Norepinephrine induces calcium spikes and proinflammatory actions in human hepatic stellate cells

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Sancho-Bru, Pau, Ramón Bataller, Jordi Colmenero, Xavier Gasull, Montserrat Moreno, Vicente Arroyo, David A. Brenner, and Pere Ginès. Norepinephrine induces calcium spikes and proinflammatory actions in human hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 291: G877–G884, 2006.—Catecholamines participate in the pathogenesis of portal hypertension and liver fibrosis through α1-adrenoceptors. However, the underlying cellular and molecular mechanisms are largely unknown. Here, we investigated the effects of norepinephrine (NE) on human hepatic stellate cells (HSC), which exert vasoactive, inflammatory, and fibrogenic actions in the injured liver. Adrenoceptor expression was assessed in human HSC by RT-PCR and immunocytochemistry. Intracellular Ca2+ concentration ([Ca2+]i) was studied in fura-2-loaded cells. Cell contraction was studied by assessing wrinkle formation and myosin light chain II (MLC II) phosphorylation. Cell proliferation and collagen-α1(I) expression were assessed by [3H]thymidine incorporation and quantitation. NE caused phosphorylation of MLC II and cell contraction. In contrast, NE did not affect cell proliferation or collagen-α1(I) expression. Importantly, NE stimulated the secretion of inflammatory chemokines (RANTES and interleukin-8) in a dose-dependent manner. Activation of human HSC expressed NE-induced liver fibrosis in rats (15, 30). Finally, genetic ablation of the sympathetic nervous system (SNS) reduces liver fibrosis in mice (32). Moreover, it has been shown that rodent hepatic stellate cells (HSC) express some adrenoceptor subtypes and that stimulation with norepinephrine (NE) induces proliferation and collagen gene expression (32, 33).

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G878  EFFECTS OF NOREPINEPHRINE IN HEPATIC STELLATE CELLS

Table 1.  PCR primer pairs for adrenoceptors and housekeeping genes

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<th>Gene</th>
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<th>Antisense</th>
<th>Product, bp</th>
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<td>5′-AAAAATGGTGTACAGATGACC-3′</td>
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<td>5′-GGGCCGCCCTCCCCCTTGG-3′</td>
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<tr>
<td>α1B</td>
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<td>5′-GGGCCGCCCTCCCCCTTGG-3′</td>
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<td>α2A</td>
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<tr>
<td>α2B</td>
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<td>β2</td>
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MATERIALS AND METHODS

Human liver specimens and HSC isolation. Human cirrhotic livers were obtained from liver explants at the time of liver transplantation from patients with end-stage liver disease due to hepatitis C virus infection (n = 5). Human control livers were obtained from resections of liver metastasis of colon cancer (n = 5). Human HSC were isolated from fragments of normal livers as described in detail previously (6). Experiments were performed with HSC activated in culture (after the second serial passage). A subset of experiments was performed in activated nonpassaged HSC. The immunocytochemistry studies were performed also in HSC freshly isolated from normal human livers (quiescent phenotype). In all cell cultures, no staining was found for CD45, factor VIII-related antigens, and cam 5.2 (Dako, Glostrup, Denmark), indicating the absence of mono/macrophagic, endothelial, and epithelial cells. Cells were cultured in standard conditions in Iscove’s modified Dulbecco’s medium (BioWhittaker, Verviers, Belgium) containing 15% FCS. Cells were serum starved for at least 12 h before the experiments. The protocol was approved by the Institutional Review Board of the Hospital Clinic of Barcelona.

Immunocytochemistry studies. Cultured HSC were fixed in methanol at −20°C for 10 min, blocked in PBS containing 0.1% BSA for 30 min, and incubated with anti-p65 or anti-α1A-adrenoceptor for 1 h (Santa Cruz Biotechnology, Santa Cruz, CA). Cells were incubated with Cy3- or fluorescein-labeled secondary antibody for 1 h. An isotype-matched antibody was used as a negative control. Cells were analyzed with a Leica TCS SL laser scanning confocal spectral microscope (Leica Microsystems Heidelberg, Mannheim, Germany) at a wavelength of 500–535 nm.

Measurement of intracellular Ca2+ concentration. Changes in intracellular Ca2+ concentration ([Ca2+]i) were measured in fura-2 (Calbiochem, San Diego, CA)-loaded cells with an inverted epifluorescence microscope as described in detail previously (6). A representative experiment was performed in flou-4-loaded cells in a confocal spectral microscope. Cells were tested with NE, prazosin, and from fragments of normal livers as described in detail previously (6).

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Fig. 1. Expression of adrenoceptors in human hepatic stellate cells (HSC). A: α1A-, α2B-, and β2-receptors were detected in HSC and whole liver, as assessed by RT-PCR. α1A-, α2A-, and β2-receptors were not detected in HSC but were amplified in mRNA isolated from human livers. GAPDH was amplified as a housekeeping gene to assess correct amplification (data not shown). B: detection of α1A-receptor in HSC freshly isolated from normal human livers (quiescent). Green staining shows α1A-receptor expression. C: detection of α1A-receptor in HSC activated after prolonged culture by immunocytochemistry. Note the α1A-adrenoceptor expression at the cell membrane. A negative control using an isotype-matched primary antibody was performed (D).

Fig. 2. Expression of α1A adrenoceptors in normal and cirrhotic human livers. A: Western blot analysis of 50 μg protein from normal and fibrotic human livers. α1A-Adrenoceptors were detected in normal livers and were overexpressed in cirrhotic livers (51.3 kDa). Actin expression was included in Western blotting to ensure equal protein loading. B: analysis of α1A-receptor expression by densitometry with respect to actin. **P < 0.01 vs. normal livers.
propranolol (Sigma, St. Louis, MO). To study the Ca\(^{2+}\) source, experiments were performed with and without extracellular Ca\(^{2+}\) ions. Cells were considered to be responders when [Ca\(^{2+}\)]i increased 50% above the resting value.†Responder cells only.‡

### Table 2. Effect of norepinephrine on [Ca\(^{2+}\)]i in human hepatic stellate cells

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Responder cells, %*</th>
<th>Peak [Ca(^{2+})]i, nM</th>
<th>Oscillations, % of cells</th>
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<tbody>
<tr>
<td>NE (10 μM)</td>
<td>51</td>
<td>84</td>
<td>317±138</td>
<td>57</td>
</tr>
<tr>
<td>Prazosin (1 μM) + NE (10 μM)</td>
<td>27</td>
<td>0</td>
<td>217±138</td>
<td>57</td>
</tr>
<tr>
<td>Propranolol (1 μM) + NE (10 μM)</td>
<td>69</td>
<td>79</td>
<td>260±170‡</td>
<td>36‡</td>
</tr>
</tbody>
</table>

Values are given as means ± SE; n, no. of cells. *Responder cells are those showing at least a 1.5-fold increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]i) compared with resting values.†Responder cells only.‡ P < 0.05 vs. cells stimulated with norepinephrine (NE).

Assessment of cellular contraction. The contractile responses of cultured HSC to NE were evaluated by wrinkle formation (19, 24). A thin layer of the polydimethyl siloxane (12,500 cP; Sigma) was spread on microscope coverslips. The silicone fluid layer was briefly heated on a low flame from a Bunsen burner to promote cross-linking of the silicone fluid surface while the overlying non-cross-linked fluid served as a lubricant and allowed the surface to move independently of the coverslip. Cells plated on the silicone rubber membrane showed basal wrinkles after 2–3 days in culture. Cells were photographed every 5 min on an Olympus IX70 inverted phase-contrast microscope with a digital charge-coupled device camera (Hamamatsu, Shizuoka, Japan) and stimulated with the agonists to assess cellular contraction by wrinkle formation. Cell contraction was considered to occur when there was a significant increase in the number of wrinkles or an increase in the length of the preexisting ones.

Gene expression assays. RNA was isolated from activated HSC with TRIzol (Life Technologies, Rockville, MD). PCR primer pairs were designed to amplify GAPDH and α\(_{1A^{-}}\), α\(_{1B^{-}}\), α\(_{2A^{-}}\), α\(_{2B^{-}}\), β\(_{1^{-}}\), and β\(_{2^{-}}\)-adrenoceptors (Table 1). Quantitative PCR was performed with predesigned Assays-on-Demand TaqMan probes and primer pairs for collagen-α\(_{1}(I)\) and ribosomal subunit 18S. Information on these Assays-on-Demand is available at [http://myscience.appliedbiosystems.com/cdsEntry/Form/gene_expression_keyword.jsp](http://myscience.appliedbiosystems.com/cdsEntry/Form/gene_expression_keyword.jsp). TaqMan reactions were carried out in duplicate on an ABI PRISM 7900 machine (Applied Biosystems, Foster City, CA).

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**Fig. 3.** Effects of norepinephrine (NE) on intraacellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]i) and cell contraction in HSC. A: representative graph showing the effect of NE in a single HSC. NE (10 μM) induced an oscillatory [Ca\(^{2+}\)]i increase (Ca\(^{2+}\) spikes). B: preincubation with prazosin (1 μM) for 10 min blocked NE-induced [Ca\(^{2+}\)]i increase. C: incubation for 10 min with propranolol (1 μM) decreased both Ca\(^{2+}\) peak and the percent of cells with oscillations. D: representative images of [Ca\(^{2+}\)]i increase induced by NE (10 μM) in HSC. Images were obtained with a confocal microscope in fluo-4-loaded HSC. Images were taken every 10 s. Color change to red reveals [Ca\(^{2+}\)]i increase. E: phosphorylation (p) of myosin light chain II (MLC II) as assessed by Western blotting (18 kDa). Stimulation with NE (10 μM) or ANG II induced MLC II phosphorylation at 5 min. F: representative images of wrinkle formation (arrows) formed by HSC stimulated with NE (10 μM).
Cell proliferation assay. DNA synthesis was estimated by [methyl-3H]thymidine (Amersham Biosciences, Little Chalfont, UK) incorporation, as described in detail previously (7). Cells were serum starved for 24 h, stimulated for 18 h with agonists, and then pulsed with 6 h with 1 μCi/ml [methyl-3H]thymidine. Results are expressed as fold stimulation compared with cells incubated with buffer.

Determination of chemokine secretion. HSC were cultured in six-well plates at a density of 4 × 10^5 cells/well for 24 h. Medium was removed, and cells were incubated in serum-free medium for 24 h in the presence of agonists. Supernatants were collected, and a sandwich ELISA for human IL-8 (BLK Diagnostics, Barcelona, Spain) and RANTES (R&D Systems, Minneapolis, MN) was performed. Cells were preincubated with SP-600125, PD-231445 (Sigma), and BAY 11-7082 (Calbiochem, Darmstadt, Germany) in indicated experiments. Results are expressed as fold increases of chemokine secretion compared with cells treated with buffer.

Recombinant adenoviral infection. Recombinant adenoviral vectors expressing a luciferase reporter gene driven by NF-kB transcriptional activation (Ad5NF-kBLuc; Ref. 39), a dominant-negative mutant form (S32A/S36A) of IkB (Ad5IkB; Ref. 23), or control green fluorescent protein (Ad5GFP; Ref. 28) were used. HSCs were infected with Ad5NF-kBLuc [multiplicity of infection (MOI) 500] and/or Ad5IkB (MOI 1,000) and Ad5GFP (MOI 1,000) for 12 h in DMEM containing 0.5% FCS. After infection, the medium was replaced with medium containing 0.5% FCS, and the culture was continued for an additional 8 h before the individual experiments were performed.

NF-kB-responsive luciferase assay. HSC were infected with Ad5NF-kBLuc for 12 h. Medium was replaced, and cells were stimulated with agonists for 8 h. NF-kB-mediated transcriptional induction was assessed with the luciferase assay system (BD Pharmingen, San Diego, CA). Luciferase activity (relative light units) was normalized to the protein concentration.

Electrophoretic mobility shift assay. Cell nuclear proteins were extracted as described previously (8). Eight micrograms of nuclear proteins were incubated with 100 pg of a 32P-labeled probe containing the activator protein-1 (AP-1) consensus site (5′-GTAAAGCATGAGTCACACACCTC-3′) in buffer containing (in mM) 10 HEPES (pH 7.8), 2 MgCl2, 50 KCl, 1 DTT, and 0.1 EDTA with 20% glycerol in the presence of single-stranded oligonucleotide (25 μg/ml) and poly(dI-dC) (25 μg/ml) for 20 min at room temperature. For the competition assay, one sample was incubated with 10 ng of unlabeled probe.

Western blot analysis. Whole cell extracts were obtained in Triton lysis buffer containing protease and phosphatase inhibitors. Twenty-five micrograms were loaded onto 10% or 15% SDS-acrylamide gels and blotted onto nitrocellulose membranes. Membranes were then incubated with antibodies against SAPK/JNK, phospho-SAPK/JNK, p44/42 MAPK (ERK), phospho-ERK, phospho-myosin light chain II (MLC II) (Cell Signaling, Beverly, MA), and α1A-adrenoceptor (Santa Cruz Biotechnology). After an extensive wash, membranes were incubated with blocking buffer containing horseradish peroxidase-conjugated secondary antibody. Proteins were detected by enhanced chemiluminescence (ECL, Amersham).

Data analysis. Data are representative of at least three experiments. Results are expressed as means + SD. Statistical analysis was performed by Student’s t-test and ANOVA. Statistical analysis was performed using SPSS software (Chicago, IL).

RESULTS

α1A-Adrenergic receptors are expressed in human HSC and are upregulated in human fibrotic livers. We first assessed the expression of adrenoceptors in human HSC by RT-PCR. Cultured human HSC expressed α1A-, α2B-, and β2-adrenoceptors (Fig. 1A). In contrast, α1B-, α2A-, and β1-adrenoceptors were not expressed in HSC but were detected in RNA obtained from the total human liver. Because α1-receptors mediate key biological effects of the SNS in fibrogenic cell types (15, 38), we further investigated their expression in HSC as well as in normal and fibrotic human liver specimens. Both quiescent and culture-activated HSC contained α1A-receptors, as assessed by immunocytochemistry (Fig. 1, B–D). Moreover, Western blot analysis of whole liver extracts revealed that normal human livers express α1A-receptors, which are markedly upregulated in livers with advanced fibrosis (Fig. 2). These results suggest that α1A-receptors are upregulated during liver fibrogenesis and that HSC are potential targets for adrenergic agonists and antagonists.

NE induces Ca2+ spikes and cell contraction in human HSC. We next stimulated HSC with NE, a major neurotransmitter of the SNS. Stimulation of cells with NE (10 μM) increased [Ca2+]i in fura-2-loaded HSC (Table 2 and Fig. 3, A and D). The typical pattern was characterized by an abrupt increase of [Ca2+]i, followed by an oscillatory response in 57% of the cells (Ca2+ spikes). NE (10 μM) induced a significant increase of [Ca2+]i in 84% of the cells and a mean Ca2+ peak of 317 ± 138 nM. Cell stimulation with 1 and 0.1 μM NE also induced a marked [Ca2+]i increase (not shown). Removal of Ca2+ from the extracellular solution did not affect the NE-induced Ca2+ response. Moreover, cell preincubation with thapsigargin, a cell-permeant sarco(endo)plasmic reticulum Ca2+-ATPase pump inhibitor that depletes intracellular Ca2+ stores, completely abolished the NE-induced Ca2+ response (data not shown). These results indicate that NE mainly releases Ca2+.

Fig. 4. Effect of NE on cell growth and collagen gene expression. A: effect of NE (10 μM) on cell growth as assessed by [3H]thymidine incorporation. HSC were treated for 24 h with NE PDGF-BB (20 ng/ml), or both. PDGF-BB induced a marked increase in DNA synthesis (P < 0.001 vs. untreated cells). NE did not stimulate cell growth or amplify PDGF-induced mitogenic effect.

B: effect of NE (10 μM) on procollagen-α1(I) gene expression, as assessed by quantitative PCR. Values are means of 5 independent experiments.
from intracellular stores. Prazosin completely prevented the NE-induced [Ca\textsuperscript{2+}] increase in all cells (Table 2 and Fig. 3B). However, preincubation of cells with propranolol only attenuated the NE-induced [Ca\textsuperscript{2+}] peak and the percentage of cells showing an oscillatory response (Fig. 3C). Similar results were obtained with nonpassaged cells activated in culture (data not shown). We next explored whether NE induces cell contraction. After 5 min, NE induced MLC II phosphorylation, which is a known prerequisite for cell contraction (20, 44) (Fig. 3E). Preincubation with 10 \mu M BAPTA-AM did not significantly modify MLC II phosphorylation, suggesting that it could be Ca\textsuperscript{2+} independent (data not shown). Moreover, we found that stimulation with NE induced cell contraction, as assessed by wrinkle formation (Fig. 3F). Together, these results indicate that NE induces cell contraction and may be involved in the vasoactive effects of HSC.

**NE does not stimulate proliferation of human HSC.** We investigated whether NE regulates human HSC growth. Different concentrations of NE (10 nM–10 \mu M) were tested. Incubation with NE did not affect DNA synthesis, as assessed by \[^3H\]thymidine incorporation (Fig. 4A). Stimulation with phenylephrine or terbutaline (\alpha- and \beta-receptor agonists, respectively) did not affect cell proliferation (data not shown).

To elucidate whether NE regulates agonist-induced cell proliferation, HSC were pretreated with NE (10 \mu M) and then challenged with PDGF-BB (20 ng/ml), a potent mitogen for these cells. NE pretreatment did not affect PDGF-induced mitogenic effects. These results indicate that NE is not mitogenic for human HSC.

**Effect of NE on gene expression of collagen-\alpha\textsubscript{1}(I) in human HSC.** We next investigated whether NE regulates the expression of collagen-\alpha\textsubscript{1}(I), the main extracellular protein found in fibrotic human livers. Stimulation of cells with NE (10 \mu M) did not affect collagen expression, as assessed by quantitative PCR (Fig. 4B). These results suggest that NE does not directly regulate collagen synthesis in activated human HSC.

**NE stimulates chemokine production by human HSC.** We next investigated whether NE regulates the inflammatory actions of HSC by stimulating chemokine secretion. NE (10 \mu M) induced a significant increase in secretion of IL-8 and RANTES, which are chemokines potentially involved in liver fibrogenesis (34, 40), to the culture medium (Fig. 5A). Preincubation of cells with prazosin markedly reduced both IL-8 and RANTES secretion. Propranolol also attenuated NE-induced chemokine production, yet only RANTES secretion was significantly reduced. These results suggest that the inflammatory

![Graph showing the effect of NE on chemokine secretion in human HSC.](image-url)
actions of NE on human HSC could be mediated by both \( \alpha_1 \)- and \( \beta \)-adrenoceptors.

Effect of NE on intracellular signaling pathways in human HSC. Finally, we explored the effect of NE on intracellular pathways known to regulate the inflammatory actions in human HSC (8, 41). These pathways include MAPK and the transcription factors NF-\( \kappa \)B and AP-1. NE (10 \( \mu \)M) induced a transient increase in ERK and SAPK/JNK phosphorylation, as assessed by Western blot analysis (Fig. 6A). NE activated NF-\( \kappa \)B, as indicated by increased NF-\( \kappa \)B-dependent gene expression as well as nuclear translocation of the heterodimer p50/p65 (Fig. 6, B and C). In addition, NE increased TNF-\( \alpha \)-induced NF-\( \kappa \)B activation, suggesting that it could amplify the inflammatory response evoked by other cytokines (data not shown). Finally, NE stimulated AP-1 DNA binding, as assessed by the electrophoretic mobility shift assay (Fig. 6D). To elucidate the role of these signaling pathways on chemokine production, HSC were preincubated with specific antagonists before NE stimulation. Preincubation of cells with BAY 11-7082 (a NF-\( \kappa \)B inhibitor), but not with SP-600125 (a JNK inhibitor) and PD-231445 (an ERK inhibitor), attenuated IL-8 secretion (Fig. 5B). Moreover, transfection with Ad5I\( \kappa \)B reduced IL-8 production, confirming NF-\( \kappa \)B involvement (Fig. 5C). These data strongly indicate that NE exerts proinflammatory actions in human HSC through NF-\( \kappa \)B activation.

DISCUSSION

In the present study, we investigated the biological effects of NE in human HSC. We provide evidence that HSC express \( \alpha_1 \)- and \( \beta \)-receptor subtypes. NE stimulates key intracellular signaling pathways in these cells and induces cell contraction and proinflammatory actions. These actions are mainly mediated by \( \alpha_1 \)-receptors, although \( \beta \)-receptors also modulate some of the effects evoked by NE. Moreover, \( \alpha_1 \)-receptors are markedly upregulated in human fibrotic livers. These results suggest that HSC are potential targets for the pathogenic effect of catecholamines in the liver, in keeping with recent data indicating that catecholamines not only regulate vascular ho-

![Fig. 6. Effect of NE on intracellular signaling pathways in human HSC. A: effect of NE on ERK and SAPK/JNK phosphorylation, as assessed by Western blotting. Stimulation with NE induced a transient phosphorylation of ERK and SAPK/JNK. Images are representative of 3 independent experiments. B: NF-\( \kappa \)B-dependent gene expression as assessed by a luciferase assay. NE stimulated NF-\( \kappa \)B-dependent gene expression that was completely blunted by prazosin but not by propranolol. * \( P < 0.05 \) vs. untreated cells; \( # P < 0.05 \) vs. cells treated with NE alone. Results are means of 4 independent experiments. C: Immunocytochemistry analysis of the p65 subunit of NF-\( \kappa \)B in HSC. Stimulation for 60 min with NE (10 \( \mu \)M) resulted in the translocation of the heterodimer p50/p65 to the nucleus, which is indicative of NF-\( \kappa \)B activation. TNF-\( \alpha \) (10 ng/ml) was used as a positive control. D: activation of the transcription factor activator protein-1 (AP-1) as assessed by electrophoretic mobility shift assay. Stimulation with NE (10 \( \mu \)M) for 60 min markedly stimulated AP-1 DNA binding. Preincubation with cold probe was performed to ensure specificity. Image is representative of 3 independent experiments.](http://ajpgi.physiology.org/)

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meostasis but also contribute to tissue repair and inflammation (11, 15, 30, 31).

The hallmark biological action of NE in vascular cell types is to induce \( \text{Ca}^{2+} \) increase and cell contraction. This effect has been extensively studied in mesangial cells and vascular smooth muscle cells (18, 26). Similarly, a large number of studies have investigated the vasoactive effects of vasoconstrictors in cultured human HSC, including angiotensin II, endothelin-1, thrombin, and leukotriene \( \text{D}_4 \) (36). Currently, studies using activated HSC are widely used to investigate the cellular basis of portal hypertension to identify potential targets for therapy. Surprisingly, little information is available on the actions of adrenergic agonists in these cells. Here, we provide evidence that NE is a potent agonist for human HSC to induce \([\text{Ca}^{2+}]_i\) increase. An intriguing finding of the present study is that the \( \text{Ca}^{2+} \) response pattern evoked by NE markedly differs from that observed with other vasoconstrictors. Previously, we (6) showed that angiotensin II induces an abrupt \([\text{Ca}^{2+}]_i\) increase followed by a sustained phase. This effect was mainly due to the entrance of \( \text{Ca}^{2+} \) from the extracellular space through voltage-operated \( \text{Ca}^{2+} \) channels. Interestingly, the effect of NE on \([\text{Ca}^{2+}]_i\) consists of numerous \( \text{Ca}^{2+} \) spikes. Baseline \( \text{Ca}^{2+} \) spikes are characterized by rapidly rising transient increases in \([\text{Ca}^{2+}]_i\), rising from a baseline that is close to the resting concentration (9). This pattern of response has been described in many cells in response to hormones and neurotransmitters (18, 25). \( \text{Ca}^{2+} \) oscillations permit cells to respond to agonists without being exposed to sustained levels of \([\text{Ca}^{2+}]_i\). It has been shown that oscillations and especially their frequency activate cell signaling like calmodulin-dependent protein kinase II and NF-\( \kappa \)-B (14, 22). Moreover, \( \text{Ca}^{2+} \) oscillations have been involved in many cellular processes like contraction, migration, cell secretion, or phagocytosis (9, 12).

The biological relevance of NE-induced \( \text{Ca}^{2+} \) spikes in HSC is unknown. Although NE induced MLC II phosphorylation, NE may promote proinflammatory actions in the liver. The proinflammatory effect of NE on HSC has not been previously described in other cell types. The finding that NE enhances chemokine secretion in a NF-\( \kappa \)-B-dependent manner. Importantly, NE enhances chemokine secretion in a NF-\( \kappa \)-B-dependent manner. Besides their role in collagen synthesis, recent data suggest that not only may an endocrine pathways regulate HSC contractility but an autocrine/paracrine system may also be involved (32). Further studies should study this hypothesis. Our finding that NE enhances chemokine secretion in HSC suggests that catecholamines may be involved in the liver. The proinflammatory effect of NE on HSC has not been previously described in other cell types. The finding that NE may promote proinflammatory actions in HSC also explains the traditional view of the SNS as a system that basically regulates systemic and hepatic hemodynamics. In summary, the results of the present study indicate that NE mediates vasoactive and proinflammatory actions in human HSC mainly through \( \alpha_1 \)-adrenoceptors. Because HSC are able to synthesize adrenergic agonists and the SNS is activated in patients with advanced liver fibrosis, these findings suggest that catecholamines may increase intrahepatic vascular resistance and promote inflammatory actions by targeting HSC. Our data explain, at least in part, the mechanisms underlying the beneficial effects of \( \alpha_1 \)-receptor agonists in the treatment of portal hypertension and suggest the potential use of these drugs to modulate hepatic inflammation and subsequent fibrogenesis.

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13. Morello had a grant from Instituto Reina Sofía de Investigación Nefrológica.


