Both Ca\(^{2+}\)-dependent and -independent pathways are involved in rat hepatic stellate cell contraction and intrahepatic hyperresponsiveness to methoxamine

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Laleman W, Van Landeghem L, Severi T, Vander Elst I, Zeegers M, Bisschops R, Van Pelt J, Roskams T, Cassiman D, Fevery J, Nevens F. Both Ca\(^{2+}\)-dependent and -independent contraction pathways in rat hepatic stellate cells are involved in rat hepatic stellate cell contraction and intrahepatic hyperresponsiveness to methoxamine. Am J Physiol Gastrointest Liver Physiol 292: G556–G564, 2007. First published September 28, 2006; doi:10.1152/ajpgi.00196.2006.—In chronic liver injury, hepatic stellate cells (HSCs) have been implicated as regulators of sinusoidal vascular tone. We studied the relative role of Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent contraction pathways in rat HSCs and correlated these findings to in situ perfused cirrhotic rat livers. Contraction of primary rat HSCs was studied by a stress-relaxed collagen lattice model. Dose-response curves to the Ca\(^{2+}\)-ionophore A-23187 and to the calmodulin/myosin light chain kinase inhibitor W-7 were used to study Ca\(^{2+}\)-dependent pathways. Y-27632, staurosporin, and calyculin (inhibitors of Rho kinase, protein kinase C, and myosin light chain phosphatase, respectively) were used to investigate Ca\(^{2+}\)-independent pathways. The actomyosin interaction, the common end target, was inhibited by 2,3-butanedione monoxime. Additionally, the effects of W-7, Y-27632, and staurosporin on intrahepatic vascular resistance were evaluated by in situ perfusion of normal and thioacetamide-treated cirrhotic rat livers stimulated with methoxamine (n = 25 each). In vitro, HSC contraction was shown to be actomyosin based with a regulating role for both Ca\(^{2+}\)-dependent and -independent pathways. Although the former seem important, an important auxiliary role for the latter was illustrated through their involvement in the phenomenon of “Ca\(^{2+}\) sensitization.” In vivo, preincubation of cirrhotic livers with Y-27632 (10⁻⁴ M) and staurosporin (25 nM), more than with W-7 (10⁻⁴ M), significantly reduced the hyperresponsiveness to methoxamine (10⁻⁴ M) by −66.8 ± 1.3%, −52.4 ± 2.7%, and −28.7 ± 2.8%, respectively, whereas in normal livers this was significantly less: −43.1 ± 4.2%, −40.2 ± 4.2%, and −38.3 ± 6.3%, respectively. Taken together, these results suggest that HSC contraction is based on both Ca\(^{2+}\)-dependent and -independent pathways, which were shown to be upregulated in the perfused cirrhotic livers, with a predominance of Ca\(^{2+}\)-dependent pathways.

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CONTRACTION PATHWAYS OF HEPATIC STELLATE CELLS

Three-Dimensional Stress-Relaxed Collagen Lattice Contraction Model

The ability of HSCs to contract three-dimensional collagen matrices was assessed as previously described with some slight modifications (29). In brief, hydrated collagen gels were prepared using rat tail tendon collagen I (Becton Dickinson Labware, Becton Dickson, Bedford, MA) and adjusted to physiological strength and pH with 1 N NaOH and 10× PBS at 4°C. Afterward, the collagen solution was mixed with a HSC suspension so that the final solution resulted in a density gradient containing HSCs remaining attached to the culture dish for 24 h, leading to a resulting cell suspension that was fractionated by density gradient centrifugation using Optiprep (Nycomed). Cells were harvested at densities of <1.053 (9% Optiprep) according to Alpini et al. (1).

Viability and purity was systematically characterized by trypan blue exclusion and morphological characterization. Cells were seeded on uncoated plastic culture dishes and washed with William’s E medium supplemented with 10% FCS, 0.6 IU/ml insulin, 2 mM glutamine, and 1% antibiotic-antimycotic solution (Invitrogen, Merelbeke, Belgium). The medium was renewed every 48 –72 h. Characterization of rat liver-derived myofibroblast-like cultures, established by cultivating enriched HSC fractions on plastic, was performed by staining with anti-α-smooth muscle actin (α-SMA), anti-desmin, and anti-synaptophysin, as previously described (3, 4). Experiments were performed between the first and third passages (1:3 split ratio) using three cell cultures from independent isolations. Rat cardiac ventricular myofibroblasts (a gift from Dr. V. Petrov, Department of Molecular and Cardiovascular Research, University of Leuven, Leuven, Belgium) and rat aortic VSMCs (a gift from Prof. Dr. S. Janssen, Center for Transgene Technology and Gene Therapy, University of Leuven, Leuven, Belgium) were isolated and cultivated as previously described (9, 20).

![Diagram](https://via.placeholder.com/150)

**Fig. 1. Model for the regulation of agonist-promoted hepatic stellate cell (HSC) contraction.** Central in this process is the actomyosin interaction, which is determined by the interaction between α-smooth muscle actin (α-SMA) and phosphorylated (P) myosin light chain (MLC), which is the rate-limiting step in this interaction. Phosphorylation of MLC is regulated on the one hand by activated (act) MLC kinase (MLCK), which is activated by Ca²⁺-dependent pathways, involving increased intracellular Ca²⁺ and calmodulin. On the other hand, inactivation (inact) of MLC phosphatase (MLCP), regulated by Ca²⁺-independent PKC and Rho kinase, also leads to sustained phosphorylated MLC and therefore increased contraction. The site-specific inhibitors and agonists used in the present study are also shown in this model: A-2387, calyculin (Caly), W-7, staurosporin (Stauro), Y-27632, and 2,3-butanedione 2-monoxime (BDM). L-Ca²⁺ channel, L-type Ca²⁺ channel; PLC, phospholipase C; PIP2, inositol bisphosphate; IP3, inositol trisphosphate; DAG, diacylglycerol.

Inhibitors and Agonists Affecting Contractility

Depending on the experiment, FCS (10%), the α₁-adrenergic agonist methoxamine (10⁻⁴ M, Sigma), or the Ca²⁺ ionophore A-23187 (Sigma) were used as agonists. Mostly, FCS was used because its ease of use, high availability, and ability to elicit as powerful a contraction as endothelin-1 (25). The actomyosin interaction, the end target of both Ca²⁺- and Ca²⁺-independent pathways, was studied with the use of 2,3-butanedione 2-monoxime (BDM), a nonmuscle myosin ATPase inhibitor (Sigma). The following kinase inhibitors were used: the calmodulin-mediated MLCK inhibitor W-7 (Sigma), the Rho kinase inhibitor Y-27632 (Calbiochem, La Jolla, CA), and the PKC inhibitor staurosporin (Sigma). Kinase inhibitors and BDM were added to agonist-free collagen lattices 5 min prior to the addition of an agonist and release. To evaluate the effect of increased intracellular Ca²⁺ on contraction in the different cell types, the Ca²⁺ ionophore A-23187 was used under agonist-free conditions. To investigate the effect of inhibition of MLCP, calyculin (Calbiochem) was added to Ca²⁺-depleted and normal (Ca²⁺ containing) lattices. The former lattices were obtained by washing lattices with 1× Ca²⁺-free PBS (3 × 2 min each), followed by 1× Ca²⁺-free PBS + 3 mM EGTA (2 × 5 min each and then 14 min) and a final set of washes with 1× Ca²⁺-free PBS + 3 mM EGTA + 0.01 μM A-23187 (3 × 2 min each). The final washing step allowed depletion of intracellular Ca²⁺.
Western Blot Analysis for rMLC and Phosphorylated rMLC
HSCs, cultured for 24 h in the presence of W-7, Y-27632, staurosporine, or FCS alone, were harvested and homogenized in lysis buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 0.02% bromophenol blue, and 0.2 M DTT). After heat denaturation (100°C for 5 min) and centrifugation (13,000 g for 10 min), equal amounts of protein (6.5 μg) were run on a 7% SDS-PAGE gel and then transferred onto a nitrocellulose Protran membrane (Schleicher & Schuell, Dassel, Germany). Membranes were blocked with 5% blocking solution (milk powder) in 1× PBS for 1.5 h at room temperature to avoid nonspecific binding. Thereafter, blots were incubated overnight with primary antibodies against rMLC and phosphorylated rMLC (1:200, Santa Cruz Biotechnology, Santa Cruz, CA). The next day, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies against rMLC and phosphorylated rMLC (1:500, Prosan, Merelbeke, Belgium), and immunoreactivity was visualized using chemiluminescence detection (ECL-plus, Amersham Biosciences). Membranes were stained with Ponceau staining to confirm equal protein loading and transfer between lanes. Densitometric quantification of Western blot signal intensity was performed with Un-Scan-IT Gel software (Silk Scientific) and calculated as percentages to mean values of HSCs treated only with FCS.

Fluo-4 Visualization of Intracellular Ca2+
HSCs, grown on glass coverslips, were incubated with 5 μM fluo-4 + 0.025% pleuronic acid (Molecular Probes) under FCS-free conditions. Coverslips were subsequently transferred to the coverglass chamber of a confocal scanning microscope (Nikon TE 300, Noran Oz). Cells were observed and photographed before, during, and after the addition of FCS-free medium containing 0.01 μM A-23187.

Animal Model of Cirrhosis
Male Wistar rats, weighing 200–250 g, were intoxicated with TAA in drinking water. The TAA concentration was adapted weekly to changes in body weight, leading to homogenous macronodular cirrhosis with all the typical characteristics of portal hypertension after 18 wk, as previously described (13, 14).

In Situ Liver Perfusion
In TAA-treated cirrhotic and control rats (n = 25 each), the effect of the α1-agonist methoxamine was studied on IHVR with and without preincubation with W-7, Y-27632, and staurosporin by in situ liver perfusion, as previously described (13, 14). Briefly, the portal vein was cannulated and perfused through a 14-gauge angiocath with oxygenated Krebs solution at 37°C. After the inferior vena cava was transected, allowing the perfusate to escape, a thoracotomy was performed to cannulate the suprahepatic inferior vena cava. Once the effluent was clear, recirculation was set up with a volume of 150 ml of buffer at a constant flow of 35 ml/min. Perfusion pressure was continuously monitored (Dataq, Akron, OH). Criteria of liver viability included the gross appearance of the liver, stable perfusion pressure (starting value ± 1 mmHg), and stable buffer pH (7.4 ± 0.1) during the initial 30-min stabilization period.

Statistical Analysis
Statistical analysis was performed using SigmaStat 2.0 (Jandel, San Rafael, CA). An unpaired Student’s t-test, Mann-Whitney rank-sum test, or ANOVA was used when appropriate. Data are given as means ± SE. P ≤ 0.05 was considered statistically significant.

RESULTS
In the first part of the experiments, HSC contraction was investigated in vitro with the use of the stress-relaxed collagen lattice contraction model. In the second part, in vitro findings were corroborated with in vivo experiments in the in situ perfused rat liver.

In Vitro Results

Relevance of intracellular Ca2+ and Ca2+-dependent pathways in HSC-mediated gel contraction. Increasing Ca2+ intracellularly under agonist-free conditions with A-23187, a Ca2+ ionophore, was demonstrated by means of confocal Ca2+ imaging, which showed a maximal rise in intracellular Ca2+ within seconds (Fig. 2). A dose-response curve with A-23187 showed maximal contraction of 54.7 ± 1.8% at 0.01 μM. Since this amount to only 75.6 ± 1.8% of FCS-promoted contraction, increasing intracellular Ca2+ appears insufficient to exclusively cause contraction (Figs. 3 and 4). The equivalent maximal contraction relative to FCS-induced contraction generated in VSMCs and cardiac myofibroblasts amounted to 85.1 ± 2.3% and 34.6 ± 2.1%, respectively (P < 0.001 vs. the equivalent effect in HSCs; Fig. 4).

MLCK, the downstream effector of the Ca2+-dependent pathway, was inhibited using W-7, an inhibitor of calmodulin-mediated activation of MLCK. In FCS-promoted HSC contraction, this resulted in an attenuation to 17.6 ± 2% at 10−4 M (vs. 77.4 ± 1.2% for control, P < 0.001; Fig. 5).

When the effect of W-7 (10−4 M) was evaluated on maximal Ca2+-induced contraction (by the addition of 0.01 μM A-23187), Ca2+-induced contraction was inhibited (16.8 ± 2.7% vs. 54.7 ± 1.8%, P < 0.001), suggesting that calmodulin is indeed the downstream target of Ca2+-dependent pathways.

Relevance of Ca2+-independent pathways in HSC-mediated gel contraction. First, MLCP, the supposed converging end point of Ca2+-independent pathways, was inhibited using calyculin, a type 1 phosphatase inhibitor. Calyculin (10−9 M) was able to induce contraction of HSC-embedded collagen lattices in the absence of FCS (53.7% vs. 15.3% under FCS-free conditions, P < 0.05). To further specify the relative role of MLCP activity in HSC-mediated contraction, HSC contraction was examined under Ca2+-depleted and Ca2+-containing conditions in the presence or absence of calyculin (10−7 M). Under similar Ca2+-depleted conditions, contraction occurred more efficient when MLCP was inhibited (4 ± 2.3% vs. 30 ± 2.7%, P = 0.002; Fig. 6). In Ca2+-containing lattices, a comparable effect was observed when MLCP was inhibited (15.3 ± 3.8% vs. 53.7 ± 5.2%, P = 0.004), but the degree of contraction was increased compared with Ca2+-depleted conditions. These data not only illustrate an auxiliary role for MLCP in the regulation of HSC contraction in addition to increasing levels of intracellular Ca2+ but also document its involvement in the phenomenon of “Ca2+ sensitization.”

Subsequently, known inactivating upstream signaling pathways of MLCP, more specifically, Rho-associated kinase and PKC-mediated pathways, were evaluated. Y-27632 and staurosporin, their respective inhibitors, dose dependently attenuated FCS-promoted HSC contraction (Fig. 7, A and B). In contrast, Y-27632 (10−4 M) and staurosporin (25 nM), which both inhibited FCS-promoted contraction by >85%, inhibited calyculin-promoted contraction by maximally 35% and 27%, respectively, strengthening the hypothesis that MLCP is their downstream target (Fig. 7C). Furthermore, W-7 (10−7 M), an inhibitor of calmodulin-mediated activation of MLCK, did not
change calycin-promoted contraction at all, suggesting its unimportance in the regulation of MLCP (Fig. 7C).

Relevance of actomyosin interaction in HSC-mediated contraction. To assess whether the end point of Ca\(^{2+}\)-dependent and -independent pathways was indeed an actomyosin interaction, BDM, a nonmuscle myosin II-Ca\(^{2+}\)-ATPase inhibitor, was added to the culture medium prior to release. This led to a dose-dependent decrease in FCS-promoted contraction, indicating the requirement of an actomyosin interaction in the contraction of stress-relaxed HSC-embedded collagen matrixes (Fig. 8A). To further substantiate the involvement of the calmodulin/MLCK-, Rho kinase-, and PKC-mediated pathways in this actomyosin interaction, we additionally examined the phosphorylation of MLC by Western blot analysis. Densitometric analysis of MLC and phosphorylated MLC (Fig. 8B) showed constitutive expression of MLC under all conditions. The addition of W-7 (10\(^{-4}\) M), Y-27632 (10\(^{-4}\) M), and staurosporin (25 nM) decreased the amount of phosphorylation of MLC compared with control conditions (FCS) (\(P < 0.001\) vs. the degree of phosphorylation under control conditions).

Relevance of Ca\(^{2+}\)-dependent and -independent pathways in the presence of the \(\alpha_1\)-adrenergic agonist methoxamine. To finally refute the possibility that these pathways were relevant exclusively to FCS stimulation, we repeated the experiments with W-7, Y-27632, and staurosporin in the presence of the \(\alpha_1\)-adrenergic agonist methoxamine, for which activated HSCs express the receptors and biosynthetic enzymes (18). Except for under basal conditions comparing only both agonists, we observed no differences to FCS-induced contraction in the presence of W-7, Y-27632, and staurosporin (Fig. 9).

Results in In Situ Perfused Normal and Cirrhotic Rat Livers

In agonist-free conditions, basal IHVR of cirrhotic rat livers was already increased compared with normal rat livers (0.22 ± 0.01 vs. 0.13 ± 0.01 mmHg·min·ml\(^{-1}\), respectively, \(P < 0.001\)). The addition of methoxamine (10\(^{-4}\) M) led to an increase in IHVR in both cirrhotic and normal perfused rat livers (0.65 ± 0.02 vs. 0.32 ± 0.02 mmHg·min·ml\(^{-1}\), respectively, \(P < 0.001\)). In the cirrhotic perfused rat liver, the response to methoxamine was aggravated compared with the normal perfused rat liver (change in increase in IHVR: 0.39 ± 0.03 mmHg·min·ml\(^{-1}\) for TAA vs. 0.21 ± 0.02 mmHg·min·ml\(^{-1}\) for control, \(P = 0.001\)), which is consistent with the phenomenon of “hyperresponsiveness” to vasocon-
strictors in cirrhosis. Preincubation with Y-27632 (10^{-4} M), staurosporin (25 nM), and W-7 (10^{-4} M) in the cirrhotic rat liver significantly decreased this hyperresponsiveness to methoxamine by −66.8 ± 1.3%, −52.4 ± 2.7%, and −28.7 ± 2.8%, respectively, suggesting a predominant involvement of Ca^{2+}-independent pathways in the increased active IHVR associated with cirrhosis (Fig. 10). In the normal liver, we noted a reduction in IHVR only after inhibition of Ca^{2+}-independent pathways and to a lesser extent than that found in cirrhosis: −43.1 ± 4.2% (P = 0.008 vs. TAA + Y-27632) and −40.2 ± 4.2% (P = 0.038 vs. TAA + staurosporin) (Fig. 10).

DISCUSSION

Accumulating evidence indicates that the contractile force generated by activated HSCs not only drives scar contraction in cirrhosis but also modulates the hepatic microcirculation (12, 19, 22–24, 26). Both aspects affect the increased IHVR to portal flow, and therefore make HSCs interesting targets for the treatment of portal hypertension. Because of their apparent key role, considerable effort has been made to elucidate the regulation that governs contractile force generation in these cells. At present, it is generally assumed that activated HSCs have a smooth muscle cell-like Ca^{2+}-dependent contraction pattern (2, 5, 22–24). This contention is founded on different observations, such as 1) a functional and ultrastructural resemblance...
to pericytes (22); 2) the expression of smooth muscle proteins (like α-SMA and myosin II) (5, 26); 3) the expression of L-type voltage-operated Ca\(^{2+}\) channels (2); and 4) the fact that agonists that are known to cause contraction in HSCs are associated with increases in intracellular Ca\(^{2+}\) (22–24). Although persuasive, these observations are challenged heretofore by a lack of direct evidence proving that an increase in intracellular Ca\(^{2+}\) indeed mediates force generation and by an apparent contradiction in terminology, as witnessed in the smooth muscle cell-like contraction pattern of a myofibroblast-like activated HSC.

In contrast to previous studies, we applied a three-dimensional stress-relaxed collagen lattice contraction model to study the contractility of HSCs (16, 19, 29, 31). The reasons herefore...
are twofold. First, cell adhesion, analogous to the in vivo situation, occurs three dimensionally to attachment sites made upon protein fibrils rather than two dimensionally along a protein-coated interface (like for contraction studies using confocal Ca\(^{2+}\) imaging or when HSCs are grown on top of polymerized collagen lattices). This model therefore mimics the reciprocal geometric and mechanical relationships with the surrounding matrix that HSCs entertain in vivo (7, 16). Second, the relevance of stressed gels follows from the observation that in the cirrhotic liver, the injured liver tissue is tethered in a way that cell contraction will inevitably increase stress in the surrounding matrix, resulting in a mechanical feedback that is missing in classically used unrestrained gels (“floating gels”) as well as in the classical two-dimensional assay, as mentioned earlier.

Using this in vitro model, we first tested the premise that force generation in HSCs is Ca\(^{2+}\)-dependent. We demonstrated that, although Ca\(^{2+}\)-dependent pathways [Y-27632 (10\(^{-4}\) M) and Stauro (25 nM)] in the presence of the \(\alpha_1\)-adrenergic agonist methoxamine (MTX; 10\(^{-4}\) M), \(\ast P < 0.05\), MTX vs. FCS.

Fig. 9. Relevance of Ca\(^{2+}\)-dependent [W-7 (10\(^{-4}\) M) and -independent pathways [Y-27632 (10\(^{-4}\) M) and Stauro (25 nM)] in the presence of the \(\alpha_1\)-adrenergic agonist methoxamine (MTX; 10\(^{-4}\) M). \(\ast P < 0.05\), MTX vs. FCS.

The inability of increased intracellular Ca\(^{2+}\) to promote maximal contraction suggests that activated HSCs have an additional mechanism to Ca\(^{2+}\)/calmodulin-dependent MLCK that is critical in regulating contractility. We therefore tested Ca\(^{2+}\)-independent pathways. Since these pathways are thought to converge in the inactivation of MLCP, resulting in delayed degradation of phosphorylated rMLC and theore decreased contraction, we first focused on this enzyme (8, 11, 19, 26, 27). We demonstrated that the phosphatase inhibitor calyculin, in the absence of any agonist, could promote HSC contraction, proving its involvement in the regulation of force generation additive to the Ca\(^{2+}\)-dependent pathway. To further specify its role in this regulation, we examined HSC contraction under Ca\(^{2+}\)-depleted or Ca\(^{2+}\)-containing conditions in the presence or absence of calyculin. These experiments showed that inhibition of MLCP increased the amount of contraction independently of the level of intracellular Ca\(^{2+}\), suggestive of a phenomenon known as Ca\(^{2+}\) sensitization (21, 27). In smooth muscle cells, this phenomenon has been extensively described and is mainly attributed to the inhibition of MLCP (21).

Several mechanisms have been identified in these cells to inactivate MLCP, the most important of which are the phosphorylation of the regulatory subunit of MLCP by the Rho/Rho kinase pathway and the inhibition of the catalytic subunit of MLCP mediated by the PKC-dependent pathway (8, 17, 21, 27). For HSCs, to our knowledge, the concept of Ca\(^{2+}\) sensitization is novel. Reviewing the two aforementioned mechanisms, which are considered to be the main regulating factors in smooth muscle cells in this process, we observed surprising parallels in HSCs. First, we and others (10, 15, 30, 31), under different conditions, could document a role for the RhoA signaling pathway since preincubation with the Rho kinase inhibitor Y-27632 attenuated contraction and decreased phosphorylation of rMLC, the rate-limiting step in the actomyosin interaction. Furthermore, we also proved that the RhoA signaling pathway acts through MLCP, since preincubation with calyculin, a phosphatase inhibitor, largely counteracted inhibition of contraction by Y-27632. This suggests that Rho kinase, a serine/threonine kinase, phosphorylates MLCP and inhibits phosphatase activity, resulting in delayed degradation of phosphorylated rMLC and thus protracted contraction. Second, we also revealed a role for the PKC-dependent pathway. Stauro-

Fig. 10. Relevance of Ca\(^{2+}\)-dependent [W-7 (10\(^{-4}\) M) and -independent pathways [Y-27632 (10\(^{-4}\) M) and Stauro (25 nM)] in situ perfused normal and thioacetamide (TAA)-induced cirrhotic rat livers (n = 5 per condition). Both Ca\(^{2+}\)-dependent and -independent pathways were shown to be involved in the hyperresponsesiveness of the cirrhotic liver to MTX because inhibition of these pathways attenuated the increased intrahepatic vascular resistance (IVR) with a predominance of Ca\(^{2+}\)-independent pathways, mediated through PKC- and RhoA-mediated signaling cascades. \(\ast P < 0.05\) vs. TAA + MTX; \(\ast P < 0.05\) vs. TAA + W-7; \(\ast P < 0.05\) vs. TAA + Y-27632; \(\ast P < 0.05\) vs. normal rat liver + MTX.
sporin, a PKC inhibitor, prevented FCS- and methoxamine-promoted contraction and decreased phosphorylation of rMLC. Similarly as for RhoA inhibition, calyculin also almost completely opposed inhibition of contraction by staurosporin, identifying MLCP as the end target of this pathway as well. These results indicate an auxiliary role for PKC to the RhoA pathway as a G protein-coupled effector of Ca\(^{2+}\) sensitization.

It should be taken into account that the inhibitors used to block the different pathways are highly specific but not exclusive to their intended target. In addition, a role for MLC phosphorylation-independent regulatory mechanisms is postulated in smooth muscle cells (21). Our data show that Ca\(^{2+}\)-dependent (MLCK related) and Ca\(^{2+}\)-independent (MLCP related) pathways are important in HSC contraction, but these findings do not exclude the presence or absence of alternative, more discrete pathways. The study of these will require more selective experimental tools and models that are lacking at the moment.

To correlate our in vitro findings to the in vivo situation, we reevaluated these pathways in the in situ perfused liver. We first confirmed the presence of an exaggerated response to methoxamine in the cirrhotic rat liver compared with the normal liver, illustrating the known phenomenon of intrahepatic hyperresponsiveness to vasoconstrictors in the cirrhotic liver (6, 14). Both Ca\(^{2+}\)-dependent and -independent pathways were shown to be involved in this hyperresponsiveness of the cirrhotic liver since inhibition of these pathways attenuated the increased IHVR. A predominance of the Ca\(^{2+}\)-independent pathways, mediated through PKC- and RhoA-mediated signaling cascades, was demonstrated.

In conclusion, we have shown that both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent pathways are necessary to raise a HSC-specific contraction pattern, which distinguishes them from both typical myofibroblasts and smooth muscle cells. These in vitro findings correlated with the data obtained in the cirrhotic liver where both pathways were involved, with Ca\(^{2+}\)-independent pathways predominating the picture. A better understanding of the intracellular signal transduction mechanisms leading to HSC contraction and the demonstration of their relevance in the establishment of the increased IHVR in the cirrhotic liver might lead to the identification of novel potential targets for the treatment of portal hypertension.

REFERENCES


