A.²²,²³ (Fig. 1b). We also determined the time dependence of cAMP levels in hepatocytes incubated with 100 µM prazosin over a 45-min period. In hepatocytes, cAMP levels increased in a time-dependent manner with a maximum of a 1.5-fold higher level than the basal level (Fig. 2a). Moreover, cAMP levels increased in a dose-dependent manner on treating hepatocytes with up to 100 µM prazosin (Fig. 2b).

cAMP levels were measured in the presence of AC inhibitors to determine whether AC was involved in the prazosin-induced increase in cAMP levels. cAMP levels in prazosin-stimulated hepatocytes were found to be suppressed by the AC inhibitors MDL-12,330A and 2',5'-dideoxyadenosine (Fig. 3).

Later, we investigated the release of HTGL in the presence of cAMP-dependent PKA inhibitors, as cAMP levels are increased due to PKA activity. Figure 4 shows prazosin-stimulated HTGL release in the presence of PKA inhibitors. HTGL release was suppressed by the PKA inhibitors H-89 and KT5720.²⁴,²⁵

Thus, we investigated whether the release of HTGL involved PKA activity in hepatocytes with or without prazosin treatment. It was observed that intracellular PKA activity increased after treatment with 100 µM prazosin in a time-dependent manner for up to 60 min (Fig. 5a). In addition, PKA activation by prazosin occurred in a dose-dependent manner on treating hepatocytes with up to 100 µM prazosin (Fig. 5b).
Several clinical reports have shown that prazosin administration alters lipid metabolism in vivo, although the detailed mechanism underlying this phenomenon remains unknown. In a previous study, we investigated the release of HTGL from rat hepatocytes after stimulation with prazosin. It was considered that PLC activation played an important role in HTGL release. However, elucidating the involvement of other protein kinases is currently in progress. PKA regulation of G-protein-mediated PLC has been previously reported by Liu and Simon. These interactions may have been involved in HTGL release from primary cultured rat hepatocytes. However, elucidating the involvement of other protein kinases is currently in progress. PKA regulation, although the underlying mechanism remains to be determined.

In another report, a cAMP analog (dibutyryl cAMP, 1 mM) was reported to suppress HTGL release from rat hepatocytes. We did not consider that a major change in HTGL release would occur with 1 mM dibutyryl cAMP. However, HTGL release was suppressed with 500 µM dibutyryl cAMP along with prazosin stimulation (data not shown). Thus, only moderate levels of cAMP seem to be necessary for HTGL release.

In contrast, intracellular cAMP levels may increase in hepatocytes on inhibition of phosphodiesterase activity. In fact, we observed that 3-isobutyl-1-methylxanthine, a PDE inhibitor, maintained HTGL release (data not shown). In addition, prazosin can inhibit PDE, and inhibiting PDE. However, an increase in cAMP levels is necessary to some extent, although not in excess amounts.

PKA is also involved in exocytosis. In this study, HTGL release from rat hepatocytes due to prazosin stimulation suggests that the release should be suppressed by PKA inhibitors, as PKA activity is closely related to secretion by hepatocytes.

In conclusion, our results suggest that prazosin-stimulated HTGL release from primary cultured rat hepatocytes is promoted by activating PKA to increase intracellular cAMP levels due to the effects of activating AC and inhibiting PDE. In addition, these effects of prazosin were probably due to prazosin uptake by hepatocytes and not due to alterations of alpha-1 adrenoceptors by prazosin. The mechanism underlying the effects of prazosin remains unclear after its uptake by hepatocytes, as almost all of it is metabolized by the cells. Thus, mechanisms other than alpha-1 adrenoceptor activation need to be investigated.

REFERENCES

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