European Journal of Pharmacology 721 (2013) 1–4

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Effects of portal hypertension on contractility of rat spleen

Jun Liong Chin [a,b], P. Aiden McCormick [a], James R. Docherty [b,*]

[a] Liver Unit, St. Vincent's University Hospital, University College Dublin, Elm Park, Dublin 4, Ireland
[b] Department of Physiology, Royal College of Surgeons in Ireland, 123, St. Stephen's Green, Dublin 2, Ireland

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
11 September 2013
Accepted 22 September 2013
Available online 17 October 2013

Keywords:
Portal hypertension
Portal hypertensive rat
Splenic contraction
Noradrenaline

ABSTRACT

Portal hypertension induces changes in vascular responses to vasoconstrictors. However, the effects of portal hypertension on splenic contraction have not previously been investigated. In partial portal vein ligated (PVL) and sham-operated rats, we examined the splenic contractile responses to cumulative concentrations of noradrenaline and KCl. In PVL rats, the potency of noradrenaline in producing splenic contraction was significantly increased (pEC_{50} of 5.88 ± 0.08), as compared to sham (5.40 ± 0.06; $p < 0.001$). In the presence of prazosin (10^{-8} M), there was a significant rightward shift in the noradrenaline concentration response curve but the shift was greater for PVL, so that in the presence of prazosin there was no significant difference between PVL and sham animals in the potency of noradrenaline. Prazosin produced a significantly greater shift of noradrenaline potency in spleen from PVL (pK_B of 8.88 ± 0.06) ($n=6$) than from sham animals (8.51 ± 0.08, $n=6$), demonstrating that the α_1-adrenoceptor mediated component is greater in spleen from PVL. In the presence of prazosin (10^{-8} M) the residual response is non-α_1-adrenoceptor mediated, presumably α_2-adrenoceptor mediated, and this response did not differ between sham and PVL. The maximum splenic contraction did not significantly differ between sham and PVL rats for either agonist. In conclusion, noradrenaline potency in contracting the rat spleen was significantly increased in tissues from PVL rats. The increased potency of prazosin suggests a greater predominance of α_1-adrenoceptors in spleen of PVL rats, as prazosin has lower potency at α_2-adrenoceptors.

1. Introduction

The portal vein-ligated (PVL) rat model of pre-hepatic portal hypertension is widely used to study the effects of portal hypertension in humans because of consistent portal-systemic shunting (Cawley et al., 1995a; Groszmann et al., 1982; Sikuler et al., 1985). Increasing portal pressure leads to the development of these shunts and ultimately a hyperdynamic circulation occurs, characterised by increased cardiac output and splanchnic blood flow.

Hypersplenism is a major complication in patients with liver disease. Patients with hypersplenism typically have cirrhosis of the liver, an enlarged spleen and low circulating platelet counts (McCormick and Murphy, 2000). The low platelet counts increase the risk of bleeding, both spontaneous bleeding and bleeding after surgery, and severely limit drug therapy in these patients. In some species the spleen has a reservoir function for circulating blood cells, and can contract to expel these; in man the spleen may contract during exercise and hypoxia (Frances et al., 2008; Richardson et al., 2008), and so plays a role in sympathetic activation for fight or flight. The effects of portal hypertension on splenic contraction have not previously been investigated.

Previous studies have reported that a component of the increase in splanchnic blood flow in portal hypertension may be attributed to increased function of the vascular endothelium (Vallance and Moncada, 1991) or decreased mesenteric vascular responsiveness to vasoconstrictors (Kiel et al., 1985; Murray and Paller, 1985; Pizcueta et al., 1990; Villamediana et al., 1988). While most *in situ* studies of pressor responses reported reduced responsiveness to vasoconstrictors in portal hypertension, the presence of circulating vasodilators may have influenced the actions of vasoconstrictors. Results from *in vitro* studies appear to be conflicting. There was no change (Leehey, 1993) or a decrease (Castro et al., 1993) in responsiveness to angiotensin in isolated aorta from cirrhotic rats and decreased sensitivity but not potency of noradrenaline (Bomzon and Blendis, 1987) or an increase in responsiveness to noradrenaline (Cawley et al., 1995b) in aorta and mesenteric arteries from portal hypertensive rats. Consistent with the latter finding, Joh et al. (1993) found a diminished vasodilator response to α-adrenoceptor antagonists in portal hypertensive rats suggesting a diminished sympathetic drive that might result in upregulation of smooth muscle receptors.

In this study, we have examined the effects of portal hypertension on the responses of rat spleen to contractile agents. Isolated

* Corresponding author.
E-mail address: docherty@rcsi.ie (J.R. Docherty).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.09.063

rat spleens from portal hypertensive and sham-operated rats were examined for their responsiveness to noradrenaline and potassium chloride (KCl) *in vitro*, and the interaction with the α_1-adrenoceptor antagonist prazosin.

2. Methods

2.1. Surgical preparation for portal vein ligation

Male Wistrar rats (250–350 g) were anaesthetized with intraperitoneal pentobarbitone (60 mg/kg). A mid-line incision was made in the abdomen and the portal vein exposed. The bile duct and hepatic artery were separated from the portal vein. A 21-gauge needle (0.8 mm outer diameter) was placed alongside the portal vein, and a 3/0 braided silk sterile suture was tied around both needle and vein. The needle was then removed, resulting in a calibrated stenosis. For sham animals, the portal vein was exposed but was not manipulated to avoid possible damage. The abdomen was closed with the same suture material and a continuous suture technique. Animals were given vetergesic (buprenorphine hydrochloride 0.05 mg/kg, subcutaneously; Schering-Plough, Welwyn, U.K.) postoperatively. After partial portal vein ligation (PVL), rats were allowed to recover for a week for the development of portal hypertension.

2.2. Splenic contractions

One week later, both PVL and sham rats were killed by pentobarbitone, stunning and exsanguination to retrieve the spleen. Prehepatic portal hypertension was confirmed by the presence of dilated collateral vessels in the rat mesentery, particularly the existence of large splenorenal shunts. The spleens were bisected transversely into two portions, and attached to myograph transducers under 9.81 mN tension in organ baths with Krebs–Henseleit solution of the following composition: (mM): NaCl 119, $NaHCO_3$ 25, D-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03, ascorbic acid 0.28. The spleens were allowed to equilibrate for 30 min at 37 °C and gassed with 5% CO_2 in O_2. The spleens were initially contracted with noradrenaline (10^{-5} M). The bathing fluid was changed every 15 min for the next hour.

Cumulative concentrations of noradrenaline or potassium chloride were added to generate full concentration contractile response curves in spleens of both PVL and sham rats. Noradrenaline was administered cumulatively in 0.5 log unit increments beginning with a concentration of 10^{-8} M. KCl was added beginning with 10 mM in increments of 10 mM until maximal response was achieved. To further investigate the differences in splenic contractions between PVL and sham rats, splenic contractions with noradrenaline were measured after 1 h exposure to, and in the continuing presence of, the non-selective α_1-adrenoceptor antagonist prazosin (10^{-8} M).

Agonist potency was assessed as a pD_2 ($-\log EC_{50}$), and antagonist potency was assessed in terms of the ability of prazosin (10^{-8} M) to produce a significant shift in agonist potency. The agonist concentration ratio was obtained using mean agonist pD_2 values obtained by non-linear regression analysis, and where there was significant shift in agonist potency, antagonist potency was calculated. Antagonist potency was expressed as an apparent dissociation constant pK_B from the equation $pK_B = [B]/(DR-1)$, where [B] is the concentration of antagonist and DR is the agonist concentration-ratio produced by the antagonist as compared to the vehicle experiment.

2.3. Comparing spleen weights

Spleen wet weights were compared between PVL and sham rats.

2.4. Drugs

The following drugs were used: noradrenaline bitartrate (Sigma, Ireland); prazosin hydrochloride (Sigma). All drugs were dissolved in distilled water.

2.5. Statistical analysis

The results are expressed as mean ± standard error of the mean. Agonist potency at producing splenic contractions was expressed as a pEC_{50} ($-\log EC_{50}$) for noradrenaline, and as an EC_{50} for KCl. pEC_{50} values were obtained by non-linear regression using GraphPad Prism 5 for MacIntosh. Agonist and antagonist potencies between PVL and sham-operated rats were compared by analysis of Variance followed by the Bonferroni test (comparison between groups) and Dunnett's test (comparison with control), or Student's *t*-test, as appropriate. *P*-value of less than 0.05 was considered to be significant. Statistical and graphical analysis was carried out using GraphPad Instat and Prism for Macintosh.

3. Results

3.1. Splenic contractions

The potency of noradrenaline in producing splenic isometric contractions was compared between PVL and sham rats ($n=6$ in each group). In PVL rats, the potency of noradrenaline was increased, with a significantly higher pEC_{50} of 5.88 ± 0.08, as compared to sham operated rats (5.40 ± 0.06; $P < 0.001$) (Fig. 1). In the presence of the α_1-adrenoceptor antagonist prazosin at a concentration of 10^{-8} M, a rightward shift was noted in the noradrenaline concentration response curve for both PVL (prazosin $pK_B = 8.88 \pm 0.06$) and sham operated rats (prazosin $pK_B = 8.51 \pm 0.08$) ($n=6$ in each group). Prazosin was significantly more potent in PVL ($P < 0.01$, Student's *t*-test). In the

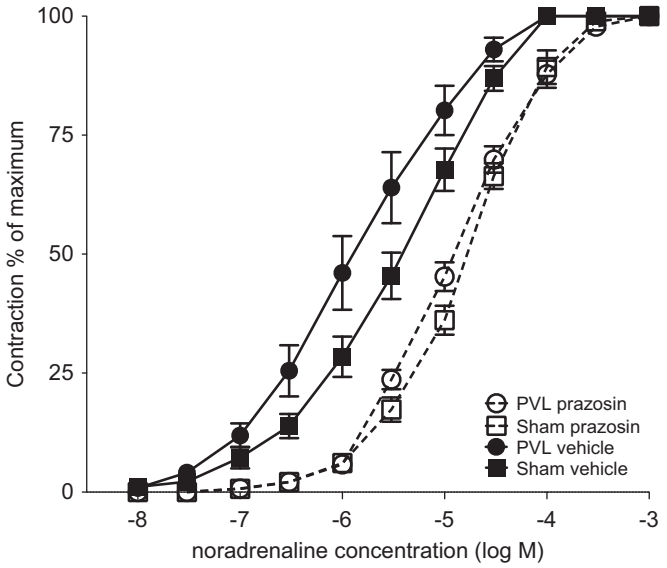

Fig. 1. Concentration response curves for isometric contractions obtained to noradrenaline, with or without prazosin, in spleen from portal vein ligated (PVL) (circles) or sham-operated (sham) (squares) rats. Responses are expressed as percentage of maximum response obtained to noradrenaline in absence (vehicle) (filled symbols), or presence of prazosin (10^{-8} M) (prazosin) (open symbols). Vertical bars represent s.e. of mean from at least 6 experiments.