Propranolol modulates the collateral vascular responsiveness to vasopressin via a $G_\alpha$-mediated pathway in portal hypertensive rats

Jing-Yi LEE*, Teh-Ia HUO††, Hui-Chun HUANG‡‡, Fa-Yauh LEE§§, Han-Chieh LIN‡‡, Chiao-Lin CHUANG‡‡‖, Ching-Chih CHANG‡‡‖, Sun-Sang WANG‡‖ and Shou-Dong LEE‡‡†

*Institute of Pharmacology, National Yang-Ming University, No. 155, Sect. 2, Li-Nong St., Taipei 11221, Taiwan, †Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sect. 2, Shih-Pai Road, Taipei 11217, Taiwan, ‡Faculty of Medicine, National Yang-Ming University, No. 155, Sect. 2, Li-Nong St., Taipei 11221, Taiwan, §Department of Medicine, Taipei Veterans General Hospital, No. 201, Sect. 2, Shih-Pai Road, Taipei 11217, Taiwan, ‖Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sect. 2, Shih-Pai Road, Taipei 11217, Taiwan, and ‡‡Department of Medical Affairs and Planning, Taipei Veterans General Hospital, No. 201, Sect. 2, Shih-Pai Road, Taipei 11217, Taiwan

ABSTRACT

Gastro-oesophageal variceal haemorrhage is one of the most dreadful complications of portal hypertension and can be controlled with vasoconstrictors. Nevertheless, sympathetic tone abnormality and vascular hyporesponsiveness in portal hypertension may impede the haemostatic effects of vasoconstrictors. Propranolol, a $\beta$-blocker binding the G-protein-coupled adrenoceptor, is a portal hypotensive agent. However, whether propranolol influences the collateral vasoresponse is unknown. Portal hypertension was induced by PVL (portal vein ligation) in Sprague–Dawley rats. In an acute study with an in situ perfusion model, the collateral responsiveness to AVP (arginine vasopressin) was evaluated with vehicle, propranolol (10 $\mu$mol/l), propranolol plus suramin (100 $\mu$mol/l, a $G_\alpha$ inhibitor) or suramin pre-incubation. $G_\alpha_q$ mRNA expression in the splenorenal shunt, the most prominent intra-abdominal collateral vessel, was measured. In the chronic study, rats received DW (distilled water) or propranolol (10 mg $\cdot$ kg$^{-1}$ $\cdot$ day$^{-1}$) for 9 days. Then the concentration–response relationship of AVP and $G_\alpha_q$ mRNA expression were assessed. Propranolol pre-incubation elevated the perfusion pressure changes of collaterals in response to AVP, which was inhibited by suramin. The splenorenal shunt $G_{a11}$ mRNA expression was enhanced by propranolol. The group treated with propranolol plus suramin had a down-regulation of $G_{a11}$ as compared with the propranolol group. Chronic propranolol treatment reduced mean arterial pressure, PP (portal pressure) and the perfusion pressure changes of collaterals to AVP. $G_{a5}$ expression was up-regulated. In conclusion, propranolol pre-incubation enhanced the portal-systemic collateral AVP responsiveness in portal hypertensive rats, which was related to $G_{a_q}$ and $G_{a11}$ up-regulation. In contrast, the attenuated AVP responsiveness by chronic propranolol treatment was related to $G_{a5}$ up-regulation. The $G_\alpha$ signalling pathway may be a therapeutic target to control variceal bleeding and PP in portal hypertension.

Key words: $G_\alpha$-mediated pathway, gastro-oesophageal variceal haemorrhage, hyperdynamic circulation, portal hypertension, propranolol, vasopressin.

Abbreviations: AVP, arginine vasopressin; BW, body weight; DW, distilled water; HR, heart rate; MAP, mean arterial pressure; PP, portal pressure; PVL, portal vein ligation; ROK, Rho-kinase.

Correspondence: Dr Fa-Yauh Lee (email fylee@vghtpe.gov.tw) or Dr Hui-Chun Huang (email hchuang2@vghtpe.gov.tw).
INTRODUCTION

Gastro-oesophageal variceal haemorrhage is one of the most dreadful complications in cirrhotic patients with portal hypertension and portal-systemic collaterals development. Vasopressin, in the past, and its long-acting analogue terlipressin, nowadays, have been used widely to control portal hypertension and variceal bleeding [1]. The haemodynamic effect of vasopressin relies mainly on splanchnic vasoconstriction, followed by decreased portal venous inflow and PP (portal pressure) [2,3]. With an in situ collateral perfusion model, it has been demonstrated that AVP (arginine vasopressin) exerts a direct vasoconstrictive effect on portal-systemic collaterals [4]. The vascular effect of AVP begins with its binding to V1a receptor, a G-protein-coupled receptor. Subsequently, activation of α-subunits of G-protein families, including Gαq and Gα11, results in vasoconstriction of vascular smooth muscle [5,6]. The V1a receptor also interacts with different isotypes of G-protein, such as Gαi, Gαs, and Gαq [7]. However, previous studies have indicated a poorer vascular responsiveness to AVP in portal hypertension during acute haemorrhage [8,9], which may adversely affect the haemostatic effect of AVP in cirrhotic patients with acute variceal haemorrhage.

An enhanced sympathetic tone accompanying portal hypertension has been reported [10]. β-Adrenoceptors couple with G-proteins of the Gαi, Gαs, and Gαq families [11] and the vascular actions are significantly influenced by G-proteins. Propranolol, a non-selective adrenergic β-blocker, ameliorates the hyperdynamic circulatory status characterized by increased HR (heart rate) and cardiac index and decreased systemic vascular resistance in portal hypertension. Such a finding suggests that an increased β-adrenergic activity plays a role in the hyperdynamic circulation [12,13]. Previous studies have noted that propranolol reduced PP in patients [14] and animals [15] with portal hypertension. In patients with cirrhosis, propranolol also decreased hepatic venous pressure gradient [16], a parameter that correlates closely with PP [17]. The effect is mediated via, firstly, reduction of HR and cardiac output by β1-adrenergic receptor inhibition [18] and, secondly, splanchnic vasoconstriction through an unopposed endogenous α-adrenergic effect following β1- and β2-blockade [19] or the blockade of vasodilator β2-adrenoceptor [20].

Regarding its influence on collateral circulation, propranolol reduces oesophageal variceal pressure [21] and azygos blood flow, an index of flow through gastro-oesophageal collaterals [13,20]. Propranolol infusion also markedly reduced the flow velocity through the varices and azygos vein [22]. Apart from the aforementioned acute vascular effect of propranolol, the long-term effect of propranolol also deserves investigation since chronic propranolol use has been applied in primary and secondary prophylaxis of gastrointestinal bleeding in cirrhotic patients [23].

As both AVP and propranolol have been widely used for the control of gastro-oesophageal variceal bleeding and portal hypertension and the potential interaction between AVP and propranolol is a concern, the present study assesses the impact of acute and long-term propranolol administration on the collateral vascular responsiveness to AVP and the roles of G-proteins in the mechanism in portal hypertensive rats.

MATERIALS AND METHODS

Animal model

Male Sprague-Dawley rats (300–350 g) were caged at 24 °C with a 12 h light/12 h dark cycle and free access to food and water. Survival surgery and a haemodynamic study were performed under anaesthesia with ketamine hydrochloride [100 mg/kg of BW (body weight)]. Portal hypertension was induced by partial PVL (portal vein ligation) as described previously [24]. The experiments followed the ‘Guide for the Care and Use of Laboratory Animals’ published by the NIH (National Institutes of Health; NIH publication number 86-23, revised in 1985). Permission was obtained from the Taipei Veterans General Hospital Animal Committee.

In situ perfusion preparation

The in situ perfusion was performed as described previously [4,25]. Briefly, both the jugular veins were cannulated with 16-gauge Teflon cannulas as outlets. Heparin (200 units/100 g) was injected. The abdomen was opened and an 18-gauge Teflon cannula was inserted into the distal superior mesenteric vein. The portal vein was ligated to exclude the liver from perfusion. The animal was transferred into a warm chamber and the perfusion area was maintained at 37 ± 0.5 °C. A non-circulating perfusion was then begun with Krebs solution (118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH2PO4, 1.2 mmol/l MgSO4, 2.5 mmol/l CaCl2, 25 mmol/l NaHCO3 and 11.0 mmol/l dextrose, pH 7.4; 37 ± 0.5 °C) containing 3 % (w/v) albumin (factor V BSA; Sigma) via the mesenteric cannula by a roller pump (model 505S; Watson-Marlow). The perfusate was equilibrated with carbogen gas (95 % O2/5 % CO2) by a silastic membrane lung [26]. Pneumothorax was created by opening slits through the diaphragm to increase resistance in pulmonary arteries and prevent the perfusate from entering left heart chambers. The collaterals were then perfused with oxygenated Krebs solution. A Spectramed DTX attached to the Gould model RS 3400 recorder was connected to monitor and record the pressure in the portal-systemic collaterals, with the zero placed at the level of the right atrium. All the experiments were...
performed 25 min after starting perfusion at a constant rate of 20 ml/min. In each individual preparation, the contracting capability of the portal-systemic collateral vessels was challenged with a 125 mmol/l KCl solution at the end of experiments.

**Measurement of systemic and portal haemodynamics**

The right femoral artery of PVL rats was cannulated with a PE-50 catheter that was connected to a Spectramed DTX transducer to measure the MAP (mean arterial pressure) and HR. A PE-50 catheter connected to a Spectramed DTX transducer to access PP was inserted into the mesenteric vein. The PP was recorded on a Gould model RS 3400 recorder [27,28].

**RNA isolation and real-time PCR analysis**

After in situ collateral perfusion, the splenorenal shunt, the most prominent intra-abdominal portal-systemic collateral vessel, was dissected and stored in liquid nitrogen. Total RNA was extracted from the splenorenal shunt with the SV Total RNA Isolation System (Promega). Total RNA (1 μg) was reverse transcribed to cDNA with ImProm-II reverse transcriptase (Promega). Quantitative RT–PCR (reverse transcription–PCR) was carried out on a LightCycler 480 (Roche Diagnostics), and a standard LightCycler amplification cycle protocol was established.

A total volume of 18 μl of GoTaq qPCR Master Mix (Promega) was pipetted into LightCycler glass capillaries and 2 μl of the cDNA product (diluted 10-fold) was added as a PCR template. Amplification cycles began with a denaturation programme for 10 min at 95 °C. In the second segment, cDNA was amplified by 40 cycles of the following time profiles and temperature: 15 s at 95 °C (denaturation), 30 s at 58 °C for G<sub>αq</sub>, G<sub>α11</sub>, G<sub>αs</sub>, G<sub>αi</sub> and β-actin (annealing) and 10 s at 72 °C (elongation). The third segment consisted of a melting curve programme (0 s at 95 °C, 15 s at 57 °C and a linear temperature transition at 0.05 °C/s from 57 to 95 °C with continuous fluorescence acquisition). The final segment consisted of a cooling programme to 40 °C. The products were standardized with a housekeeping gene, β-actin, from the same RNA samples. Quantitative analysis was performed with LightCycler analysis software (Roche Diagnostics).

**Table 1** Primators of target and housekeeping genes used for real-time PCR analysis

<table>
<thead>
<tr>
<th>Names</th>
<th>Sense (forward)</th>
<th>Antisense (reverse)</th>
<th>GenBank accession number</th>
</tr>
</thead>
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<tr>
<td>G&lt;sub&gt;αq&lt;/sub&gt;</td>
<td>5′-GCAATGGGGCTAGCTAGG-3′</td>
<td>5′-GATAGGAAGGGTGTCACA-3′</td>
<td>NM031036</td>
</tr>
<tr>
<td>G&lt;sub&gt;α11&lt;/sub&gt;</td>
<td>5′-CGGTGGAACCTGGAAGATC-3′</td>
<td>5′-TGACAGGAGTGTGGAAAC-3′</td>
<td>NM031033</td>
</tr>
<tr>
<td>G&lt;sub&gt;αs&lt;/sub&gt;</td>
<td>5′-CTGTGGAACCTTGGACT-3′</td>
<td>5′-TGGAGTCAATGTTAGAGGC-3′</td>
<td>NM021845</td>
</tr>
<tr>
<td>G&lt;sub&gt;αi&lt;/sub&gt;</td>
<td>5′-ATACAGCAACACCATCCAGTC-3′</td>
<td>5′-AACGGGTTTCTGATGACC-3′</td>
<td>NM017327</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5′-GCGCTAGGGCAAGGGTG-3′</td>
<td>5′-GCTGGAAGGTGAGTTGCTCA-3′</td>
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</tr>
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</table>

Primer sequences of target and housekeeping genes are listed in Table 1.

**Experimental design**

**Acute effects of propranolol administration on portal-systemic collaterals**

On the 8th day after PVL, rats were anaesthetized with ketamine hydrochloride (100 mg/kg of BW). The BW, MAP, HR and PP were measured [28]. Portal-systemic collaterals of PVL rats were pre-incubated with Krebs solution (n = 11), propranolol (10 μmol/l, n = 11), propranolol (10 μmol/l) plus suramin (100 μmol/l, n = 7), a G<sub>α</sub> inhibitor) or suramin (100 μmol/l, n = 6). Cumulative concentration–response curves of collateral vessels were determined by graded final concentrations of AVP in escalation with a constant flow rate (20 ml/min). The final concentrations in perfusate were from 0.1 to 100 nmol/l of AVP in perfusate. Each new concentration was allowed to stabilize for 3 min before the next higher concentration was added. After the perfusion experiments, splenorenal shunt was isolated and dissected for real-time PCR analysis.

**Chronic effects of propranolol administration on portal-systemic collaterals**

Two groups of PVL rats received either propranolol (10 mg·kg<sup>−1</sup>·day<sup>−1</sup>, oral gavage, n = 8) or DW (distilled water) (n = 8) from 2 days prior to ligation until 7 days after the operation. On the 8th day after PVL, rats were anaesthetized with ketamine hydrochloride (100 mg/kg of BW) and the BW and baseline haemodynamics were measured [28]. Cumulative concentration–response curves of collateral vessels were determined. After the perfusion experiments, splenorenal shunt was isolated and dissected for real-time PCR analysis from rats with or without propranolol treatments.

**Drugs**

Propranolol, the reagents for preparing Krebs solution, and AVP were purchased from Sigma. Suramin was purchased from Tocris Bioscience (Tocris Cookson). All solutions were freshly prepared on the days of each experiment.
**RESULTS**

**Acute effects of propranolol or propranolol plus suramin on portal-systemic collaterals**

**Baseline haemodynamics**
The BW, MAP, HR and PP before pre-incubations were similar among the Krebs, propranolol, propranolol plus suramin and suramin groups ($P > 0.05$, Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krebs solution</td>
<td>11</td>
<td>316.4 ± 5.7</td>
<td>89.6 ± 4.0</td>
<td>311.3 ± 16.3</td>
<td>13.45 ± 0.8</td>
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<tr>
<td>Propranolol</td>
<td>11</td>
<td>326.6 ± 7.3</td>
<td>94.5 ± 4.2</td>
<td>320.5 ± 23.1</td>
<td>13.49 ± 0.7</td>
</tr>
<tr>
<td>Propranolol plus suramin</td>
<td>7</td>
<td>331.6 ± 6.8</td>
<td>95.1 ± 3.7</td>
<td>249.9 ± 23.7</td>
<td>15.09 ± 0.9</td>
</tr>
<tr>
<td>Suramin</td>
<td>6</td>
<td>321.5 ± 4.8</td>
<td>96.8 ± 2.0</td>
<td>346.0 ± 14.3</td>
<td>15.71 ± 0.7</td>
</tr>
<tr>
<td>Chronic treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>8</td>
<td>328.1 ± 6.2</td>
<td>102.1 ± 3.9</td>
<td>367.9 ± 20.4</td>
<td>14.97 ± 0.6</td>
</tr>
<tr>
<td>Propranolol</td>
<td>8</td>
<td>326.3 ± 4.3</td>
<td>89.0 ± 4.0*</td>
<td>332.6 ± 16.6</td>
<td>12.86 ± 0.5*</td>
</tr>
</tbody>
</table>

Table 2 Baseline haemodynamics of portal hypertensive rats with acute and chronic propranolol treatments

* $P < 0.05$ compared with the chronic DW group.

**Porto-systemic collateral responsiveness to AVP of portal hypertension rats**
Figure 1 shows the constrictive effects of AVP on portal-systemic collateral perfusion. Compared with Krebs group, propranolol (10 μmol/l) pre-incubation enhanced the perfusion pressure changes of the portal-systemic collaterals in response to AVP (10 μmol/l, 4.44 ± 0.41 compared with 6.00 ± 0.38, $P = 0.011$; 30 μmol/l, 5.22 ± 0.15 compared with 6.64 ± 0.28, $P = 0.003$; and 100 μmol/l, 3.56 ± 0.18 compared with 5.27 ± 0.38 mmHg, $P = 0.002$). However, pre-incubation with propranolol and suramin (100 μmol/l) alleviated the enhanced perfusion pressure changes in response to AVP elicited by propranolol (30 μmol/l, 6.64 ± 0.28 compared with 4.57 ± 0.53, $P = 0.000$; and 100 μmol/l, 5.27 ± 0.38 compared with 3.43 ± 0.48 mmHg, $P = 0.002$). Perfusion pressure changes of portal-systemic collaterals to AVP were similar among the groups pre-incubated with propranolol plus suramin, suramin or Krebs ($P > 0.05$).

**RNA expression of $G_{\alpha q}$, $G_{\alpha 11}$, $G_{\beta q}$ and $G_{\beta 11}$ (Figure 2)**
Compared with PVL rats with Krebs solution pre-incubation, splenorenal shunt mRNA expression of $G_{\alpha q}$ and $G_{\alpha 11}$ were significantly higher in the propranolol pre-incubation group ($G_{\alpha q}$/β-actin, 0.024 ± 0.003 compared with 0.013 ± 0.002, $P = 0.010$; $G_{\alpha 11}$/β-actin, 0.010 ± 0.001 compared with 0.005 ± 0.001, $P = 0.001$). $G_{\alpha 11}$ expression was attenuated in the propranolol plus suramin pre-incubation group compared with the propranolol group ($G_{\alpha 11}$/β-actin: 0.010 ± 0.001 compared with 0.007 ± 0.001, $P = 0.040$). No significant changes were found in $G_{\alpha q}$ and $G_{\beta 11}$ expression ($G_{\alpha q}$/β-actin, 1.010 ± 0.109 compared with 1.007 ± 0.251; $G_{\beta 11}$/β-actin, 1.006 ± 0.099 compared with 1.000 ± 0.049).

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**Data analysis**
All results are expressed as means ± S.E.M. The changes in perfusion pressure ($\Delta$mmHg) over the baseline were calculated for each concentration. Statistical analyses were performed using an unpaired Student’s $t$ test or one-way ANOVA as appropriate. Results were considered statistically significant at a two-tailed $P$ value of less than 0.05. Correlation of $G_{\alpha q}$ mRNA expression and the maximal perfusion pressure change were analysed by simple linear regression.
Propranolol and a \( \alpha \)-mediated pathway

Figure 2 Real-time PCR analysis of \( \alpha_q \), \( \alpha_{11} \), \( \alpha_s \) and \( \alpha_i \) mRNA expression in splenorenal shunts of PVL rats pre-incubated with Krebs, propranolol or propranolol plus suramin

\( \alpha_q \) and \( \alpha_{11} \) mRNA expression levels were significantly increased in propranolol group. In addition, \( \alpha_{11} \) mRNA expression in the propranolol plus suramin group was significantly lower than that in the propranolol group (\( P < 0.05 \)).

\( \alpha_s/\beta \)-actin, 0.016 ± 0.002 compared with 0.010 ± 0.001, \( P > 0.05 \).

Correlation between RNA expression of \( \alpha_q \) and \( \alpha_{11} \) and the maximal perfusion pressure change

Figure 3 depicts simple linear regression of \( \alpha_q \) and \( \alpha_{11} \) RNA expression and the maximal perfusion pressure change of the collateral vascular response to AVP in portal hypertensive rats. Linear correlation of \( \alpha_q \) and \( \alpha_{11} \) expression with the maximal perfusion pressure change is shown and the \( P \) values were 0.007 and 0.006 respectively. Furthermore, positive correlations between \( \alpha_q \) (\( R = 0.756 \)) and \( \alpha_{11} \) (\( R = 0.762 \)) RNA expression and the maximal perfusion pressure changes were noted. Expression of \( \alpha_s \) (\( P = 0.312, R = 0.336 \)) and \( \alpha_{11} \) (\( P = 0.331, R = 0.324 \)) was not correlated with the maximal perfusion pressure changes of the collateral vascular bed in response to AVP.

Chronic effects of propranolol on portal-systemic collaterals

Baseline haemodynamics

Table 2 shows the baseline haemodynamics of the two groups of PVL rats with long-term DW (as control) or propranolol treatments. There were no significant differences in BW and HR between DW and propranolol groups (\( P > 0.05 \)). However, compared with DW group, propranolol significantly decreased MAP (\( P = 0.035 \)) and PP (\( P = 0.019 \); Figure 4).

Portal-systemic collateral responsiveness to AVP of portal hypertension rats

Figure 5 reveals the concentration–response curves of AVP in PVL rats with or without propranolol treatment. Long-term administration of propranolol decreased portal-systemic collateral response to AVP at the concentrations of 10 nmol/l (7.63 ± 0.60 compared with 6.00 ± 0.46 mmHg, \( P = 0.049 \)) and 30 nmol/l (8.00 ± 0.53 compared with 5.75 ± 0.70 mmHg, \( P = 0.023 \)).

RNA expression of \( \alpha_q \), \( \alpha_{11} \), \( \alpha_s \) and \( \alpha_i \) (Figure 6)

In splenorenal shunt of PVL rats receiving propranolol for 9 days, the mRNA expression of \( \alpha_s \) protein was significantly higher than that of the DW-treated rats (\( \alpha_s/\beta \)-actin, 3.236 ± 0.477 compared with 2.032 ± 0.194, \( P = 0.022 \)). There were no significant changes in \( \alpha_q \), \( \alpha_{11} \) and \( \alpha_i \) mRNA expression (\( \alpha_q/\beta \)-actin, 0.179 ± 0.003 compared with 0.145 ± 0.001; \( \alpha_{11}/\beta \)-actin, 0.010 ± 0.002 compared with 0.006 ± 0.001; \( \alpha_i/\beta \)-actin, 0.115 ± 0.014 compared with 0.085 ± 0.024, \( P > 0.05 \)).

Correlation between the RNA expression of \( \alpha_s \) and the maximal perfusion pressure change

Figure 7 reveals the negative correlation between \( \alpha_s \) expression and maximal perfusion pressure changes to AVP in PVL rats (\( P = 0.007, R = -0.612 \)). Expression of \( \alpha_q \) (\( P = 0.184, R = -0.328 \)), \( \alpha_{11} \) (\( P = 0.155, R = -0.350 \)) and \( \alpha_i \) (\( P = 0.997, R = 0.000 \)) were not correlated with the maximal perfusion pressure changes of collateral vasculature.

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Figure 3  Linear regression analysis of $G_{\alpha q}$ and $G_{\alpha 11}$ mRNA expression in splenorenal shunts and the maximal perfusion pressure changes of collateral vascular response to AVP in PVL rats

The maximal perfusion pressure change to AVP was positively correlated with $G_{\alpha q}$ ($P = 0.007$, $R = 0.756$) and $G_{\alpha 11}$ ($P = 0.006$, $R = 0.762$) expression.

DISCUSSION

The results of the present study indicate that acute administration of propranolol in the perfusate significantly strengthened portal-systemic collateral vascular response to AVP in portal hypertensive rats. The result is compatible with the finding of a previous study that intravenous propranolol infusion induces an increase in hepatocollateral vascular resistance in portal hypertensive animals [29,30]. Propranolol also induces an increase in portal-systemic collateral venous tone in patients with portal hypertension [12]. Furthermore, the involvement of $\beta$-adrenoceptors in portal-systemic collaterals of portal hypertensive rats has been demonstrated by the observation that isoprenaline (isoproterenol)-induced collateral vascular relaxation could be reversed by propranolol [25].

The addition of suramin almost abolished the enhanced collateral AVP vascular response exerted by propranolol. This is reflected by the result that perfusion pressure changes of portal-systemic collaterals to AVP were similar between the groups pre-incubated with propranolol plus suramin or with vehicle. Previous studies have shown that AVP induces vasoconstriction by binding $V_{1a}$ vasopressin receptors in vascular smooth muscle cells [31] and rat mesenteric arteries [32]. $V_{1a}$ activation leads to phospholipase C production and Ca$^{2+}$ release [33,34]. ROK (Rho-kinase) signalling pathway activation is also involved in vasopressin-induced rat renal arterial vasoconstriction [35]. Regardless of how vasopressin affects Ca$^{2+}$ release and ROK activity, the coupling of vasopressin receptor to G-proteins, especially $G_{\alpha q}$ and $G_{\alpha 11}$, is the initial signal inducing vascular contraction [5]. However, the relevant survey in this distinct vascular bed has not been performed. The current finding that propranolol pre-incubation significantly enhances the mRNA expression of $G_{\alpha q}$ and $G_{\alpha 11}$ in splenorenal shunt vessel suggests that an enhanced collateral AVP responsiveness by propranolol is related to $G_{\alpha q}$ and $G_{\alpha 11}$ activation. Furthermore, this notion is supported by a positive correlation between the collateral maximal perfusion pressure changes and mRNA expression of $G_{\alpha q}$ and $G_{\alpha 11}$.

Pre-incubation with suramin alone, a non-selective $G_{\alpha}$ inhibitor, did not affect vascular responsiveness to AVP in the portal-systemic collateral vascular bed. A previous study in isolated perfused guinea-pig hearts demonstrated that suramin attenuated the perfusion pressure increases in response to AVP [36]. The different results may be due to the use of different species, experimental models and vascular beds. Besides, the maximal perfusion pressure change of perfused heart is much higher than that of portal-systemic collaterals. The low amplitude of perfusion pressure in the present study may limit the detection of suramin effect on portal-systemic collaterals. For further clarification, we performed real-time PCR analysis of splenorenal shunt G-protein expression in the propranolol plus suramin group. The results show that, compared with propranolol, incubation with propranolol plus suramin elicits a significantly lower $G_{\alpha 11}$ level. Taken together, the results allow us to conclude that, in this distinct vascular bed, suramin does not influence the AVP vasoresponse itself, but alleviates the effect of propranolol by $G_{\alpha 11}$ down-regulation.

It is worth noting that suramin has been identified as a G-protein inhibitor by interfering with GDP/GTP exchange and blocking receptor–G-protein coupling [37]. Suramin also non-competitively decreases GTPase activation and completely prevents GTP hydrolysis increased by opioid peptide in NG-108-15 cell membranes [38]. On the other hand, although experiments surveying the influences of suramin on G-protein expression have rarely been reported, an earlier study indicated that suramin incubation markedly reduced, by approx. 80%, DNA synthesis in human $G_{\alpha 12}$ transfectants [39]. The present results also show that suramin down-regulates changes of portal-systemic collaterals to AVP were similar between the groups pre-incubated with propranolol plus suramin or with vehicle. Previous studies have shown that AVP induces vasoconstriction by binding $V_{1a}$ vasopressin receptors in vascular smooth muscle cells [31] and rat mesenteric arteries [32]. $V_{1a}$ activation leads to phospholipase C production and Ca$^{2+}$ release [33,34]. ROK (Rho-kinase) signalling pathway activation is also involved in vasopressin-induced rat renal arterial vasoconstriction [35]. Regardless of how vasopressin affects Ca$^{2+}$ release and ROK activity, the coupling of vasopressin receptor to G-proteins, especially $G_{\alpha q}$ and $G_{\alpha 11}$, is the initial signal inducing vascular contraction [5]. However, the relevant survey in this distinct vascular bed has not been performed. The current finding that propranolol pre-incubation significantly enhances the mRNA expression of $G_{\alpha q}$ and $G_{\alpha 11}$ in splenorenal shunt vessel suggests that an enhanced collateral AVP responsiveness by propranolol is related to $G_{\alpha q}$ and $G_{\alpha 11}$ activation. Furthermore, this notion is supported by a positive correlation between the collateral maximal perfusion pressure changes and mRNA expression of $G_{\alpha q}$ and $G_{\alpha 11}$.

Pre-incubation with suramin alone, a non-selective $G_{\alpha}$ inhibitor, did not affect vascular responsiveness to AVP in the portal-systemic collateral vascular bed. A previous study in isolated perfused guinea-pig hearts demonstrated that suramin attenuated the perfusion pressure increases in response to AVP [36]. The different results may be due to the use of different species, experimental models and vascular beds. Besides, the maximal perfusion pressure change of perfused heart is much higher than that of portal-systemic collaterals. The low amplitude of perfusion pressure in the present study may limit the detection of suramin effect on portal-systemic collaterals. For further clarification, we performed real-time PCR analysis of splenorenal shunt G-protein expression in the propranolol plus suramin group. The results show that, compared with propranolol, incubation with propranolol plus suramin elicits a significantly lower $G_{\alpha 11}$ level. Taken together, the results allow us to conclude that, in this distinct vascular bed, suramin does not influence the AVP vasoresponse itself, but alleviates the effect of propranolol by $G_{\alpha 11}$ down-regulation.

It is worth noting that suramin has been identified as a G-protein inhibitor by interfering with GDP/GTP exchange and blocking receptor–G-protein coupling [37]. Suramin also non-competitively decreases GTPase activation and completely prevents GTP hydrolysis increased by opioid peptide in NG-108-15 cell membranes [38]. On the other hand, although experiments surveying the influences of suramin on G-protein expression have rarely been reported, an earlier study indicated that suramin incubation markedly reduced, by approx. 80%, DNA synthesis in human $G_{\alpha 12}$ transfectants [39]. The present results also show that suramin down-regulates
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Figure 4 Haemodynamic changes elicited by chronic propranolol treatment

Compared with DW-treated PVL rats, propranolol significantly reduced the MAP and PP ($P < 0.05$).

Figure 5 Concentration–response curves to AVP in the portal-systemic collateral vascular bed of PVL rats with chronic propranolol or DW treatment, expressed as absolute increase over the baseline value

Vascular responsiveness to AVP in the propranolol group was lower than that in the DW group ($* P < 0.05$).

$G_{\alpha_{11}}$ mRNA expression enhanced by propranolol. Although the underlying mechanism deserves further exploration, our data disclose the potential roles of $G$-proteins in modulating the collateral AVP vascular response influenced by propranolol.

In patients suffering from cirrhosis and portal hypertension, propranolol exerts the portal hypotensive effect via the reduction of splanchnic blood inflow and consequently the decrease of portal vein blood flow [23,40]. Consistently, our experiment with chronic propranolol treatment also significantly resulted in the reduction of MAP and PP. In contrast with the finding that acute administration of propranolol in the perfusate enhances the collateral vascular responsiveness to AVP, chronic propranolol administration attenuates the collateral AVP vascular response. This result is different from a similar study on superior mesenteric artery of portal hypertensive rats [41]. However, the discrepancy may have resulted from different vascular beds, dosage and the timing of propranolol administration. The divergent reactions in arterial and venous sites also suggest the complicated vascular pathophysiology in portal hypertension.

It may be of interest to find out whether desensitization of AVP receptors or antagonism of AVP signalling pathway develops as a result of chronic propranolol administration. However, desensitization of AVP receptors alone should shift the AVP response curve of the collateral vascular bed, but not the amelioration of AVP responsiveness found in the present study. Instead, the current result indicates that $G_{\alpha}$ are up-regulated by chronic propranolol treatment. It has been noted that AVP decreases isoprenaline-stimulated cAMP accumulation via its effector enzyme adenylate cyclase [42]. On the other hand, chronic propranolol treatment sensitizes adenylate cyclase [43], which increases cAMP production and induces vasodilatation. It seems that AVP decreases cAMP production and then induces vascular constrictive effects that are compromised by adenylate cAMP.
Figure 6  Real-time PCR analysis of $G_{\alpha q}, G_{\alpha 11}, G_{\alpha s}$ and $G_{\alpha i}$ mRNA expression in splenorenal shunts of PVL rats with chronic propranolol or DW treatment

$G_{\alpha s}$ mRNA expression level was increased in propranolol-treated PVL rats ($P < 0.05$).

Figure 7  Linear regression analysis of $G_{\alpha s}$ mRNA expression in splenorenal shunts and the maximal perfusion pressure changes of the collateral vascular bed to AVP in PVL rats

The maximal perfusion pressure change to AVP was negatively correlated with $G_{\alpha s}$ expression ($P = 0.007, R = -0.612$).

Since the AVP receptor $V_1a$ is G-protein-coupled [7], $G_{\alpha s}$ enhancement after chronic propranolol treatment may contribute to the hyporesponsiveness of portal-systemic collaterals to AVP. This is supported by the negative correlation between $G_{\alpha s}$ mRNA expression and the maximal collateral perfusion pressure changes to AVP in PVL rats. It is worth noting that dl-propranolol dilates aortic and mesenteric arterial smooth muscle via a mechanism involving activation of the NO/cGMP pathway and calcium influx blockade, which is independent of $\beta$-adrenoceptor blockade [47]. Chronic propranolol treatment also amplifies relaxation in response to acetylcholine of mesenteric artery and aortic rings in NO-deficient rats, which results from blockage of calcium entry in the vascular smooth muscle and elevation of NO bioavailability [48]. Although different mechanisms have been implicated, our novel findings suggest that changes in $G_{\alpha}$ families at least partly explain the different effects of acute and chronic propranolol administration on the portal-systemic collateral responsiveness to AVP.

In conclusion, the enhanced vascular responsiveness to AVP of propranolol pre-incubation is related to activation of $G_{\alpha q}$ and $G_{\alpha 11}$. However, chronic propranolol treatment attenuates the portal-systemic collateral vascular response to AVP, which is associated with $G_{\alpha s}$ overexpression. Propranolol seems to time dependently modulate AVP responsiveness in portal-systemic collateral vasculature in portal hypertensive rats. The $G_{\alpha}$ signalling pathway may be a potential therapeutic target in the control of PP and variceal bleeding in portal hypertension.
AUTHOR CONTRIBUTION

Fa-Yauh Lee and Hui-Chun Huang were the principal investigators responsible for this study and take full responsibility for the paper. Jing-Yi Lee performed most of the experiments described and contributed to the data acquisition, data analysis and writing of the paper. Teh-La Huo contributed to the data analysis and writing of the paper. Han-Chieh Lin contributed to revision of the paper. Chiao-Lin Chuang and Ching-Chih Chang contributed to the experimental design, data acquisition, data analysis and writing of the paper. Sun-Sang Wang and Shou-Dong Lee supervised this study and contributed to revision of the paper.

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