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Spontaneous activity of rat portal vein at normal and elevated hydrostatic pressure

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Sigurdsson SB, Örnhagen H Ch. Spontaneous activity of rat portal vein at normal and elevated hydrostatic pressure. Undersea Biomed Res 1980; 7(3):171–181.—The effects of high hydrostatic pressure on the spontaneous contractile activity of isolated rat portal veins were studied. During compression, an increase of activity was seen, whereas stable elevated hydrostatic pressure gave a decrease of both frequency and time-integrated force. Decompression further reduced the activity, but all changes were reversible upon return to control pressure. During sustained high pressure the frequency of contractions was reduced by 15.9% at 25 atm, 26.4% at 50 atm, and 45.8% at 100 atm. The corresponding reductions in integrated active force were 13.7%, 16.7%, and 40.7%, respectively. Contractions caused by electrical stimulation of nerve endings left in the preparation were reduced by 44.1%, and potassium contractures were reduced by only 15.3% at 100 atm. It is concluded that inhibition of activity in rat portal vein at high hydrostatic pressure is due in part to effects on the smooth muscle membrane.

hydrostatic pressure
compression rate
phenoxybenzamine
propranolol
smooth muscle
contracture

A number of biological processes are known to be affected by high ambient pressure. This includes those processes in the cell membrane in which pressure seems to alter macromolecular structures and change dynamic components of membrane function (1). These effects are considered to be responsible for changes in excitability of isolated nervous tissue (2) and heart muscle (3) seen at high pressure. Increased ambient pressure causes bradycardia in human beings (e.g., 4, 5, 6), experimental animals (7), and isolated sinus node preparations (8). Also, in spontaneously active ventricular Purkinje fibers pressure decreases the firing frequency. In these cells the reduced firing frequency is caused by a slowing of the spontaneous diastolic depolarization (8). A similar mechanism for the sinoatrial node has been suggested as the cause of the hyperbaric bradycardia. The contractile force of cardiac muscle is increased by hydrostatic pressure as described by Edwards and Cattell (9).

Like cardiac muscle preparations, the portal vein from the rat contracts spontaneously, but its contraction frequency is much lower, only about three contractions per minute (10). The amplitude and frequency of these contractions changed if the vein was stretched (11) or exposed to changes in osmolality (12, 13). These effects resulted largely from changes in

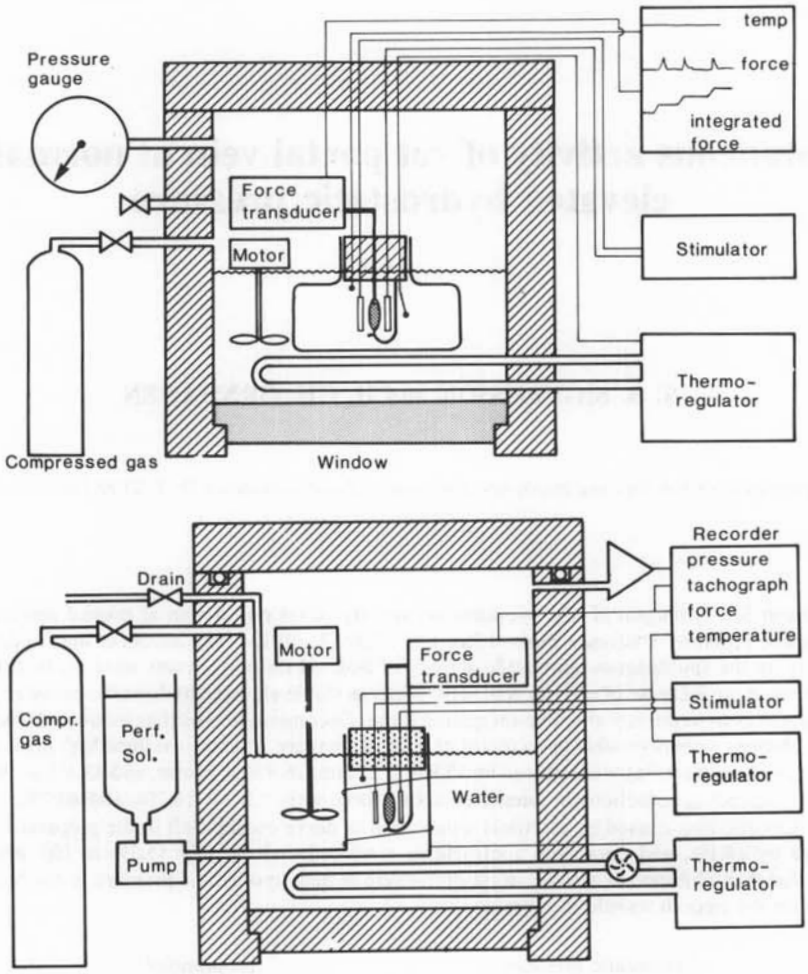

Fig. 1. Schematic of chambers and subsystems used in experiments. (See text for details.)

membrane function and were not believed to be caused by some kind of staircase phenomenon. It was therefore of interest to determine if increased hydrostatic pressure also affects the pacemaker mechanism of the smooth muscle cells in the rat portal vein.

METHODS

The experiments were performed on portal vein preparations from Sprague-Dawley rats weighing between 200 and 300 g. The animals were killed by a blow on the neck, and the portal vein was dissected. A 5-mm strip of the vein was mounted in a 45-ml organ bath made of glass (Fig. 1 *top*) and connected to a force transducer (Grass model FT 03) and stretched to a passive tension of about 4×10^{-3} N. (The transducer was tested for linearity at pressure with the help of an electromagnet.) The response of the transducer was found to be unaffected by pressure. The isometric force was recorded directly on a polygraph (Grass model 7 D) and could also be quantitated by integrating the active tension curve for each contraction with an electronic

integrator device (14). The preparations were allowed to accommodate in the tissue bath for at least 60 min before the first compression. The bathing medium was a Tris-buffered solution with the following millimolar composition: NaCl 120, KCl 6.0, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11.5, and Tris(hydroxymethyl)aminomethane 21 (Trizma base, Sigma Chemical Co). The solution was titrated with HCl to a pH of 7.4 at 37°C and bubbled with O_2 before the experiment. According to calculations based on the results from Hellstrand (15) the amount of O_2 in the bath was more than enough for the metabolism of the preparations during the experiments.

To minimize diffusion of gases from the chamber atmosphere to the solution the liquid surface was reduced to 7 mm^2 with a Plexiglas stopper. In spite of this precaution, in some experiments small bubbles could be seen in the solution toward the end of decompressions from 100 atm. Compressions were made with air from cylinders. To avoid liberation of O_2 on the temperature control thermistor when the Tris buffer solution was heated from room temperature to 37°C, the base-line (control) pressure was 2 atm. The bath temperature was kept at 37°C ± 1°C.

Separate experiments showed that neither the slight pressure increase at control pressure (2 atm) nor temperature variations of the magnitude ± 1°C appreciably affected frequency or force of spontaneously contracting portal veins. These experiments were performed with an improved perfusion and temperature control system (Fig. 1, *bottom*), a 2.5-ml tissue bath being kept at 37.0°C ± 0.2°C during all phases of the experiments. A high pressure pump (Constametric I, Laboratory Data Control, USA) gave a continuous flow of Tris buffer solution in these experiments. This method assured an inert gas free compression of the preparations. (Since no difference in results were seen between experiments in the two different setups, any possible effect of dissolved N_2 or O_2 in the older setup must be of minor importance, and the results are presented together.) The continuous-flow setup allowed changes of the perfusion solution at pressure and was therefore used to study potassium contractures. Perfusion solution with high concentrations of K^+ (120 m*M*) depolarizes the cell membrane and allows Ca^{2+} to enter the cells and a contracture to develop (16, 17). For this purpose a solution with all NaCl replaced by equimolar amounts of KCl was used. This solution is hereafter in this paper called K^+-high Tris buffer.

To avoid a possible influence of norepinephrine leaking from nerve ending during the experiment, α- and β-receptors were blocked by 10^{-6} *M* of phenoxybenzamine and propranolol (18)

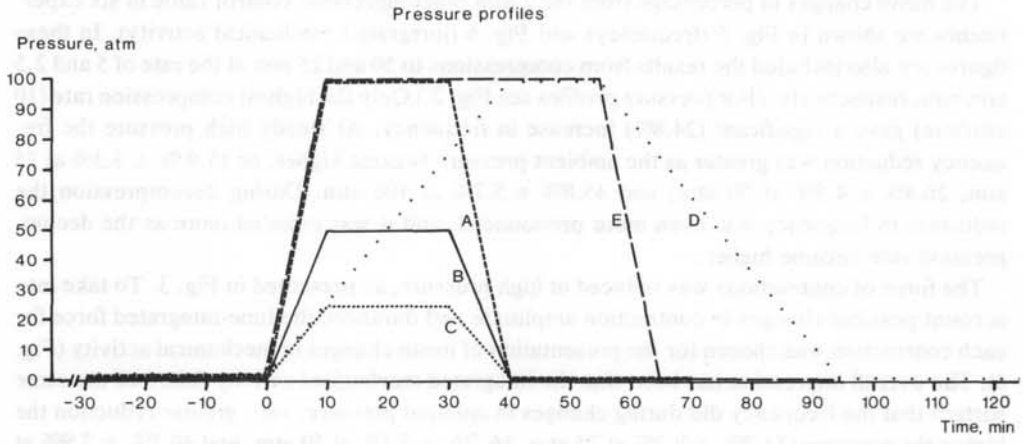

Fig. 2. Pressure profiles for the different compression experiments A–E. (See text for details.)

in all experiments except those in which effects of electrical field stimulation were studied. No effect was seen on spontaneous activity after addition of blockers. For each preparation, compressions were made over 10 min at the rates 10, 5, and 2.5 atm/min, giving final pressures of 100, 50, and 25 atm, respectively (pressure profiles A, B, and C in Fig. 2). Time of exposure to the sustained high pressure in most experiments was 20 min, and at least 30 min were allowed for recovery at control pressure between a decompression and a new compression.

External field stimulation of the preparation was made via two platinum electrodes placed on each side of the muscle. Square-wave pulses (1 ms, 20 V) at the rate of 16/s gave tetanic contractions (10). These contractions were the result of release of norepinephrine from sympathetic nerve endings, since they could be blocked by phenoxybenzamine at the dose used.

The order of compression rates and maximum pressures were randomly changed between preparations. Results presented are means and SE except when individual preparations are discussed. Students's t test of paired data has been used for statistics.

RESULTS

The spontaneous isometric contractions from one rat portal vein in normal Tris buffer solution under different conditions with regard to pressure are shown in Fig. 3. The first recording (I) was made at 2 atm and shows normal spontaneous activity. The next recording (II) shows the effects of compression at a rate of 10 atm/min. The amplitude of the contractions was reduced as the pressure increased, and there was a small increase in frequency. When 100 atm was reached, the amplitude was further reduced, and frequency started to decrease. Recording III shows the final 10-min period at this pressure. During the decompression back to 2 atm at 10 atm/min the frequency was even more reduced, and in this preparation the spontaneous activity stopped completely (recording IV). After a couple of minutes at 2 atm, the contractile activity started again, and the muscle showed essentially normal activity within 10 min (recording V). In some experiments a short period of increased contraction force could be seen immediately after return to control pressure.

In order to see if the effects of high ambient pressure was due to change in resting tension, the muscle was exposed to Ca^{2+}-free solution at pressure (Fig. 4). The spontaneous activity disappeared, but no change in resting tension was seen. Neither was there any major change in resting tension as a result of compression.

The mean changes in percentage from the 2-atm precompression control value in six experiments are shown in Fig. 5 (frequency) and Fig. 6 (integrated mechanical activity). In these figures are also included the results from compressions to 50 and 25 atm at the rate of 5 and 2.5 atm/min, respectively. (For pressure profiles see Fig. 2.) Only the highest compression rate (10 atm/min) gave a significant (24.8%) increase in frequency. At steady high pressure the frequency reduction was greater as the ambient pressure became higher, or 15.9 % ± 3.3% at 25 atm, 26.4% ± 4.5% at 50 atm, and 45.8% ± 5.7% at 100 atm. During decompression the reduction in frequency was even more pronounced, and it was reduced more as the decompression rate became higher.

The force of contractions was reduced at high pressure, as presented in Fig. 3. To take into account possible changes in contraction amplitude and duration, the time-integrated force for each contraction was chosen for the presentation of mean changes in mechanical activity (Fig. 6). The overall impression has been that the integrated mechanical activity followed the same pattern that the frequency did during changes in ambient pressure, viz., greater reduction the higher the pressure (13.7% ± 8.3% at 25 atm, 16.7% ± 7.1% at 50 atm, and 40.7% ± 7.9% at 100 atm).

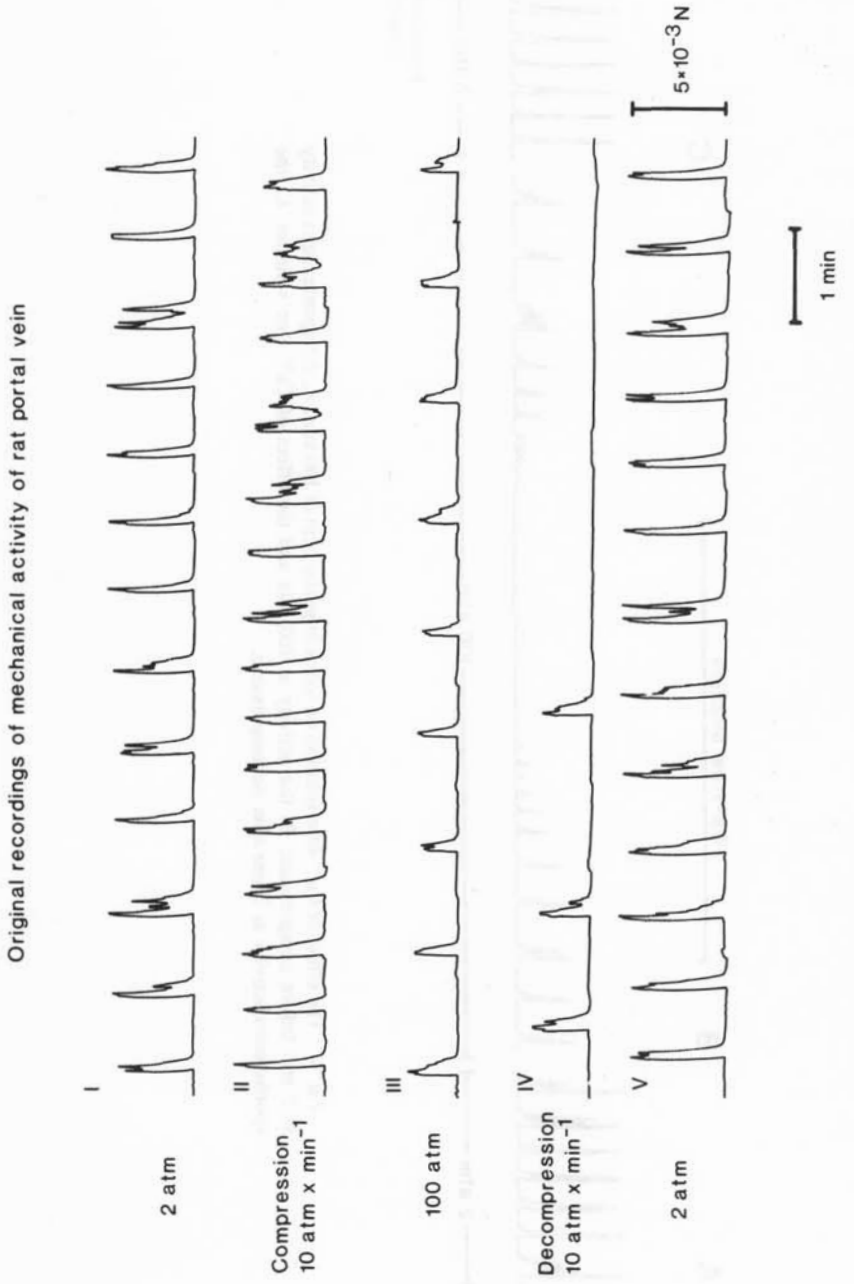

Fig. 3. Original recordings from one portal vein preparation. I, at 2 atm before compression; II, during compression at 10 atm/min; III, at 100 atm stable pressure; IV, during decompression at 10 atm/min; V, at 2 atm after decompression. Time bar, 1 min; force bar 5×10^{-3}N.

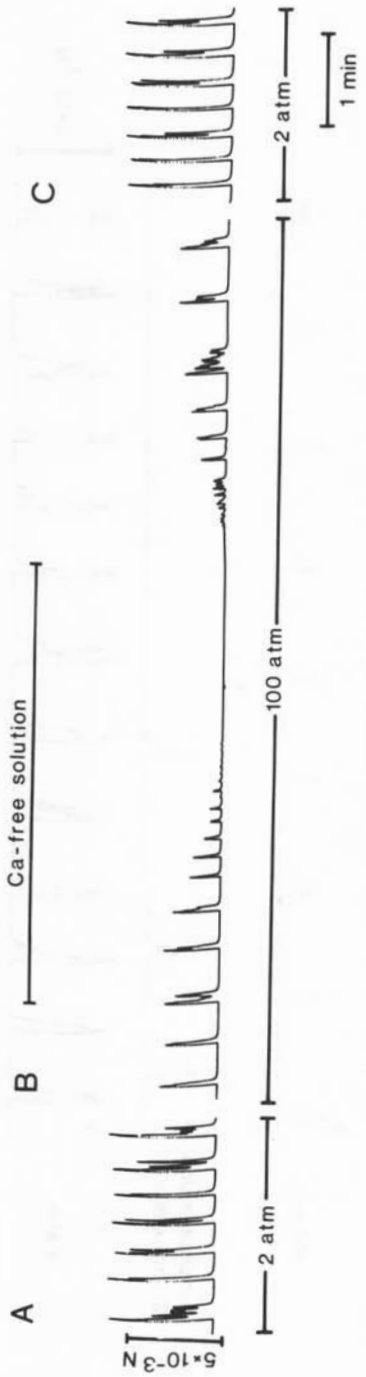

Fig. 4. The effect of Ca^{2+}-free solution on tonus in portal vein at 100 atm. A, the spontaneous activity at 2 atm before compression; B, the activity at 100 atm and the effect of Ca^{2+}-free solution; C, the spontaneous activity at 2 atm after decompression.

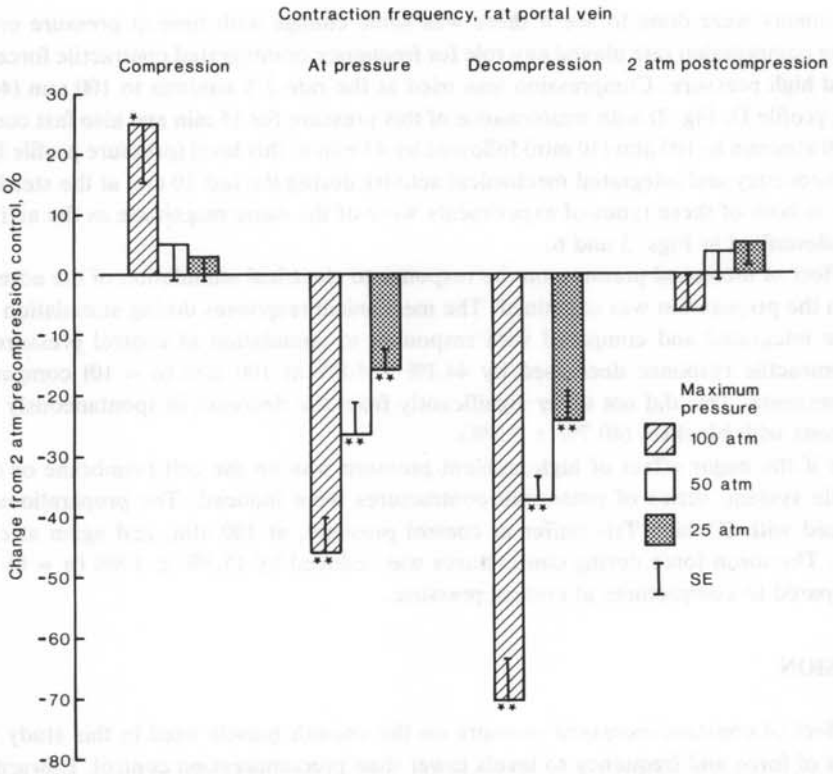

Fig. 5. Change of mean contraction frequency in percentage of precompression control for six rat portal vein preparations compressed to 100, 50, and 25 atm. *Bars*, 1 SE; significance levels are indicated (* = $P<0.05$; ** = $P<0.01$).

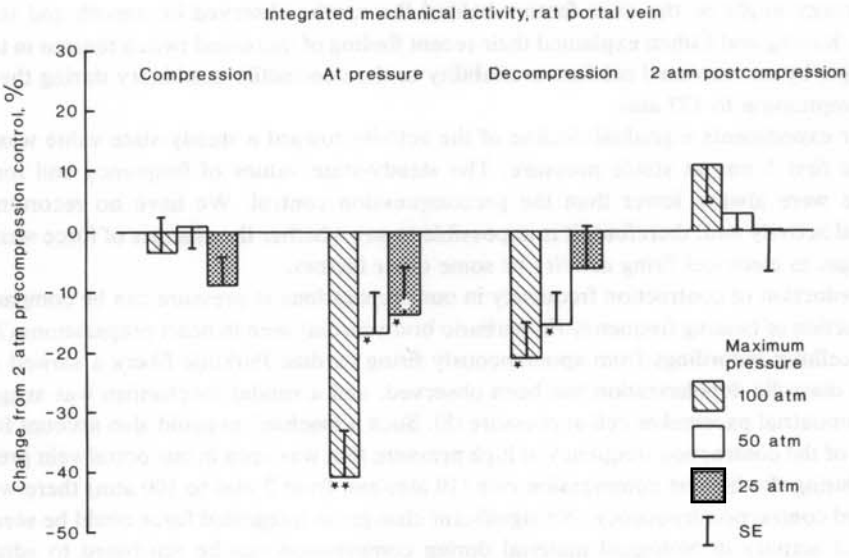

Fig. 6. Change of mean integrated mechanical activity in percentage of precompression control for six rat portal vein preparations compressed to 100, 50, and 25 atm. *Bars*, 1 SE; significance levels are indicated (* = $P<0.05$; ** = $P<0.01$).

Experiments were done to see if there was some change with time at pressure or if the preceding compression rate played any role for frequency or integrated contractile force at the sustained high pressure. Compression was tried at the rate 2.5 atm/min to 100 atm (40 min, pressure profile D, Fig. 2) with maintenance of this pressure for 15 min and also fast compression at 10 atm/min to 100 atm (10 min) followed by 45 min at this level (pressure profile E, Fig. 2). The frequency and integrated mechanical activity during the last 10 min at the steady high pressure in both of these types of experiments were of the same magnitude as the activity at 100 atm described in Figs. 5 and 6.

The effect of increased pressure on the response to electrical stimulation of the adrenergic nerves in the preparation was examined. The mechanical responses during stimulation at 100 atm were integrated and compared with responses to stimulation at control pressure. The mean contractile response decreased by 44.1% ± 4.8% at 100 atm (n = 10) compared to control pressure. This did not differ significantly from the decrease in spontaneously active preparations with blockers (40.7% ± 7.9%).

To see if the major effect of high ambient pressure was on the cell membrane or on the contractile system, series of potassium contractures were induced. The preparations were depolarized with K^+-high Tris buffer at control pressure, at 100 atm, and again at control pressure. The mean force during contractures was reduced by 15.3% ± 3.8% (n = 6) at 100 atm compared to contractures at control pressure.

DISCUSSION

The effect of constant increased pressure on the smooth muscle used in this study was a reduction of force and frequency to levels lower than precompression control. Edwards (19) and Cattell (20) observed decreased force in smooth muscle, whereas increased force has been observed in striated muscle at pressure by Cattell (20), Brown (21), and Kendig and Cohen (22). Edwards and Cattell and Brown suggested that increased viscosity and conversion of more energy might be the main factors behind the results observed in smooth and striated muscle. Kendig and Cohen explained their recent finding of increased twitch tension in the rat diaphragm by an increased calcium availability to the contractile machinery during the stepwise compression to 137 atm.

In our experiments a gradual decline of the activity toward a steady-state value was seen over the first 5 min at stable pressure. The steady-state values of frequency and force at pressure were always lower than the precompression control. We have no recordings of electrical activity and, therefore, it is impossible to say whether the changes of force were due to changes in electrical firing activity or some other factors.

The reduction of contraction frequency in our preparations at pressure can be compared to the reduction of beating frequency (hyperbaric bradycardia) seen in heart preparations (7, 23). In intracellular recordings from spontaneously firing cardiac Purkinje fibers a slowed spontaneous diastolic depolarization has been observed, and a similar mechanism was suggested for the sinoatrial pacemaker cell at pressure (8). Such a mechanism could also account for the slowing of the contraction frequency at high pressure that was seen in our portal vein preparations. During the highest compression rate (10 atm/min from 2 atm to 100 atm) there was an increased contraction frequency. No significant changes in integrated force could be seen. An increased activity in biological material during compression can be attributed to adiabatic temperature increase, but separate experiments showed that force and frequency of these smooth muscles are, however, not markedly sensitive to temperature changes of the order of ± 1°C, which were seen in the bath during compression in our experiments. The increase in

frequency seems therefore to be caused by a stimulating effect of high compression rates. During decompression the frequency was reduced below levels at pressure. This further reduction during decompression could be the reversed "compression phenomenon."

The spontaneous activity at 2 atm was not changed when α- and β-adrenergic blocking agents (phenoxybenzamine $10^{-6}M$ and propranolol $10^{-6}M$) were added to the perfusion solution. The responses to increases in pressure were also the same in the presence of blockers as without, indicating a direct effect of pressure on the smooth muscle cell and not an effect mediated via changes in spontaneous release of transmitter from nerve terminals in the portal vein preparation. A further support for this is that electrically driven contractions, caused by an increased release of norepinephrine during stimulation, are at pressure reduced as much as the spontaneous contractions. Akers and Carlsson (24) concluded from experiments on smooth muscle from rabbit duodenum that the conformation of the receptors at the membrane surface was changed by pressure so that the affinity for the transmitter was decreased. In our experiments we have not found any support for such a phenomenon but rather an effect by pressure on a later step in the chain of events leading to contractions.

The contractile machinery itself seemed relatively unaffected by pressure in our experiments. No change in passive tension (elastic properties) or resting tension tested by elimination of Ca^{2+} (25) was seen at compression. The maximum force the preparation could develop was reduced by only 15.3% at 100 atm compared to 2 atm, tested by chemical depolarization of the membrane by K^+-high Tris buffer. Because resting tension is almost unchanged at pressure compared to control, it is unlikely that an increased resting tension (Ca^{2+}-dependent tone) at pressure should be the mechanism behind the reduced activity. Not only hydrostatic pressure but other environmental and physical factors affect the spontaneous activity of the portal vein. Thus, for example, increase in osmolality (13) and decrease in passive tension (11) decrease the electrical and mechanical activity. In both these situations the decreases in activity are short lasting, and some kind of adaptation takes place. At stable, increased pressure for 45 min no change in activity was seen. The portal vein is normally exposed to changes in stretch and osmolality, and adaptation to such changes therefore seems to be a physiologic reaction. High hydrostatic pressure is a nonphysiologic stimulus, which could be an explanation for the lack of adaptation. However, upon arrival at control pressure a transient phase of hyperactivity was seen. Such overshoot reactions after decompression have been observed earlier in heart muscles (8) and have been explained as effects of adaptation taking place at pressure. In our experiments the overshoot therefore seems to be caused by some other mechanism.

In conclusion: Compression gave a small increase of spontaneous activity in the rat portal vein, whereas stable, elevated hydrostatic pressure gave a decreased spontaneous activity. The inhibition of activity could in part be explained by effects on the smooth muscle membrane.

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Sigurdsson SB, Örnhagen H Ch. L'activité spontanée de la veine porte du rat sous une pression normale et une pression hydrostatique élevée. Undersea Biomed Res 1980; 7(3):171–181.—Les effets d'une pression hydrostatique élevée ont été étudiés sur l'activité contractile des veines porte isolées du rat. Sous compression, il a été observé une augmentation d'activité, alors qu'une pres-

sion stable hydrostatique élevée engendrait une diminution et de la fréquence et de la force intégrée dans le temps. La décompression produisit une réduction ultérieure de l'activité, mais tous les changements étaient réversibles lors du retour de contrôle de la pression. Sous une pression forte soutenue, il y eut une diminution de la fréquence des contractions de 15,9% à 25 atm, 26,4% à 50 atm, et 45,8% à 100 atm. Les réductions correspondantes en force active intégrée équivalaient 13,7%, 16,7%, et 40,7% respectivement. Des contractions dûes à une stimulation électrique des pointes de cellules nerveuses qui avaient été laissées dans la préparation, furent réduites de 44,1%, et les contractions de potassium diminuèrent seulement de 15,3% à 100 atm. Il en ressort que l'inhibition de l'activité dans la veine porte du rat sous une forte pression hydrostatique résulte en partie des effets sur la membrane douce du muscle.

pression hydrostatique
taux de compression
phénoxybenzamine
propranolol
contracture
muscle lisse

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