Short Communication

Mechanisms Involved in the Contraction of Intrahepatic Portal Vein Branches by Clomipramine and Oxethazaine in Isolated Perfused Rat Livers

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Abstract. Clomipramine (CLM) and oxethazaine (OXZ) were previously reported to increase portal pressure by contracting portal vein branches (PVBs) in isolated perfused rat liver. In the present study, to characterize the contractile mechanisms, the effects of Y27632, HA1077, staurosporine, papaverine, SKF96365, and sulindac sulfide on the portal pressure increase induced by CLM and OXZ were examined comparatively with those induced by endothelin-1. The results suggest that 1) intrahepatic PVBs employ a Rho-kinase-dependent pathway for sustained contraction, 2) CLM contracts PVBs by activating a Rho-kinase pathway and Ca²⁺-channels, and 3) OXZ acts primarily by promoting Ca²⁺ entry through its ionophore-like action.

Keywords: intrahepatic portal vein branch, Rho kinase, clomipramine

In previous studies (1, 2), we showed that the tricyclic antidepressant clomipramine (CLM) and the topical anesthetic oxethazaine (OXZ) increased portal pressure (PP) in isolated perfused rat liver (IPRL) independent of their pharmacological actions, and this PP increase was accompanied by marked intrahepatic flow redistribution as characterized by vital staining with fluorescent dyes, that is, peripheral mal-perfusion and short-circuited flow in the central portion of the liver. This flow redistribution was related to contraction of the intrahepatic portal vein branches (PVBs), but not to contraction of the hepatic vein. Endothelin-1 (ET-1) exhibited a similar intrahepatic flow disturbance in the comparative study (1, 2).

Such intrahepatic flow redistribution, if induced in vivo by drugs, could lead to adverse effects due to a decrease in hepatic extraction and metabolism of drugs as well as hepatotoxicity due to hypoxia. Although drug toxicity based on such a mechanism has not been realized, it may be latently involved. Therefore, it is important to examine the mechanism of contraction of PVBs by drugs.

IPRL is an indispensable tool to characterize contrac-

tile mechanisms of PVBs since the extrahepatic portal vein preparation, which is generally used in pharmacological studies, is much less sensitive to these drugs than IPRL (1, 2). At present, the exact localization of the contraction of PVBs by these drugs remains to be determined, although ET-1 is reported to cause localized constriction in the distal segments of preterminal portal venules (3).

In the present study, to gain some insight into contractile mechanisms of intrahepatic PVBs by these drugs, we examined the effects of several smooth muscle relaxants and related compounds on the PP increase induced by CLM and OXZ in comparison to the effects on ET-1-induced PP increase.

The method for preparation of IPRL and the perfusion system is the same as described in a previous paper (1). Male Sprague-Dawley rats (Japan SLC, Shizuoka) weighing 220–240 g were used. The left and median lobes of the liver were excised and perfused with Krebs-Henseleit bicarbonate buffer (containing 1.3 mM CaCl₂) saturated with 95% O₂–5% CO₂, in a non-recirculating constant flow (20 ml/min) system, and PP and oxygen uptake were monitored. Drugs or inhibitors (Sigma, St. Louis, MO, USA) were dissolved in water or dimethyl sulfoxide at 5 or 10 mM and infused into the perfusion line. ET-1 (Peptide Institute, Minoh) was

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dissolved as indicated by the supplier. Appropriate concentrations of the inhibitors were determined with CLM using 4–5 livers, and the experiments with OXZ and ET-1 were conducted usually at one inhibitor concentration using 2–3 livers. The animal experiments were performed in accordance with the guidelines issued by The Japanese Pharmacological Society.

As shown in Fig. 1 (top panel), CLM and OXZ produced a gradual and steady increase in PP in the IPRM similar to the effects of ET-1 which was most potent, but unlike the effects of norepinephrine and high KCl-depolarization which provoked a rapid increase in PP (data not shown).

Firstly, a RhoA/Rho-kinase signal transduction system is considered to play an important role in the tonic contraction of vascular smooth muscle cells, especially through the use of the specific Rho-kinase inhibitors, Y27632 and HA1077 (4, 5). As shown in Fig. 1 (2nd panel), Y27632 (10 μ M) almost completely inhibited the PP increase induced by CLM. The inhibition was gradually reduced after removal of the inhibitor, and at a second challenge, it again decreased the PP. It also inhibited OXZ- and ET-1-induced PP increases to a lesser extent. Similarly, HA1077 suppressed the PP increase by CLM, OXZ, and ET-1, although less potent than Y27632 (Fig. 1, 3rd panel).

Since a high concentration of indomethacin (100 μ M) inhibited the PP increase by CLM in a previous study (1), we tested another non-steroidal anti-inflammatory drug, sulindac, with its active metabolites sulindac sulfide and sulindac sulfone. Among them, sulindac sulfide (3 μ M) was found to markedly suppress the CLM-induced PP increase (4th panel), but sulindac itself and sulindac sulfone (10 μ M) had no inhibitory effect (data not shown). Sulindac sulfide also inhibited the ET-1-induced PP increase to a lesser extent, but in contrast, enhanced the OXZ-induced increase.

Staurosporine (0.1 μ M), a non-selective protein kinase C (PKC) inhibitor, was the most potent and markedly inhibited the PP increase induced by CLM, OXZ, and ET-1 (5th panel). Papaverine, a phosphodiesterase inhibitor, was also effective at 10–50 μ M (6th panel).

SKF96365, a non-selective inhibitor of Ca^{2+} entry, slightly inhibited CLM- and ET-1-induced PP increases, but rather enhanced the OXZ-induced PP increase (7th panel). This inhibitor was tested at 3 μ M, since its higher concentration (10 μ M) gradually increased PP by itself.

Contraction of vascular smooth muscle consists of two components: a rapid phasic component and a sustained tonic component. The former component is activated by the release of Ca^{2+} from intracellular stores, which activates a calmodulin (CaM)-myosin light chain

kinase (MLCK) pathway. In the latter, in addition to the partial activation of MLCK by an influx of extracellular Ca^{2+} , a signal transduction system leading to the inhibition of myosin light chain phosphatase (MLCP) is involved, i.e., receptor-coupled activation of the $\text{G}_{12/13}$ family of heteromeric G proteins, activation of Rho guanine nucleotide exchange protein, GTP binding to RhoA, activation of Rho-kinase by GTP-RhoA, and inhibition of MLCP (4, 5). ET-1 binds to ET_A receptors to increase $[\text{Ca}^{2+}]_i$ through activation of G_q protein and, in addition, simultaneously sensitizes the calcium effect through activation of the Rho-kinase pathway (4, 6).

The selective Rho-kinase inhibitor Y27632 inhibits contraction of aortic smooth muscle by phenylephrine at concentrations of 1–10 μ M, but not by a KCl-induced contraction (7). It also inhibits contraction by a variety of agonists, including ET-1, angiotensin, and the thromboxane (TXA_2) analogue U46619 (4, 5, 7). In the present study, Y27632 and HA1077 inhibited not only the ET-1-induced PP increase but also the PP increases by CLM and OXZ. This indicates that the contraction of PVBs involves a Rho-kinase pathway, which is activated by CLM and OXZ. However, the mechanism of the activation remains unclear.

Sulindac sulfide is reported to inhibit proliferation of human colon cancer cells by impairing the nucleotide exchange on p21Ras by CDC25 and accelerating the p21Ras GTPase reaction by p120GAP as well (8) and by inhibiting store-operated calcium entry (at 60 μ M) (9). Zhou et al. (10) recently reported that a subset of non-steroidal anti-inflammatory drugs, including sulindac sulfide and indomethacin, reduce amyloidogenic $\text{A}\beta_{42}$ secretion by inhibiting Rho activity. These reports are in favor of the present finding that sulindac sulfide inhibited the CLM- and ET-1-induced PP increase, especially the former, at lower concentrations (3 μ M) than those reported to inhibit cell growth. However, OXZ-induced PP increase was enhanced by sulindac sulfide. Thus, further experiments are necessary to clarify the mechanism of action of sulindac disulfide in PVBs.

Activation of PKC, like Rho-kinase, leads to phosphorylation of a phosphatase-1 inhibitor protein (CPI-17) and inactivation of MLCP (4). However, PKC has isoforms and their roles in the Rho-kinase mediated Ca^{2+} sensitization is not universal. The non-selective PKC inhibitor staurosporine is also a potent inhibitor of Rho-kinase with an IC_{50} value 215-fold more potent than that of Y27632 (11). Thus, the strong inhibition of the CLM-, OXZ-, and ET-1-induced PP increases by staurosporine may further support the involvement of Rho-kinase in their action, although involvement of PKCs cannot be ruled out.

Fig 1. Effects of some smooth muscle relaxants and related compounds on the portal pressure increase induced by clomipramine, oxethazaine, and endothelin-1 in isolated perfused rat livers. Clomipramine (10 μ M), oxethazaine (3 μ M), and endothelin-1 (1 nM) were infused as indicated by the white column shown on the top of each panel. Inhibitors were infused as indicated by dark columns with their micromolar concentrations shown. Typical traces are shown.

Smooth muscle relaxation induced by cyclic AMP and protein kinase A is proposed to involve phosphorylation of effector proteins, including MLCK and ion channels that regulate cytosolic Ca^{2+} concentration, and activation of protein kinase G by cyclic AMP (12). The regulation of the Rho-dependent pathway, for example, via phosphorylation of Rho, is also proposed (4). Thus, papaverine, a phosphodiesterase inhibitor, may affect the PP increase by CLM, OXZ, and ET-1 through its multiple cyclic AMP-dependent mechanisms.

Next, involvement of Ca^{2+} movement was examined using SKF96365, which is reported to non-specifically inhibit store-operated, receptor-operated, and voltage-operated calcium influx (13). SKF96365 is also reported to increase $[\text{Ca}^{2+}]_i$ at concentrations above $15\text{ }\mu\text{M}$ through activation of Ca^{2+} -permeable channels (14). This agrees with our observation that $10\text{ }\mu\text{M}$ SKF96365 increased PP by itself. Inhibition of CLM- and ET-1-induced PP increase by SKF96365 ($3\text{ }\mu\text{M}$) indicates that influx of extracellular calcium occurs in both cases. However, the same concentration of the inhibitor did not inhibit but rather enhanced the PP increase by OXZ. This could be due to a synergistic $[\text{Ca}^{2+}]_i$ -increasing action of SKF96365 and OXZ. Considering together that the PP increase by OXZ was also exaggerated by sulindac sulfide, OXZ may have a mechanism distinct from those of CLM and ET-1. As reported previously, OXZ has a Ca^{2+} -ionophore-like action at micromolar concentrations (15), and thus, the action of OXZ may be primarily related to a calcium influx.

In conclusion, intrahepatic PVBs may respond not only to vasoconstrictors but also to certain drugs independently of their expected pharmacological actions. PVBs have a Rho-kinase-dependent contractile mechanism. Inhibitors of the Rho-kinase system may be useful for improving intrahepatic flow disturbance. CLM may activate a Rho-kinase pathway and enhance calcium entry through nonspecific interaction with certain receptors or proteins upstream of the Rho-kinase, whereas OXZ may act primarily by promoting the entry of extracellular calcium. Sulindac sulfide inhibited CLM- and ET-1-induced, but not OXZ-induced, contraction of PVBs by still undefined mechanisms.

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