Membrane potential in smooth muscle cells from hypertrophic rat portal vein[1]

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Summary. Portal hypertension was induced in rats by partial ligation of the hepatic branches of the portal vein. After 5 days the vein was removed and mounted in vitro. In contrast to control (C) veins, hypertensive (H) vessels were almost devoid of spontaneous contractions. Microelectrode recordings showed that the smooth muscle cells of H vessels were hyperpolarized. If $[K^+]_o$ was increased, or if Ba^{2+} was added spontaneous activity could be initiated in H vessels. A relation in H cells between hyperpolarization and increased aerobic glycolysis (suggesting an increased electrogenic Na^+-K^+-pumping) is proposed.

The smooth muscle of the rat portal vein is mainly longitudinal[3], and has a myogenic spontaneous contractile activity[4,5]. Bursts of action potentials occur at regular intervals (2–3 per min), followed by phasic contractions.

Hypertension in the portal venous bed can be induced by partial ligation of the hepatic branches of the vein[6]. After 5 days the cross-sectional area of the longitudinal muscle is more than doubled[3], mainly as a result of hypertrophy of the muscle cells. Such vessels show a marked decrease in rhythmic contractile activity, but contract in high-K^+ solution or in response to noradrenaline[3,6,7]. In the present study we have investigated the nature of this decreased spontaneous activity by intracellular recording of membrane potentials.

Material and methods. Male Sprague-Dawley rats weighing about 250 g were used. Under ether anesthesia the 2 branches of the portal vein entering the liver were partially ligated with surgical silk. Sham-operated rats with loose loops of silk around the branches served as controls. After 5 days the animals were anesthetized, and the portal venous pressure was recorded by a Statham P23DG pressure transducer via a catheter inserted into the superior mesenteric vein. Transmural pressure was less than 10 cm H_2O for controls, and more than 15 cm H_2O for veins with portal ligatures. The portal veins were then removed. In experiments with only force recording the preparations were mounted in a 50 ml mantled organ bath for longitudinal isometric force registration. The preload was 5 mN for controls, and 10 mN for hypertrophic vessels (corresponding to lengths for optimal active force[3]). For electrical and mechanical recordings the vessels were mounted horizontally in a continuously perfused 2 ml chamber. One end of the vessel was partly immobilized over a truncated cone, and the other end connected to a force transducer. Membrane potentials were recorded in the immobilized part of the vein using intracellular microelectrodes filled with 3 M KCl, with a resistance between 20 and 30 MΩ. The signals were recorded on a storage oscilloscope, and photographed. The vessels were left to recover for 1 h before onset of the experiments. During this period the vessels were exposed for 10 min to phenoxybenzamine (10^{-6} M) to obtain total, irreversible α-adrenergic blockade[8]. The bathing medium was a Krebs solution of the following composition in mM: NaCl 122, KCl 4.73, $CaCl_2$ 2.49, $MgCl_2$ 1.19, $NaHCO_3$ 15.5, KH_2PO_4 1.19, glucose 11.5, and $CaNa_2$-versenate 0.026. The temperature was kept at 37 °C, and the solutions were constantly aerated by 4% CO_2 in O_2, giving pH 7.4. High-K^+ solution was made by substituting 122 mM NaCl with equimolar amounts of KCl. Statistical significance was tested using Student's t-test for unpaired data.

Results. Figure 1 shows representative recordings of spontaneous contractile activity in control (C) and hypertrophic (H) portal vein. The C vein contracts rhythmically with a frequency of about 2–3 per min, whereas the H vessel contracts with a very low frequency, if at all.

Recordings of membrane potential were performed on 6 C and 6 H veins. Resting membrane potential in 'non-pacemaker' cells was -61 ± 1 mV for C, and -67 ± 1 mV for H veins (SD, 6–12 cells from each preparation). Only cells which were impaled for 2 min or more were considered. The difference is significant ($p < 0.01$). Figure 2 (upper panels) shows that the quiescent periods in a control vessel are interrupted by bursts of action potentials, followed by contraction. Cells that depolarized between the bursts were considered to be pacemaker cells, and were not included. The hypertrophic vein (lower panel) seems to be devoid of spontaneous contractions at $[K^+]_o$ 6 mM, raising the $[K^+]_o$ to 15 mM depolarizes the cell and a burst of action potentials is induced followed by a contraction of an apparently normal appearance. The return of the $[K^+]_o$ to its original value results in a hyperpolarization of the membrane and the loss of spontaneous contractile activity. When $[K^+]_o$ had been increased to 15 mM for a prolonged period the vessels contracted rhythmically, and resting membrane potential was -45 ± 3 mV (SD, 12 cells from 2 H veins).

The results so far suggest the cells in the H veins are hyperpolarized, but that these cells can, when depolarized, generate action potentials, and contract. Cumulative $[K^+]_o$ dose-response curves were determined for 6 C and 6 H veins to see if a normal contraction pattern could be

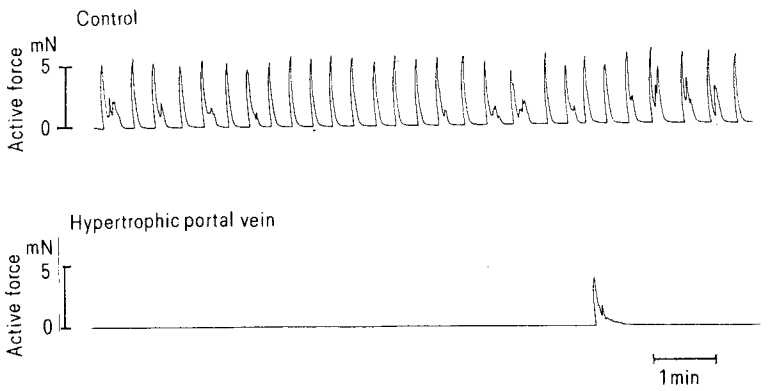

Figure 1. Mechanical activity of a control and a hypertrophic rat portal vein. Note the marked difference in contraction frequency.

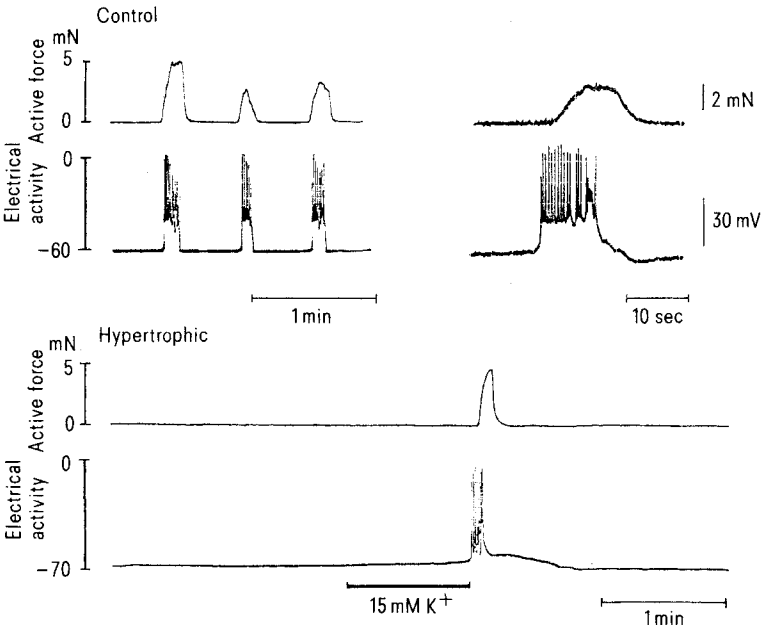

Figure 2. Electrical and mechanical activity of 2 portal vein preparations. Upper panel: Recordings from a control vein displayed on 2 different time bases. Lower panel: Recordings from a hypertrophic vein. The K^+ concentration in the bath was increased to 15 mM for 1 min.

initiated in H veins. The time in each $[K^+]_o$ was 15 min. Figure 3 (upper panels) shows that the rhythmic contractions in a C vein is increased up to 12 mM $[K^+]_o$. At higher $[K^+]_o$ the contractions become continuous, and at $[K^+]_o$ 50 mM a smooth contracture is developed. The H vein in figure 3 is quiescent up to $[K^+)_o$ 18 mM. At this concentration a high-frequent rhythmic activity is developed. At higher $[K^+]_o$ the H vein behaves in a similar way to the control. The mechanical activity during the last 5 min in each $[K^+]_o$ was quantitated with an electronic integrator device. In figure 3 (lower panel) this integrated activity is given for C and H veins relative to maximum response. It can be seen that in order to obtain a comparable relative mechanical activity a considerably higher $[K^+]_o$ is needed for the H than for the C veins. Maximal response was reached at $[K^+]_o$ 50 mM for both groups. It amounted to 17.2 ± 1.3 mN for C, and 24.8 ± 1.9 mN (SE, $n = 6$ for each) for H veins. The difference was significant ($p < 0.01$).

Induction of rhythmic spontaneous activity in H veins could also be elicited by addition of Ba^{2+} (0.2–0.5 mM) to the bathing solution (not shown in the figs).

Discussion. The electrical and contractile activity of the

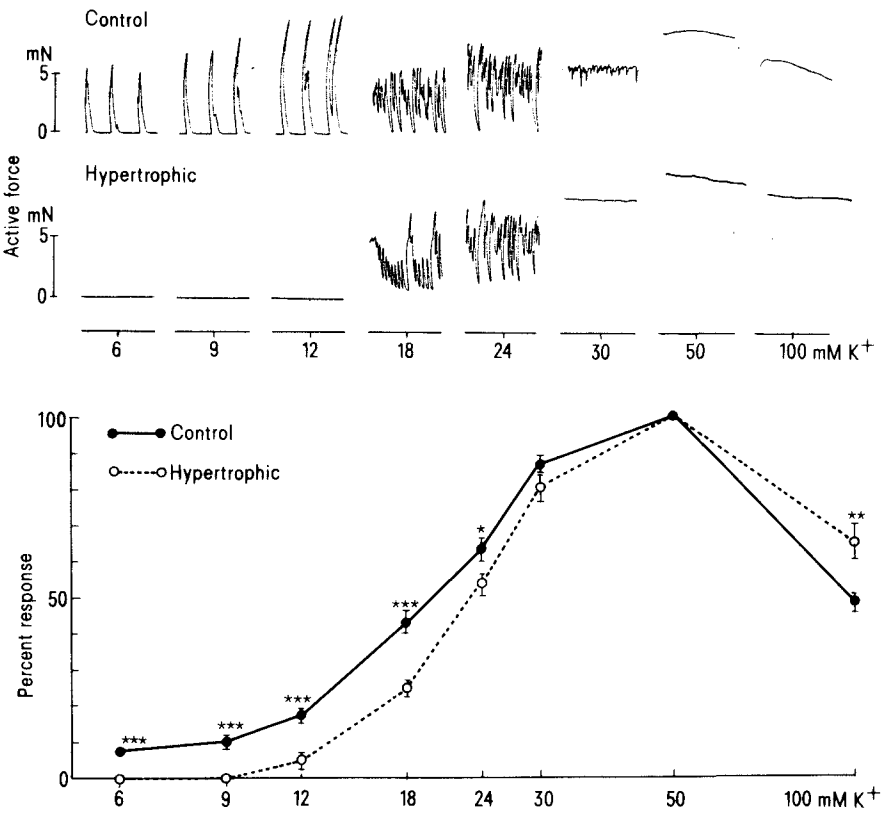

Figure 3. First panel: Contractile activity of a control and a hypertrophic portal vein in solutions with different K^+ concentrations. Second panel: Integrated mechanical activity (expressed in percent of maximum) vs $[K^+]_o$, $n = 6$ for both groups.

normal rat portal vein is well characterized[4,5]. Bursts of action potentials appear at a frequency of 2–3 per min, followed by phasic contractions (see figs 1 and 2). In previous studies[3,6,7] it has been shown by mechanical recording that rat portal veins lose their spontaneous contractile activity when subjected to 5 days increased transmural pressure. The present study confirms this (fig. 1). We have also shown that the smooth muscle cells of such hypertensive (H) vessels have a more negative resting potential than cells from normotensive control (C) veins. If the H cells are depolarized by an increase of $[K^+]$ in the bathing media to 15 mM (fig. 2) bursts of action potentials followed by contraction are elicited. This contraction pattern is very similar to that found in C veins at normal (5.9 mM) $[K^+]_o$. Phasic contractions can also be initiated if Ba^{2+} (up to 0.5 mM) is added to the Krebs solution. This ion is known to decrease K^+ conductance in the rat portal vein[9]. Figure 3 illustrates that comparable integrated mechanical activity can be recorded from C and H veins if $[K^+]_o$ is increased for the latter. The difference in K^+ sensitivity becomes less pronounced in higher $[K^+]_o$, and maximum contractile response is reached at similar concentrations (50 mM $[K^+]_o$). Maximum response was significantly higher in H veins. A tendency to[3] or a clear increase[6,7] in maximum active force in H veins have been reported earlier. This, and also the increased dry weight[3] of the H veins, suggest a considerable synthesis activity during the hypertensive period.

Smooth muscle cells from tail arteries in spontaneously hypertensive rats (SHR) are found to have similar transmembrane potentials[10] (although an increased electrogenic component is suggested) to those of cells from normotensive control animals. The development of hypertension in SHR is, however, slower than in the animal model used in the present study. Rats subjected to normobaric hypoxia (which induces pulmonary hypertension) have already after 10 days[11] an increased membrane polarization in smooth muscle cells of small pulmonary arteries (SPA). The authors of that study proposed that the hyperpolarization in SPA cells may be a result of an increased activity of the electrogenic Na^+-K^+-pump, possibly caused by an increase in the coupled influx[12] of Na^+ and amino acids (the latter necessary for the synthesis activity of the hypertrophying cell). The energy metabolism of the portal vein preparation is well characterized[7,13] and in a previous study[7] an increased basal lactate production was found in H compared to C veins, despite optimum O_2 supply. As there are indications[14] of a coupling in smooth muscle between aerobic glycolysis and the Na^+-K^+-pump, this might well suggest an increased activity of that pump, which then leads to the observed hyperpolarization of the H cells.

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Increased plasma concentration of cyclic GMP in atrial fibrillation

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Summary. Plasma concentration of cyclic nucleotides in patients with paroxysmal atrial fibrillation was determined by ultrasensitive radioimmunoassay on the day of attack and also the next day, following recovery to sinus rhythmus. The concentration of cyclic GMP in the plasma of patients with attacks of paroxysmal atrial fibrillation was significantly higher than in those with sinus rhythmus, but no significant difference in plasma concentration of cyclic AMP was observed.

The accurate determination of plasma cyclic nucleotides appears to be very useful for assessing responses to hormones and neurotransmitters in clinical studies. With cyclic AMP, which is the 2nd messenger for β-adrenergic agents and many hormones, the plasma concentration appears to reflect changes in the tissues[1], because the nucleotide in plasma is in a dynamic steady-state relationship with its intracellular pools[2]. Cyclic GMP, like cyclic AMP, is present in plasma[3], and the plasma concentration of cyclic GMP could serve as a good index for cholinergic activity[4].

Material and methods. 100 and 2 normal subjects (48 males, 54 females; mean age, 52.8 ± 9.1) and 5 patients with paroxysmal atrial fibrillation, on the day of attack as well as the next day on recovery to sinus rhythmus, were studied. Blood (1–2 ml) was collected into a chilled tube in ice with 2 µl of 0.5 M EDTA and immediately centrifuged at 4 °C. Plasma was separated and frozen at −20 °C until assayed. Cyclic nucleotides were simultaneously measured in duplicate by the radioimmunoassay method of Cailla et al.[5,6], as modified by Honma et al.[7]. The recovery of plasma cyclic AMP was $105 \pm 5.5\%$ with 15 pmoles added, and that of cyclic GMP was $93 \pm 2.0\%$ with 121 pmoles added[7]. All values are expressed as mean ± SEM. Statistical analysis was performed with Student's t-test. Regression lines were fitted by the method of least squares.

Results and discussion. The mean plasma concentrations of cyclic AMP and cyclic GMP in 102 normal subjects were 17.0 ± 0.8 pmoles/ml, and 4.2 ± 0.2 pmoles/ml, respectively.