Characteristics of Contractile Response of Isolated Portal Veins from Chronic Portal Hypertensive Rats under Altered Levels of External K^+ , Ca^{2+} , and Norepinephrine Concentrations: A Comparison with Normal Wistar Rats

Takahisa YOSHIMURA, Makoto ARITA, and Michio KOBAYASHI*

*Department of Physiology and * Department of Surgery, Faculty of Medicine, Medical College of Oita, Oita-gun, Oita, 879–56 Japan*

Abstract We studied contractile properties of portal veins isolated from chronic portal hypertensive rats (PHR) resulting from liver cirrhosis, a model obtained by repeated subcutaneous injections of CCl_4 (2 mg/kg) twice weekly for over 45 weeks. Portal venous pressure *in vivo* was significantly higher in PHR (167.0 ± 38.7 mmH₂O) than in the control normal Wistar rats (NWR) (102.0 ± 25.5 mmH₂O). A pair of portal veins from PHR and NWR were mounted longitudinally in an organ bath and perfused with Tyrode's solution with different K^+ , Ca^{2+} , and norepinephrine concentrations. The isometric tension was measured by a strain-gauge. Under control conditions, spontaneous phasic contractile force, corrected by cross-sectional area, was greater, and the frequency was lower in PHR than in NWR preparations. The averaged peak contractile force measured at different $[K]_o$ (5.4–86.4 mM) was also greater in PHR than in NWR. Force of the tonic contraction measured at different $[Ca]_o$ (0.45–5.4 mM), under conditions of 86.4 mM $[K]_o$ was significantly larger in PHR than in NWR preparations. However, the Ca^{2+} sensitivity of both preparations was the same. D-600 ($\geq 0.1 \mu M$) inhibited the tonic contraction in both preparations with an identical sensitivity to the drug. In the presence of norepinephrine (10 μM), the Ca^{2+} sensitivity of the tonic contraction increased both in PHR and NWR preparations. The increase was more pronounced in PHR and was completely reversed in the presence of the α_1 -adrenoceptor blocker, prazosin (0.1 μM). The α_1 -adrenoceptor sensitivity to norepinephrine was not altered in PHR preparations. The rate of Ca^{2+} release and uptake of intracellular Ca^{2+} seemed identical in both preparations. Thus, in the absence of norepinephrine, the phasic and tonic contractile forces of portal veins from PHR are larger than that of NWR, probably due to increased membrane Ca^{2+} permeability. The PHR preparations have a higher affinity for external Ca^{2+} in the presence of norepinephrine, an additional factor contributing

to elevation of portal blood pressure in the presence of chronic liver cirrhosis.

Key words: rat portal vein, contraction, portal hypertension, α_1 -adrenoceptor, Ca^{2+} sensitivity.

Portal hypertension associated with liver cirrhosis is a common disease occurring worldwide (CONN and GROSZMANN, 1982). The possible rupture of esophageal varices is hazardous. Portal venous pressure can be elevated either by increased vascular resistance or by increased blood flow in chronic liver cirrhosis (SIBAYAMA and NAKATA, 1981; OHNISHI *et al.*, 1986). At least two mechanisms can explain the increased vascular resistance. One is organic narrowing of the portal veins due to structural alterations of the venous wall itself and/or the compression by surrounding pathologic tissues. The other is functional change, e.g., altered contractility or altered responsiveness to vasoactive agents of the portal smooth muscles (SAITOH, 1983). In liver cirrhosis, structural changes in the small hepatic portal veins may be a main factor responsible for the increased blood pressure. However, functional alteration of portal smooth muscle cannot be ignored, because, in the portal vein, a considerable vasomotor control is evidenced with much less vasomotor control in hepatic arteries and hepatic veins (SAITOH, 1983).

In the isolated portal vein from spontaneously hypertensive rats, spontaneous contractions developed with a higher frequency and a greater active force as compared to findings in normotensive controls (PANG and SUTTER, 1980, 1981). Similar findings were obtained also in portal veins isolated from portal hypertensive rats, the portal vein of which had been partially occluded for a period of time to elevate the blood pressure (SIGURDSSON and UVELIUS, 1983). However, the models were the acute pre-hepatic type of the portal hypertension and obviously different from the chronic portal hypertension due to liver cirrhosis often seen clinically. Therefore, we investigated the characteristics of contractile response of isolated portal veins from rats with a chronic liver cirrhosis.

The liver was made cirrhotic by long-term injections of carbon tetrachloride (CCl_4). This CCl_4 -induced cirrhosis is similar to the liver cirrhosis in man (TAMAYO, 1983), with apparent increases in the portal venous pressure (SAITOH, 1983). We obtained evidence for the existence of abnormalities in membrane Ca^{2+} permeability in the portal veins from portal hypertensive rats. This seems to be the first report which describes the differences in contractile responses of isolated portal veins between normal and chronic portal hypertensive rats secondary to liver cirrhosis. Some of the results have been reported in abstract form (YOSHIMURA *et al.*, 1986).

MATERIALS AND METHODS

Portal hypertensive rat (PHR) model. About 8-week-old male Wistar rats (150–200 g) were given subcutaneous injections (2 ml/kg) of carbon tetrachloride (CCl_4) mixed with an equivolume of olive oil twice weekly for 45–50 weeks. All these rats were fed laboratory chow and allowed free access to tap water. Only animals treated for more than 45 weeks were used as PHR rats, since we confirmed that after more than 45 weeks of CCl_4 treatment, the liver cirrhosis was established, both macroscopically and microscopically (YOSHIMURA *et al.*, 1986). The body weight was measured by a spring balance before starting the experiment. After laparotomy of the animals anesthetized with secobarbital sodium (Yoshitomi Pharmaceutical Industries, 20 mg/kg, i.p.), we first measured the portal venous pressure using a low pressure transducer (MPU-0.5A; Nihon Kohden) connected to a polyethylene tube inserted into the superior mesenteric vein. The pressure was amplified by the pre-amplifier (AP621-G; Nihon Kohden) and recorded on a pen recorder (WT645-G; Nihon Kohden). After this measurement, the portal vein was excised for the *in vitro* study (see below). Thereafter the liver and spleen were removed and weighed.

The normal Wistar rats (NWR) were also fostered on the same chow and under the same conditions for identical periods (45–50 weeks) and were used as the controls. Table 1 compares weights of body, liver, and spleen, and portal venous pressure measured from 21 NWRs and 21 PHRs.

Portal vein preparation. The portal veins from PHR and NWR were removed carefully using fine scissors, placed in warm (37°C) oxygenated Tyrode's solution, dissected free of connective tissue, and cut 4.0–5.0 mm in length. Both veins were cut longitudinally for ready access of the perfusate to the luminal surface of portal vein. A pair of preparations from a NWR and a PHR were always mounted simultaneously in the same organ bath for isometric tension recording, using double force displacement transducers (TB621-T; Nihon Kohden). A passive force was always set at 1 g, according to the method used by HARRIS *et al.* (1983). The isometric force was amplified by pre-amplifier systems (AP621-G; Nihon Kohden) and displayed on a chart paper mounted in a pen-recorder (WT645-G; Nihon Kohden). On some occasions, the signals were tape-recorded (RMG5204; Nihon Kohden). The pair of preparations was perfused with Tyrode's solution bubbled with 97% O_2 and 3% CO_2 and the temperature was maintained at 37°C . A quantitative comparison of the developed force was conducted by dividing the amplitude of the contraction displayed on a pen-recorder by the cross-sectional area of the vein, which was later measured on a microscope photograph. In some experiments (Fig. 2), the magnitude of contraction was expressed as "integrated force" obtained by integrating the area intervening between the contraction trace and the resting level ($=1\text{ g}$) for a period of 3 min (Fig. 2A) divided by 3, with the resultant value expressed as the unit of $\text{mN}\cdot\text{min}$ (YOSHIMURA and ARITA, 1987). The medium frequency of spontaneous phasic contraction was obtained by analyzing the power density histogram. All

these calculations were made using a data processor (ATAC-450; Nihon Kohden).

Drugs and solutions. The composition of the Tyrode's solution (mM) was as follows: NaCl, 136.7; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; NaH₂PO₄, 0.42; NaHCO₃, 11.9; glucose, 11.1. Changes in K⁺ concentrations ([K]_o) were made by replacing NaCl with equimolar concentrations of KCl to make 10.8, 21.6, 43.2, and 86.4 mM [K]_o. The changes in Ca²⁺ concentrations ([Ca]_o) in high (86.4 mM) K⁺-Tyrode's solution were made by simply adding or removing appropriate amounts of CaCl₂, making 0, 0.45, 0.9, 1.8, 2.7, 3.6, and 5.4 mM [Ca]_o. The drugs used in the study include: D-600 (Sigma), norepinephrine (Sigma), and prazosin hydrochloride (Tokyo Kasei). They were added to either control or modified Tyrode's solutions, at appropriate concentrations.

Histology. After terminating the experiments on contractions, the portal vein preparations were fixed overnight in 10% formalin with phosphate buffered solution. The cross-sectional preparations were prepared and stained with hematoxylin-eosin. The cross-sectional area was measured by applying a digitizer (PC9801; Nihon Denki) on an enlarged microscopic photograph.

Statistics. Results are expressed as the mean $\pm$ standard error of the mean (S.E.M.). Student's *t*-test was used to evaluate the significance of difference between two values. A *p* value of 0.05 or less was regarded as being statistically significant.

RESULTS

Comparisons of various parameters

Table 1 shows a comparison of various parameters between normal Wistar rats (NWR) and portal hypertensive rats (PHR). The PHR did not differ from NWR in body, liver, and spleen weights. However, the portal venous pressure was significantly elevated in PHR (167.0 ± 38.7 mmH₂O) as compared to NWR (102.0 ± 25.5 mmH₂O). Histologic studies of portal veins from both muscle preparations showed that the longitudinal muscle layer is 3 to 4 times thicker than the circular muscle layer, consistent with the finding by BROWN *et al.* (1982). Cross-sectional area of the portal vein tended to be larger than that of NWR, albeit with no statistical significance.

Table 1. Comparisons of various parameters measured *in situ* and *in vitro* in normal Wistar rats (NWR) and chronic portal hypertensive rats (PHR).

Parameter	NWR (n = 21)	PHR (n = 21)	Difference
Body weight (g)	523.3 $\pm$ 60.2	440.0 $\pm$ 88.7	NS
Liver weight (g)	18.9 $\pm$ 1.87	26.7 $\pm$ 8.56	NS
Spleen weight (g)	1.15 $\pm$ 0.25	1.61 $\pm$ 0.5	NS
Portal venous pressure (mmH ₂ O)	102.0 $\pm$ 25.5	167.0 $\pm$ 38.7	<i>p</i> < 0.01
Cross-sectional area of portal veins (mm ²)	1.35 $\pm$ 0.28	1.43 $\pm$ 0.35	NS

All values are expressed as mean $\pm$ S.E.M.

Effect of altered external K^+ concentrations

The isolated portal veins from rats showed spontaneous contractions in modified Krebs' solution. The contractions have been reported to be triggered by a burst of calcium-dependent action potentials (JOHANSSON *et al.*, 1967; AXELSSON *et al.*, 1967). Since those spontaneous contractions seemed to occur by Ca^{2+} influx through so-called potential- (or voltage-) operated Ca^{2+} channels (CAUVIN *et al.*, 1983), we compared the contraction change of portal veins in NWR and PHR under conditions of increased $[K]_o$ which may continuously activate the potential-operated Ca^{2+} channels in portal veins (HARRIS *et al.*, 1983).

Fig. 1. A: typical records of spontaneous phasic contractions in a pair of isolated preparations of portal veins dissected from a normal Wistar rat (NWR, top trace) and a portal hypertensive rat (PHR, bottom trace) under various concentrations of extracellular K^+ ($[K]_o$). Zero level of tension scale (broken lines) corresponds to the resting tension of approximately 1 g applied to the muscle by a passive stretch. B: relationship between phasic contractile force corrected by the cross-sectional area (ordinate) and $[K]_o$ (abscissa) in NWR (open circles, $n=10$) and PHR (closed circles, $n=10$) preparations. Points and vertical bars denote mean $\pm$ S.E.M. * $p < 0.05$, significant difference from corresponding values in NWR.

After equilibration with the control Tyrode's solutions ($[K]_o = 5.4$ mM) for 60 min, phasic contractions occurred at a frequency of 4–5/min, with a slightly different amplitude. The amplitude of contractions in PHR preparations seemed greater than that of NWR (Fig. 1A). The phasic contractile force changed in a biphasic manner when $[K]_o$ was increased stepwise from 5.4 to 86.4 mM i.e., the contraction reached a maximum at 10.8 mM $[K]_o$ and then decreased toward 86.4 mM $[K]_o$. The phasic contraction disappeared with development of a sustained tension (tonic contraction). Figure 1B summarizes these data. The averaged amplitude of phasic contractile force at control $[K]_o$ (5.4 mM) was 42.5 ± 8.9 mN/mm² in NWR, and 78.0 ± 15.8 mN/mm² in PHR. The phasic contractions were greater at all $[K]_o$ tested although the difference at 43.2 mM $[K]_o$ was not significant. At 10.8 mM $[K]_o$, the averaged amplitude of phasic contractions became maximum, as predicted by Fig. 1A.

Portal vein pressure undulates periodically *in situ* and the frequency was reported to increase in the case of portal hypertension (NAKAGAWA, 1982). Therefore, we compared the frequency of contraction of isolated PHR preparations with that of NWR under normal as well as increased $[K]_o$ conditions, with the use of power density analysis. The frequency at which the maximum power was registered was increased as $[K]_o$ was increased from 5.4 to 43.2 mM in both NWR (from 5.6 ± 1.2 to 24.8 ± 5.6 /min) and PHR (from 4.8 ± 1.8 to 16.4 ± 5.2 /min). The frequency in PHR tended to be less at all $[K]_o$ tested, thus behaving opposite to that expected from *in situ* experiments.

We then measured the integrated force of preparations in NWR and PHR. Figure 2B compares the integrated force of the portal vein preparations from NWR with that from PHR in various $[K]_o$. Both integrated forces increased as $[K]_o$ was increased (5.4–43.2 mM), and the force from PHR was greater than that from NWR in all $[K]_o$ tested. However, when both forces were compared as a relative response of each maximum contraction (100%), there was no significant difference between these preparations. These findings suggest that the absolute contractile force developed per unit sectional area (1 mm²) of the preparation is greater in PHR, as compared to NWR, in a wide range of external K^+ concentrations, but that the sensitivity of the contraction to altered external K^+ concentration is approximately the same among PHR and NWR preparations.

Effect of altered external Ca^{2+} concentrations on K^+ depolarized preparations and the effect of Ca^{2+} antagonist

The increased contractile force of PHR so far demonstrated in Figs. 1B and 2B prompted us to investigate contraction responses to altered $[Ca]_o$. There is the possibility that increased membrane permeability for Ca^{2+} underlies this greater amplitude of contraction of portal veins derived from PHR.

Potential-operated Ca^{2+} channel of portal vein may be activated at $[K]_o = 50$ mM (SIGURDSSON and UVERIUS, 1983). Thus we used 86.4 mM K^+ -Tyrode's solution to activate the Ca^{2+} influx through the potential-dependent Ca^{2+} channels.

Fig. 2. A: a method for measurement of mean integrated mechanical force (mN·min). Total area of tension developed during consecutive 3 min (hatched) was divided by 3, to obtain the mean integrated force for 1 min. B: relationship between integrated force of portal vein preparations from NWR (open circles, $n=6$) and PHR (closed circles, $n=6$) and $[K]_o$. C: normalized percent response of integrated force, reproduced from panel B. In both PHR and NWR, the force recorded at 43.2 mM $[K]_o$ is taken as 100%. Points and vertical bars in panels B and C denote mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, significant difference from corresponding values in NWR.

In high K^+ -Tyrode's solution, the spontaneous phasic force disappeared (Fig. 3A). In the depolarized portal vein, contracture occurred and was dependent on $[Ca]_o$, the threshold concentration of which was close to 0.1 to 0.3 mM, a concentration reported by BIAMINO and JOHANSSON (1970).

In Fig. 3A, after equilibration with normal Tyrode's solution, portal vein preparations were perfused with 86.4 mM K^+ -Tyrode's solution containing nominally free Ca^{2+} for 30 min. The $[Ca]_o$ was then changed stepwise within the range from 0.45 to 5.4 mM (protocol illustrated at the bottom of Fig. 3A). Higher

Fig. 3. A: typical tonic contractile responses of portal veins from NWR (upper trace) and PHR (lower trace) as external Ca^{2+} concentrations ($[\text{Ca}]_o$) were increased, under conditions of high $[\text{K}]_o$ (86.4 mM). The experimental protocol appears at the bottom. B: averaged tonic force (mN/mm²) of portal veins from NWR (open circles, $n=6$) and PHR (closed circles, $n=6$) versus different $[\text{Ca}]_o$ in 86.4 mM $[\text{K}]_o$. C: normalized percent response reproduced from the tonic force in panel B. In both PHR and NWR, the force recorded at 5.4 mM $[\text{Ca}]_o$ is taken as 100%. Points and vertical bars in panels B and C denote mean $\pm$ S.E.M. * $p < 0.05$, a statistical difference from corresponding values in NWR.

concentrations of $[\text{Ca}]_o$ were not applied until the previous response had reached a quasi-steady state level (about after 20 min).

Thus obtained averaged responses of NWR and PHR preparations to altered $[\text{Ca}]_o$ are shown in Fig. 3B and C. The maximum tonic contractile force induced by increasing $[\text{Ca}]_o$ was significantly greater in PHR than in NWR at all but a lower range of $[\text{Ca}]_o$ (0 and 0.9 mM, Fig. 3B). However, when the force was normalized taking the maximum force at 5.4 mM $[\text{Ca}]_o$ as 100%, the 2 curves appeared to be identical ($\text{EC}_{50} = 1.8$ mM in both preparations), indicating no significant change with regard to the sensitivity of the tonic contractile force to $[\text{Ca}]_o$ between NWR and PHR (Fig. 3C).

To ensure that the positive inotropic effect induced by the increased $[\text{Ca}]_o$ is mediated by the increased Ca^{2+} influx through the potential-operated Ca^{2+} channels, we checked the effect of Ca^{2+} channel blocker, D-600 (1 μM), on the $[\text{Ca}]_o$ -induced positive inotropy (Fig. 4A). D-600 is suitable for this purpose, because this drug blocks the potential-operated Ca^{2+} channels from the outer surface of the

Fig. 4. A: effects of D-600 ($1 \mu\text{M}$) on the relationship between tonic contractile force and $[\text{Ca}]_o$ in portal veins from NWR (top panel, $n=8$) and PHR (bottom panel, $n=8$). The experiments were conducted in $86.4 \text{ mM } [\text{K}]_o$. B: summarized effects of different concentrations of D-600 (abscissa; scaled using $-\log[\text{D-600}]$) on tonic force of portal veins (ordinate; scaled as relative values to the control recorded without D-600) from PHR and NWR under conditions of $1.8 \text{ mM } [\text{Ca}]_o$ and $86.4 \text{ mM } [\text{K}]_o$. Points and vertical bars denote mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, significant difference from corresponding control values.

membrane, in vascular smooth muscle (OHYA *et al.*, 1987).

D-600 at a concentration of $1 \mu\text{M}$ shifted the force- $[\text{Ca}]_o$ relationship downward to the right in both NWR and PHR but the effect was much greater in PHR (Fig. 4A). In $3.6 \text{ mM } [\text{Ca}]_o$, D-600 inhibited the contractile force by $18.8 \pm 2.5\%$ in NWR while by $12.2 \pm 1.4\%$ in PHR, thereby suggesting that a component of potential-operated Ca^{2+} influx is much greater in PHR than in NWR. Figure 4B shows effects of different concentrations of D-600 on the tonic contractile force measured at $1.8 \text{ mM } [\text{Ca}]_o$ and $86.4 \text{ mM } [\text{K}]_o$. In both preparations, the depressant effect of D-600 was enhanced as the concentration was increased. Normalized relationships between the tonic force and D-600 concentrations were identical (Fig. 4B), thereby indicating that the sensitivity of the tonic force to D-600 is approximately the same among NWR and PHR. It seems that the total amount of Ca^{2+} -influx through the potential-operated Ca^{2+} channel is increased in PHR; however, we found no difference in the Ca^{2+} sensitivity of the tonic force between the portal veins obtained from NWR and PHR.

Fig. 5. A: typical tonic contractile response of portal veins from NWR (upper trace) and PHR (lower trace) to altered norepinephrine (NE) concentrations under conditions of 1.8 mM $[\text{Ca}]_o$ and 86.4 mM $[\text{K}]_o$. The protocol is shown at the bottom. B: averaged tonic force of portal veins from NWR (open circles, $n=8$) and PHR (closed circles, $n=8$) at different NE concentrations (scaled using $-\log[\text{NE}]$). C: normalized percent response reproduced from the tonic force in panel B. In both PHR and NWR, the force which develops at 10^{-4}M norepinephrine is taken as 100%. Points and vertical bars in panels B and C denote mean $\pm$ S.E.M. ** $p < 0.005$, significant difference from corresponding values in NWR.

Effect of norepinephrine in K^+ depolarized preparations

Recently two types of membrane Ca^{2+} channel were evidenced using patch clamp techniques: one is a potential-operated Ca^{2+} channel and the other a receptor-operated Ca^{2+} channel (BENHAM and TSIEN, 1987). While adrenergic, cholinergic, and histaminic receptors may be present on cell membrane, the portal veins are reported to be mostly innervated with sympathetic neurons and have exclusively α_1 -adrenergic receptors and no α_2 - or β -receptors (MILLS *et al.*, 1984). Norepinephrine (NE) increases contractile tension of portal veins in high K^+ media (JOHANSSON *et al.*, 1967). Therefore, we speculated that increased contractile responses hitherto observed in PHR preparation might be, at least in part, related to an increased activity of receptor-operated Ca^{2+} channels; therefore we compared

the effects of norepinephrine on the tonic contraction in PHR and NWR preparations. Figure 5 shows one such experiment conducted using 1.8 mM $[Ca]_o$ and 86.4 mM $[K]_o$. After equilibration with normal Tyrode's solution, high K^+ -Tyrode's solution was introduced (Fig. 5A). The pattern of contraction changed from phasic to tonic. After 30-min exposure to the high K^+ -Tyrode's solution, norepinephrine of increasing concentrations (1, 10, 100 μM) was applied, and concentration-dependent increases in the tonic force occurred. In Fig. 5B, the maximum-developed force per unit cross-sectional area was averaged in both preparations and plotted against each norepinephrine concentration. It is evident that the averaged tonic force of portal vein preparations in PHR is greater than that in NWR, at all the concentrations of norepinephrine tested. However, as shown in Fig. 5C, when the developed force was normalized taking the values at 100 μM norepinephrine as 100%, the 2 curves overlapped, thereby indicating no significant changes in the sensitivity to norepinephrine between NWR and PHR. The 50% effective concentration (EC_{50}) of norepinephrine is $0.71 \pm 0.05 \mu M$ in NWR and $0.65 \pm 0.06 \mu M$ in PHR, with no significant difference between these values. The result suggests that the Ca^{2+} influx via receptor-operated Ca^{2+} channel may be greater in PHR, but that the sensitivity to external norepinephrine concentration is much the same between PHR and NWR.

Effect of altered $[Ca]_o$ on norepinephrine-induced tonic force

Figure 6A shows the effect of altered $[Ca]_o$ in the presence of norepinephrine (10 μM) in both NWR and PHR preparations. Norepinephrine (10 μM) produced small contractions even in the absence of external Ca^{2+} ($[Ca]_o$ = nominally 0 mM), in both preparations. In all $[Ca]_o$ tested, the force was greater in the PHR. In Fig. 6B, the averaged contractile force obtained from similar experiments is plotted against different $[Ca]_o$. The force developed at moderate $[Ca]_o$ (0.9, 1.8, and 2.7 mM) was significantly larger in PHR than in NWR. In both preparations, the contractile force increased as $[Ca]_o$ was increased, with the augmentation saturated at 2.7 mM $[Ca]_o$ in the case of PHR, while at 5.4 mM $[Ca]_o$ in the case of NWR. The finding indicates that, in the presence of norepinephrine, Ca^{2+} sensitivity of the contractile force is not identical in PHR and NWR preparations; the PHR has a greater sensitivity. The finding sharply contrasts to that observed in the absence of norepinephrine (Fig. 3C), i.e., no difference in the Ca^{2+} sensitivity between PHR and NWR.

Effect of prazosin on norepinephrine-induced, $[Ca]_o$ -dependent contractions

The positive inotropic effect of norepinephrine demonstrated in Fig. 5 could be due to an increased Ca^{2+} influx via receptor-operated Ca^{2+} channels or due to direct increases in intracellular Ca^{2+} concentrations via pharmacomechanical coupling (TIMMERMANS *et al.*, 1987). All these effects may be mediated by the stimulation of α_1 -receptor (MILLS *et al.*, 1984). Therefore, in Fig. 7A, we studied the effect of α_1 -antagonist (prazosin) on the $[Ca]_o$ -dependent tonic force in the presence

Fig. 6. A: typical tonic contractile response of portal veins from NWR (upper trace) and PHR (lower trace) to stepwise increases in $[Ca]_o$ in the presence ($10 \mu M$) of norepinephrine (NE) and 86.4 mM $[K]_o$. The experimental protocol is shown at the bottom. B: summarized relationship between tonic contractile force and $[Ca]_o$ in the presence of $10 \mu M$ NE and 86.4 mM $[K]_o$, in portal veins from PHR (closed circles, $n=6$) and NWR (open circles, $n=6$). Data points to the left of the vertical broken line indicate the values recorded without NE under conditions of nominally free $[Ca]_o$ and 86.4 mM $[K]_o$. Points and vertical bars denote mean $\pm$ S.E.M. * $p < 0.05$, significant difference from corresponding values in NWR.

of norepinephrine, in both NWR and PHR preparations. Prazosin ($0.1 \mu M$) significantly depressed the tonic force at all levels of $[Ca]_o$. Figure 7B compares the relative force developed in various $[Ca]_o$, in which the 4 curves in panel A are reproduced by adjusting the force at the highest $[Ca]_o$ ($= 5.4 \text{ mM}$) as 100%. It is noteworthy that, in the absence of prazosin, the curves of PHR (●) and NWR (○) apparently differ with a half maximal percent force (EC_{50}) at about 0.6 mM $[Ca]_o$ in the case of PHR versus 1.0 mM $[Ca]_o$ in the case of NWR. In the presence of prazosin (α_1 -receptor blocked), however, the curves of PHR (▲) and NWR (△) were

Fig. 7. A: effects of prazosin ($0.1 \mu\text{M}$) on the relationship between tonic contractile force and $[\text{Ca}]_o$ in portal veins from NWR (top, $n=8$) and PHR (bottom, $n=8$). Experiments conducted in the presence of $10 \mu\text{M}$ NE and 86.4 mM $[\text{K}]_o$. Points and vertical bars denote mean $\pm$ S.E.M. * $p < 0.05$, significant difference from corresponding control values. B: normalized response reproduced from panel A. The force developed at 5.4 mM $[\text{Ca}]_o$ was regarded as 100%, in all relationships. In both A and B, solid symbols refer to PHR; open symbols, NWR, while circles and triangles refer to absence and presence of prazosin, respectively (see inset to the right).

practically the same with their EC_{50} at about 1.8 mM $[\text{Ca}]_o$, a finding which suggests an abolition of the difference in Ca^{2+} sensitivity.

In Fig. 8, the prazosin-sensitive component of the total tonic force is plotted against various $[\text{Ca}]_o$. The component was obtained from Fig. 7A by subtracting the tonic force in the presence of prazosin from that in the absence of prazosin, for both NWR and PHR. Prazosin-sensitive components of PHR preparations significantly exceeded those of NWR at $[\text{Ca}]_o$ ranging from 0.9 to 2.7 mM , that is close to physiologic extracellular Ca^{2+} concentrations. This finding explains a much greater increase of tonic force in the PHR preparation produced by an application of norepinephrine, at physiologic $[\text{Ca}]_o$ ($= 1.8 \text{ mM}$) (Fig. 5A and B).

Contractions related to direct Ca^{2+} release

The increased contractile force so far observed in PHR preparations could be due to some changes in intracellular Ca^{2+} traffic. For instance, either an increased capacity of Ca^{2+} stores or an increased ability of Ca^{2+} uptake or release may result in increased phasic or tonic contractions, as evidenced in the PHR preparation.

Fig. 8. $[Ca]_0$ -dependence of the prazosin-sensitive component of tonic force of portal veins from PHR (broken line) and NWR (solid line). Prazosin-sensitive force (ordinate) is expressed as the difference between a pair of 2 curves, i.e., in the absence (control) and presence of prazosin ($10 \mu M$) in Fig. 7A. Points and vertical bars denote mean $\pm$ S.E.M. * $p < 0.05$, significant difference from corresponding values in NWR.

Thus, we explored possible differences in the function of Ca^{2+} stores between NWR and PHR preparations. Portal veins hold much of Ca^{2+} at the intracellular Ca^{2+} store or sarcoplasmic reticulum (SR) (BOND *et al.*, 1984). Norepinephrine is considered to release the stored Ca^{2+} from the SR via pharmacomechanical coupling (LEIJTEN *et al.*, 1985; TIMMERMANS *et al.*, 1987).

Figure 9A shows the experimental protocol we used to study the effect of norepinephrine ($100 \mu M$) in Ca^{2+} -free (with 2 mM EGTA), high (86.4 mM) K^+ -Tyrode's solution. Both preparations from NWR and PHR were equilibrated in this solution for 20 min after switching from the normal Tyrode's solution. The preparations were then repeatedly exposed to Ca^{2+} -free, high K^+ -Tyrode's solution containing norepinephrine ($100 \mu M$) for 2 min, once every 10 min (arrows in Fig. 9A). On application of norepinephrine, a tonic contraction of a small amplitude developed in both NWR and PHR preparations. However, the amplitude of the contraction decreased with repetition of norepinephrine application in both preparations. This norepinephrine-induced tonic force was considered as an indicator of the amount of Ca^{2+} released from the SR (BOND *et al.*, 1984). The released Ca^{2+} might have been partially re-uptaken by the SR during the 8-min period of norepinephrine-free perfusion and contribute to the subsequent release upon stimulation by norepinephrine.

Figure 9B compares the summarized results obtained from similar experiments in NWR and PHR. There seemed to be no difference between the amplitude of contraction at any time of repeated norepinephrine application.

Fig. 9. A: typical contractile response of portal veins from NWR (upper trace) and PHR (lower trace) to repeated application of 100 μ M norepinephrine (NE) under conditions of 86.4 mM $[K]_o$ and nil $[Ca]_o$ plus 2 mM EGTA. Arrows indicate the onset of a 2-min period of NE application, at 10-min intervals. B: the averaged NE-induced contractile force in portal veins from PHR (closed circles) and NWR (open circles), summarized from similar a experiment ($n=4$) as shown in panel A. Points and vertical bars denote mean $\pm$ S.E.M. C: time course of decay in NE-induced contractile response during 6 repeated applications of NE. Note that the slopes of 2 lines are approximately identical, having $t_{0.5} = 21.5$ (PHR) and 22.2 min (NWR).

In Fig. 9C, a typical time course of decay in the norepinephrine-induced contraction is plotted on a semilogarithmic scale. The time required for the contractile force to reach the half maximum force ($t_{0.5}$) was 21.5 min in the case of NWR, and 22.2 min in the case of PHR, thereby indicating no difference in the half decay time between NWR and PHR. This finding suggests that kinetics of Ca^{2+} release or uptake may not be altered between the PHR and NWR preparations.

DISCUSSION

There is a "diversity" in the regulatory mechanism of the contraction-

relaxation cycle in smooth muscle (KURIYAMA, 1981; WEISS, 1981; YABU, 1986). Calcium has various and complex actions on the physiological processes of smooth muscle contractions, and the calcium ion is considered to be delivered to the contractile protein via the following mechanisms: 1) opening of potential-operated Ca^{2+} channel, 2) opening of receptor-operated Ca^{2+} channel, 3) opening of "leak" Ca^{2+} channel, 4) Na^+ - Ca^{2+} exchange, and 5) direct release of Ca^{2+} from intracellular Ca^{2+} store via stimulation of membrane receptor (KURIYAMA, 1981; WEISS, 1981; BENHAM and TSIEN, 1987).

The present study was undertaken to elucidate differences in regulatory mechanisms of contraction in portal veins obtained from normal and chronic portal hypertensive rats, and to obtain clues to the mechanism of the development of portal hypertension. We found differences in *in vitro* responses of portal veins from PHR, as compared to those of NWR, which include: 1) a decreased frequency and increased magnitude of spontaneous phasic contractions in PHR, under normal and high K^+ media (Fig. 1); 2) the integrated force is also greater in PHR than that of NWR, at all levels of $[\text{K}]_o$ (Fig. 2); 3) a larger tonic contractile force in PHR for most levels (1.8–5.4 mM) of $[\text{Ca}]_o$ (Fig. 3); 4) much greater development of tonic force in PHR in response to norepinephrine (Fig. 5); 5) much greater prazosin-sensitive component of the tonic force in PHR (Figs. 6–8). Thus, portal vein preparations from PHR developed greater phasic and tonic contractions at various combinations of $[\text{K}]_o$ and $[\text{Ca}]_o$.

The increased phasic and tonic contractile force of PHR in the absence of norepinephrine can be explained by assuming that the Ca^{2+} permeability of portal veins is increased in PHR, as compared to NWR. Altered function of intracellular Ca^{2+} pools or increased Ca^{2+} sensitivity of contractile protein may be another cause of the increased contractility in PHR. However, altered SR function seemed less likely since there was no difference in the release and uptake kinetics of SR between PHR and NWR preparations (Fig. 9). Increased Ca^{2+} sensitivity of contractile protein in portal veins of PHR is equivocal. There was no difference in the calcium requirement for activation of skinned vascular (rat tail artery) smooth muscles obtained from spontaneously hypertensive rats (MRWA *et al.*, 1986), while the Ca^{2+} reactivity of portal veins from spontaneously hypertensive rats was increased, as compared to that of NWR (HARRIS *et al.*, 1983). Therefore, we tentatively attribute the enhanced contractile response of PHR observed in the absence of norepinephrine mostly to an increased Ca influx, probably via potential-operated Ca^{2+} channels.

Although the tonic contraction measured under high K^+ condition was greater in PHR than NWR at all $[\text{Ca}]_o$ tested (0–5.4 mM), the sensitivity of this tonic force to $[\text{Ca}]_o$ was identical between PHR and NWR ($\text{EC}_{50} = 2.2$ mM, in both preparations, Fig. 3C). In the presence of norepinephrine (10 μM), the Ca^{2+} sensitivity seemed to be increased both in PHR and NWR because the % force- $[\text{Ca}]_o$ relationship of each preparation was shifted to higher $[\text{Ca}]_o$ after application of prazosin, a specific α_1 -receptor blocker (Fig. 7B). However, this increase in sensitivity was much greater in

PHR ($EC_{50} = 0.6$ mM) than in NWR ($EC_{50} = 1.0$ mM) and the effects were completely reversed in the presence of prazosin, an α_1 -blocker ($EC_{50} = 1.8$ mM in both preparations, Fig. 7B). This EC_{50} value was close to the EC_{50} ($= 2.2$ mM) measured under control conditions (in the absence of norepinephrine and prazosin) (cf. Fig. 3C).

Thus, between PHR and NWR, there seem to be significant differences in the kinetics of α_1 -receptor-activated Ca^{2+} channels. The difference seemed especially important because the α_1 -activated component of the tonic contraction in PHR becomes most prominent under physiologic extracellular concentrations of Ca^{2+} ($= 1.8$ mM, Fig. 8).

It has been demonstrated that K^+ -dependent contraction of smooth muscle depends almost entirely upon the extracellular Ca^{2+} ; however, during norepinephrine stimulation, both extracellular and intracellular sources of Ca^{2+} are utilized (CASTEELS and DROOGMANS, 1984; SAIDA and VAN BREEMEN, 1984). Therefore, our results, which showed increased Ca^{2+} sensitivity of PHR preparations during norepinephrine stimulation, could be due to any of the following alterations: 1) increased influx of Ca^{2+} through receptor-operated Ca^{2+} channels (as discussed above); and 2) increased release of Ca^{2+} from intracellular Ca^{2+} stores, as a result of increased inositol phospholipids turnover.

The increased Ca^{2+} sensitivity in PHR as compared to NWR was not due to increased norepinephrine sensitivity of the α_1 -receptor, because the α_1 -receptor sensitivity of PHR, measured as an amplitude change of tonic tension, was the same as that of NWR (Fig. 5C). However, in the portal veins dissected from the acute portal hypertension rats (veins which had been partially ligated for a certain period of time), the sensitivity of the contractile force to norepinephrine was decreased (KIEL *et al.*, 1985) inconsistent with the present finding in chronic portal hypertensive rats.

On the other hand, an identical finding, e.g., increased Ca^{2+} sensitivity, without changes in α_1 -receptor sensitivity has been noted in rings of the caudal artery of spontaneously hypertensive rats (AQUEL *et al.*, 1986). It is interesting that the contractile response of the systemic artery in spontaneously hypertensive rats shares similar responses with the portal veins of chronic portal hypertensive rats, thereby indicating similar local mechanisms underlying the development of respective hypertension.

Finally, the increased phasic and tonic contraction found in PHR portal veins could not be attributed to direct vascular effects of CCl_4 repeatedly injected to the animals for production of liver cirrhosis, because direct application of CCl_4 (dissolved in phospholipid micelles) to isolated portal veins of NWR did not enhance the contractile force but rather suppressed it (YOSHIMURA and ARITA, 1987).

Whether or not the increased phasic and tonic contractions of PHR portal veins are the cause of chronic portal hypertension or a mere result, i.e., an adaptation to the increased blood pressure within the portal veins, remains equivocal.

In clinical situations, esophageal varices secondary to portal hypertension are prone to rupture after drinking alcohol, probably due to transient increases in portal venous pressure (SIBAYAMA *et al.*, 1982). Alcohol and its metabolite acetaldehyde are known to release norepinephrine from sympathetic nerve endings, thereby increasing norepinephrine concentrations of the blood (SIBAYAMA *et al.*, 1982). The elevated levels of blood norepinephrine may increase the blood pressure of portal veins by stimulating the α_1 -adrenoceptor, since stimulation of the α_1 -adrenoceptor increases Ca^{2+} influx via activation of receptor-operated Ca^{2+} channels and/or accelerating inositol phospholipids turnover. In portal hypertensive patients, this increase in blood pressure may be far greater than in healthy persons, because Ca^{2+} sensitivity of the portal vein of the former is increased, resulting in a rupture of varices. Indeed, the α -adrenergic receptor blocker, phenoxybenzamine, decreased portal venous pressure in portal hypertensive patients (IKEDA, 1979).

Vasopressin and propranolol have been used to alleviate portal hypertension (LEBREC *et al.*, 1980; OKUMURA *et al.*, 1984; MILLS *et al.*, 1984). However, our results suggest that a Ca^{2+} -antagonist may be another choice of treatment, and that an α_1 -adrenoceptor antagonist such as prazosin would be more useful in preventing norepinephrine-related increases in portal blood pressure, provided that a component of tonic contraction (as shown here in rat portal veins) plays a role in elevating portal blood pressure of patients with liver cirrhosis.

We are grateful to Dr. T. Kiyosue for kind help with measurement of frequency and integrated force of phasic contractions of portal veins, Misses K. Takahashi and K. Goto for secretarial and technical services, and to M. Ohara for comments.

REFERENCES

- AQUEL, M. B., SHARMA, R. V., and BHALLA, C. (1986) Increased Ca^{2+} sensitivity of α_1 -adrenoceptor-stimulated contraction in SHR caudal artery. *Am. J. Physiol.*, **250**: C275-C282.
- AXELSSON, J., WAHLSTROM, B., JOHANSSON, B., and JONSSON, O. (1967) Influence of the ionic environment on spontaneous electrical and mechanical activity of rat portal vein. *Circ. Res.*, **21**: 609-619.
- BENHAM, C. D. and TSIEN, R. W. (1987) Calcium-permeable channels in vascular smooth muscle: Voltage-activated, receptor-operated, and leak channels. *In*: Progress in Cell Calcium and the Control of Membrane Transport, ed. by MANDEL, L. J. and EATON, D. C., Rockefeller University Press, New York, pp. 45-64.
- BIAMINO, G. and JOHANSSON, B. (1970) Effects of Ca^{++} and Na^+ on contractile tension in smooth muscle of the rat portal vein. *Pflügers Arch.*, **321**: 143-158.
- BOND, M., KITAZAWA, T., SOMLYO, A. V., and SOMLYO, A. P. (1984) Release and recycling of calcium by the sarcoplasmic reticulum in guinea pig portal vein smooth muscle. *J. Physiol. (Lond.)*, **355**: 677-695.
- BROWN, B. P., ANURAS, S., and HEISTED, D. D. (1982) Responsiveness of longitudinal and circular muscle layers of the portal vein. *Am. J. Physiol.*, **242**: G498-G503.

- CASTEELS, R. and DROOGMANS, G. (1984) Cell membrane responsiveness and excitation-contraction coupling in smooth muscle. *J. Cardiovasc. Pharmacol.*, **6** (Suppl. 2): S304-S312.
- CAUVIN, C., LOUTZENHISER, R., and VAN BREEMEN, C. (1983) Mechanism of calcium antagonist-induced vasodilation. *Annu. Rev. Pharmacol. Toxicol.*, **23**: 373-396.
- CONN, H. O. and GROSZMANN, R. J. (1982) The pathophysiology of portal hypertension. *In: The Liver: Biology and Pathobiology*, ed. by ARIAS, I., POPPER, H., SCHACHTER, D., and SHAFRITZ, D. A., Raven Press, New York, pp. 821-848.
- HARRIS, A. L., SWAMY, V. C., and TRIGGLE, D. J. (1983) Calcium reactivity and antagonism in portal veins from spontaneously hypertensive and normotensive rats. *Can. J. Physiol. Pharmacol.*, **62**: 146-150.
- IKEDA, M. (1979) Effect of phenoxybenzamine (POB) on portal venous pressure in patients with portal hypertension. *Am. J. Gastroenterol.*, **71**: 389-394.
- JOHANSSON, B., JOHSSON, O., and AXELSSON, J. (1967) Electrical and mechanical characteristics of vascular smooth muscle response to norepinephrine and isoproterenol. *Circ. Res.*, **21**: 619-633.
- KIEL, J. W., PITTS, V., BENOIT, J. N., GRENGER, D. N., and SHEPHERD, A. P. (1985) Reduced vascular sensitivity to norepinephrine in portal hypertensive rats. *Am. J. Physiol.*, **248**: G193-G195.
- KURIYAMA, H. (1981) Excitation-contraction coupling in various visceral smooth muscles. *In: Smooth Muscle*, ed. by BULBRING, A. F., JONES, A. W., and TOMITA, T., Arnold, London, pp. 171-197.
- LEBREC, D., NOUEL, O., and CORBIC, M. (1980) Propranolol-A medical treatment for portal hypertension? *Lancet*, **2**: 180-182.
- LEIJTEN, P., SAIDA, K., and VAN BREEMEN, C. (1985) Norepinephrine-induced intracellular Ca^{2+} release from vascular smooth muscle. *J. Cardiovasc. Pharmacol.*, **7** (Suppl. 6): S38-S42.
- MILLS, P. R., RAE, A. P., FARAH, D. A., RUSSEL, R. I., LORIMER, A. R., and CARTER, D. C. (1984) Comparison of three adrenoceptor blocking agents in patients with cirrhosis and portal hypertension. *Gut*, **25**: 73-78.
- MRWA, U., GUTH, K., HAIST, C., TROSCKA, M., HERRMANN, R., WOJCIECHOWSKI, R., and GAGELMANN, M. (1986) Calcium requirement for activation of skinned vascular smooth muscle from spontaneously hypertensive (SHRSP) and normotensive control rats. *Life Sci.*, **38**: 191-196.
- NAKAGAWA, H. (1982) Effects of physiological vasoactive substances and gastrointestinal hormone on spontaneous mechanical activity in the canine portal vein. *Shinshu Med. J.*, **30**: 634-649.
- OHNISHI, K., SAITOH, M., TERABAYASHI, H., CHIN, N., and OKUDA, K. (1986) Portal hemodynamics in cirrhosis. *Acta Hepatol. Jpn.*, **26**: 915-923.
- OHYA, Y., TERADA, K., and KURIYAMA, H. (1987) D-600 blocks the Ca^{2+} channel from the outer surface of smooth muscle cell membrane of the rabbit intestine and portal vein. *Pflügers Arch.*, **408**: 80-82.
- OKUMURA, H., SEKIYAMA, T., KATSUTA, Y., ARAMAKI, T., SATOMURA, K., AKAIKE, M., TERADA, H., and SEKINO, M. (1984) Effect of propranolol on portal venous pressure in patients with chronic liver disease: Comparison with vasopression. *Acta Hepatol. Jpn.*, **22**: 1534-1540.
- PANG, C. C. Y. and SUTTER, M. C. (1980) Hydralazine prevents changes in the contractile response of aortic but not portal vein strips in hypertensive rats. *Blood Vessels*, **17**: 293-301.

- PANG, C. C. Y. and SUTTER, M. C. (1981) Differential effects of D-600 on contractile responses of aorta and portal vein from SHR. *Blood Vessels*, **18**: 120–127.
- SAIDA, K. and VAN BREEMEN, C. (1984) Characteristics of the norepinephrine-sensitive Ca^{2+} store in vascular smooth muscle. *Blood Vessels*, **21**: 43–52.
- SAITOH, M. (1983) Vasomotor responses of the normal and cirrhotic livers to hepatic nerve stimulation. *Acta Hepatol. Jpn.*, **24**: 740–751.
- SIBAYAMA, Y. and NAKATA, K. (1981) Significance of connective tissue proliferation on portal hypertension in liver cirrhosis. *Acta Hepatol. Jpn.*, **22**: 690–700.
- SIBAYAMA, Y., SAITOH, M., SAKAGUCHI, Y., HASHIMOTO, K., and NAKATA, K. (1982) Upper gastrointestinal bleeding in liver cirrhosis and alcohol ingestion. *Acta Hepatol. Jpn.*, **23**: 1158–1165.
- SIGURDSSON, S. B. and UVELIUS, B. (1983) Membrane potential in smooth muscle cells from hypertrophic rat portal vein. *Experientia*, **39**: 1288–1290.
- TAMAYO, R. P. (1983) Is cirrhosis of the liver experimentally produced by CCl_4 an adequate model of human cirrhosis? *Hepatology*, **3**: 112.
- TIMMERMANS, P. B. M. W. M., CHIU, A. T., and THOOLEN, M. J. M. C. (1987) Calcium handling in vasoconstriction to stimulation of alpha-1 and alpha-2 adrenoceptors. *Can. J. Physiol. Pharmacol.*, **65**: 1649–1657.
- WEISS, G. B. (1981) Calcium and excitation-contraction coupling in vascular smooth muscles. *Can. J. Physiol. Pharmacol.*, **60**: 483–488.
- YABU, H. (1986) Regulatory mechanism of contractile-relaxation cycle in smooth muscle cells. *J. Physiol. Soc. Jpn.*, **48**: 453–465 (in Japanese).
- YOSHIMURA, T. and ARITA, M. (1987) Carbon tetrachloride depresses the contractile tension of portal veins isolated from rats. *J. Clin. Exp. Med. (Igakunoayumi)*, **143**: 909–910 (in Japanese, abstract in English).
- YOSHIMURA, T., KIYOSUE, T., and ARITA, M. (1986) Contractile response of portal veins from chronic portal hypertensive rats to external potassium and calcium concentrations. *J. Physiol. Soc. Jpn. (Suppl.)*, **48**: 316.