EFFECTS OF DILTIAZEM ON GUINEA PIG PORTAL VEIN IN HYPERTONIC SOLUTION

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Abstract—Effects of diltiazem were investigated on the isolated portal vein of the guinea pig in hypertonic solution. Hyperosmolarity was produced by sucrose. In contrast to a small and somewhat irregular spontaneous contraction in isotonic solution, the portal vein in hypertonic solution elicited a spontaneous contraction at regular intervals and with a large amplitude and low frequency. The spontaneous phasic contraction in hypertonic solution was not affected by tetrodotoxin, atropine and propranolol. Phentolamine also had no significant influence in some preparations. On the contrary, remarkable changes in the contractile activity were found when the extracellular concentration of CaCl₂ was reduced from 2.5 to 1.3 or 0.6 mM. In calcium-free solution, the spontaneous contraction was completely abolished. In addition, the phasic contraction was suppressed by CoCl₂. Thus, it is assumed that the spontaneous contractile activity of the guinea pig portal vein in hypertonic solution is myogenic in nature and Ca⁺⁺ plays an essential role in the contraction. On the other hand, diltiazem suppressed the spontaneous phasic contraction in both isotonic and hypertonic solutions. The spontaneously generated and electrically evoked spikes in hypertonic solution were also inhibited by diltiazem. Furthermore, the effects of diltiazem on the mechanical activity and evoked spike were reversed by the addition of CaCl₂. Therefore, it is inferred that diltiazem suppresses the spontaneous phasic contraction by inhibiting the mobilization of Ca⁺⁺ which triggers the generation of the contraction.

A new coronary vasodilator, diltiazem, possesses a calcium antagonistic property in the cardiac ventricular (1, 2) and various smooth muscles (3–8). As to the vascular smooth muscle, it has been demonstrated that diltiazem suppresses the K-contracture of the isolated coronary artery (3, 4) and this effect is reversed by increasing extracellular concentration of CaCl₂ (4). In the present experiments, the isolated portal vein of the guinea pig was used, since the portal vein is known to exhibit spontaneous electrical and mechanical activities (9–11). It has been reported that verapamil, a calcium antagonist, inhibits the spontaneous activities of the portal vein isolated from the guinea pig (12). During the course of the present experiments, it was noted that when the extracellular osmolarity was increased with sucrose, the guinea pig portal vein elicited a spontaneous phasic contraction at regular intervals with a large amplitude and low frequency, as compared with a small and somewhat irregular contraction in isotonic solution. Therefore, the effects of diltiazem on the isolated portal vein were mainly examined in hypertonic solution. The contractile properties of the portal vein in response to hyperosmolarity are also described herein.
MATERIALS AND METHODS

Male guinea pigs, weighing 250-300 g, were stunned and bled. The portal vein was isolated and longitudinal strips, approx. 25 mm long and 5 mm wide, were prepared. The preparation was suspended in an organ bath with 10 ml of modified Krebs solution and the isometric contraction was recorded on an ink-writing oscillograph through a strain gauge transducer. The initial tension applied to the muscle was adjusted to approx. 300 mg. Experiments were started 60-90 min after the preparation was equilibrated in the isotonic Krebs solution. Transmembrane potential of the smooth muscle of the portal vein was recorded intracellularly (13) with glass microelectrodes (resistance 40-80 megohms), filled with 3 M KCl. The recording electrode was inserted from the outer surface of the preparation. In experiments with evoked spike activity, the preparation was driven extracellularly (14) at a constant rate of 0.03 Hz by suprathreshold rectangular current pulse (duration 200 msec). The recording electrode was placed within 0.5 mm from the stimulating electrode. The composition of modified Krebs solution was as follows (mM): NaCl 122.0, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, KH₂PO₄ 1.2, NaHCO₃ 15.5, glucose 11.5. The solution was aerated with 95% O₂ and 5% CO₂. The solution was made approx. twice as hypertonic by adding 9.25 g of sucrose to 100 ml of the solution. The calcium-free solution was prepared by omitting CaCl₂ and EGTA was not added. For experiments with CoCl₂, KH₂PO₄ was eliminated from the modified Krebs solution and the concentration of NaHCO₃ was reduced from 15.5 to 8.0 mM in order to avoid the precipitation of the insoluble cobalt.

Hyperosmolarity was achieved by replacing the isotonic Krebs solution with the hypertonic solution at the same temperature. After the phasic contraction attained equilibration in hypertonic solution (usually 60 min), 0.1 ml of the test compound solution was added to the organ bath to give a final concentration. In the microelectrode experiments, the test compound dissolved in hypertonic solution was prepared in a reservoir and applied to the preparation through a small vinyl tubule connected to the recording chamber. When the extracellular concentration of CaCl₂ was reduced, the solution was substituted by a solution containing a lower concentration of CaCl₂. Experiments were carried out at 32±1°C. The following compounds were used: diltiazem hydrochloride (Tanabe Seiyaku Co., Ltd.), tetrodotoxin (Sankyo Co., Ltd.), atropine sulfate (Wako Pure Chem. Ind., Ltd.), phentolamine mesylate (CIBA-Geigy Ltd.), propranolol hydrochloride (Sumitomo Chem. Ind., Ltd.).

RESULTS

Spontaneous contraction of isolated portal vein in hypertonic solution

The isolated portal vein of the guinea pig elicited somewhat irregular spontaneous phasic contraction with a small amplitude in isotonic solution (Figs. 1 and 3). As shown in Fig. 1, hyperosmolarity caused a considerable increase in the amplitude of spontaneous phasic contraction, the amplitude being 315±34 mg (mean±SE, n=21) 90 min after the increase in osmolarity. The basal tension also increased from 185±16 to 330±26 mg (n=21). In addition, it was evident that the phasic contraction in hypertonic solution
Fig. 1. Effects of hyperosmolarity on the spontaneous contractile activity of the isolated guinea pig portal vein. In A, continuous recording was carried out for approx. 30 min and then a record was taken 60 min after the increase in osmolarity. In B, each record in hypertonic solution was obtained 60 min after the increase in osmolarity. Records were of the fast sweep speed.

Fig. 2. Effects of CaCl₂ on the spontaneous contractile activity in hypertonic solution. Left: Experimental records. Results were obtained 15 min after the change in extracellular concentration of CaCl₂. Right: Dose-response relationship for the effect of CaCl₂. Each point is the mean of 4–5 experiments and the vertical bars represent SE of the mean. Results were expressed as the percent of the activity in 2.5 mM CaCl₂.

Effects of tetrodotoxin, atropine, phentolamine and propranolol: Tetrodotoxin (10⁻⁶ g/ml), atropine (10⁻⁶ g/ml) and propranolol (10⁻⁶ g/ml) had no significant influence on the spontaneous contractile activity of the portal vein in hypertonic solution. Phentolamine (10⁻⁵ g/ml) also produced no remarkable change in the contractile activity in 4 out of 7 preparations. In the remaining 3 preparations, the amplitude of phasic contraction was appeared at regular intervals of lower frequency (1.01±0.03 cycle/min, n=21). Fig. 1B illustrates that the effects of hyperosmolarity on the contractile activity were almost reversible and reproducible in the same preparation.
slightly reduced and the frequency was slightly increased by phentolamine.

**Effect of CaCl₂**: Fig. 2 represents the effect of CaCl₂ on the spontaneous contraction in hypertonic solution. When the extracellular concentration of CaCl₂ was reduced from 2.5 to 1.3 and 0.6 mM, the amplitude of phasic contraction was decreased dose-dependently, while the basal tension and frequency of the contraction were increased. A small oscillatory contraction of higher frequency was recognized in 0.6 mM CaCl₂. In calcium-free solution, the phasic contraction was abolished and the basal tension decreased. On the other hand, an increase in the extracellular concentration of CaCl₂ from 2.5 to 5.0 mM produced no significant change in the amplitude of phasic contraction. The frequency of the contraction was slightly increased while the basal tension was decreased to some extent in 5.0 mM CaCl₂.

**Effect of CoCl₂**: CoCl₂ (1.2 mM) reduced the basal tension as well as the amplitude of phasic contraction and finally abolished the contractile activity.

**Effects of diltiazem on the spontaneous contractile activity of the portal vein**

**Experiments in isotonic solution**: Fig. 3A shows the effect of diltiazem on the spontaneous contractile activity in isotonic solution. Diltiazem at the concentration of $2.2 \times 10^{-7}$ M reduced the amplitude of phasic contraction by approx. 50% of the control, while the frequency of the contraction increased. In the presence of $2.2 \times 10^{-6}$ M diltiazem, the phasic contraction was almost totally abolished with a slight decrease in the basal tension. As shown in Fig. 3A, the effects of diltiazem ($2.2 \times 10^{-6}$ M) were partly reversed by the addition of 3 mM CaCl₂.

**Experiments in hypertonic solution**: Effects of diltiazem on the spontaneous contractile activity in hypertonic solution (Fig. 3B) were qualitatively similar to those in isotonic solution (Fig. 3A). Fig. 4 illustrates the dose-response relationship for the effects of diltiazem in hypertonic solution. Diltiazem at the concentration of $2.2 \times 10^{-5}$ M produced a slight decrease in the amplitude of spontaneous phasic contraction. This effect was remarkable as the concentration was increased and the phasic contraction was completely abolished at $2.2 \times 10^{-5}$ M. In parallel with the decrease in the amplitude, frequency of the phasic contraction increased dose-dependently and at $2.2 \times 10^{-6}$ M irregular contraction with a small

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**Fig. 3.** Effects of diltiazem on the spontaneous contractile activity of the isolated guinea pig portal vein. A: Experiments in isotonic solution. B: Experiments in hypertonic solution. Diltiazem was added cumulatively and each record was taken 15 min after the addition. CaCl₂ (3 mM) was added to the preparation exposed to $2.2 \times 10^{-6}$ M diltiazem and the record was taken 15 min after the addition.
amplitude and high frequency was observed. The basal tension was not affected significantly at doses up to $2.2 \times 10^{-6}$ M, while it was reduced at $2.2 \times 10^{-5}$ M. As represented in Fig. 3B, addition of 3 mM CaCl$_2$ to the preparation exposed to $2.2 \times 10^{-6}$ M diltiazem partly recovered the original phasic contraction.

**Effects of diltiazem on the transmembrane potential in hypertonic solution**

Fig. 5 shows an example of the effect of diltiazem ($2.2 \times 10^{-5}$ M) on the spontaneously generated and electrically evoked action potentials, recorded intracellularly. As reported by Ito and Kuriyama (9), isolated portal vein of the guinea pig generated spontaneous action potential with a hump during the falling phase in hypertonic solution. One min after administration of $2.2 \times 10^{-5}$ M diltiazem, the spontaneous action potential was completely suppressed, whereas the action potential evoked by electrical stimulation remained unchanged. The evoked action potential, however, was gradually inhibited and finally abolished (5.2 min in Fig. 5). The resting potential was not affected significantly. When CaCl$_2$ (5 mM) was added, the inhibitory effect of diltiazem on the evoked action potential was completely recovered.

![Dose-response curves for the effect of diltiazem on the spontaneous contractile activity in hypertonic solution.](image)

**Fig. 4.** Dose-response curves for the effect of diltiazem on the spontaneous contractile activity in hypertonic solution. Results were expressed as the percent change to the control. Each point is the mean of 5 experiments and the vertical bars represent SE of the mean.

![Effects of diltiazem (2.2 × 10⁻⁵ M) on the spontaneous and evoked electrical activities of the isolated portal vein in hypertonic solution.](image)

**Fig. 5.** Effects of diltiazem ($2.2 \times 10^{-5}$ M) on the spontaneous and evoked electrical activities of the isolated portal vein in hypertonic solution. Upper trace, intensity of applied current pulse; lower trace, membrane potential. Depolarizing current pulse (200 msec, 0.6 V/cm) was applied to the preparation which exhibited the spontaneous activity. CaCl$_2$ (5 mM) was added to the preparation in the presence of diltiazem.
DISCUSSION

In contrast to a small and somewhat irregular spontaneous contraction generated in isotonic solution, the isolated guinea pig portal vein in hypertonic solution (sucrose 270 mM) elicited a regular spontaneous phasic contraction with a large amplitude and lower frequency. These effects of hyperosmolarity on the guinea pig portal vein were different from those on the rat portal vein, in which hyperosmolarity inhibited the spontaneous contractile activity and the muscle developed a tonic increase in tension (15).

As demonstrated in the present experiments, the contractile activity in hypertonic solution was not affected by tetrodotoxin, atropine and propranolol. Phentolamine also had no significant influence on the contraction in some preparations. The results suggest that in the guinea pig portal vein, the spontaneous contractile activity in hypertonic solution is myogenic in nature and neural mechanisms including cholinergic and adrenergic activities are not involved. A slight change in the contractile activity caused by phentolamine, as observed in some preparations, may not be ascribed to the inhibition of α-adrenergic mechanism but due to some non-specific direct action of phentolamine (16). On the other hand, the spontaneous contractile activity in hypertonic solution changed remarkably in response to an extracellular concentration of CaCl₂ less than 2.5 mM: the amplitude of phasic contraction was reduced while the frequency and basal tension were increased when the concentration of CaCl₂ was decreased from 2.5 to 1.3 or 0.6 mM. In calcium-free solution, the spontaneous phasic contraction was completely abolished. Furthermore, Co²⁺, which is known to inhibit the transmembrane influx of Ca²⁺ (17-19), suppressed the spontaneous phasic contraction. Thus, it is assumed that Ca²⁺ plays an essential role in the generation of spontaneous contractile activity of the guinea pig portal vein in hypertonic solution. Increase in the frequency and basal tension in a lower concentration of CaCl₂ may be ascribed to a depolarization of cell membrane (20) or to a decrease in membrane stability.

The membrane properties of guinea pig portal vein in hypertonic solution have been described (9, 10) and the generation of the spontaneous spike discharge was also demonstrated in the present experiments. It has been reported that the automaticity and propagation of excitation in the guinea pig portal vein are not affected by tetrodotoxin (9). In the rat portal vein, Ca²⁺ is assumed to be essential for activation of the contractile mechanism and for maintenance of the spontaneous spike discharge in isotonic solution (21).

As shown in the present experiments, the contractile response to diltiazem of the isolated guinea pig portal vein in hypertonic solution was similar to that in isotonic solution. In addition, in hypertonic solution, the portal vein generated a spontaneous phasic contraction at regular intervals and with a large amplitude. Thus, the effect of diltiazem on the spontaneous contractile activity can be examined more quantitatively in hypertonic solution than in isotonic solution. It was found that in hypertonic solution, diltiazem reduced dose-dependently the amplitude of spontaneous phasic contraction, while the frequency of the contraction was increased. At the high concentration of diltiazem (2.2 × 10⁻⁵ M), the reversed, while the spontaneous spike activity suppressed by diltiazem was not recovered.
contractile activity was completely abolished with a decrease in the basal tension. It was also evident that the effects of diltiazem (2.2 x 10^{-6} M) were partly reversed by the addition of CaCl₂ (3 mM). Since Ca^{++} is assumed to be essential for the spontaneous contractile activity in hypertonic solution as described above, the present results suggest that diltiazem inhibits the mobilization of Ca^{++} which triggers the generation of phasic contraction in the guinea pig portal vein. The mechanism by which diltiazem increases the frequency of phasic contraction has not been elucidated. On the other hand, both spontaneous electrical and mechanical activities of the portal vein were completely inhibited at the high concentration of diltiazem (2.2 x 10^{-5} M). It has been reported that the spontaneous phasic contraction of the portal vein is associated with spike activity, although the recording of synchronized electrical and mechanical activities was rather difficult due to the segmental arrangement of the structures (11). Therefore, the inhibitory effect of diltiazem on the spontaneous phasic contraction may be ascribed, at least partly, to suppression of the spike activity.

As shown in the present experiments, the action potential evoked by electrical stimulation was inhibited by diltiazem and this effect was completely reversed by the addition of CaCl₂. Essentially similar effects have been demonstrated in the guinea pig taenia coli (5), whose action potential is mainly due to Ca^{++} entry (22-26). Thus, it is suggested that diltiazem suppresses the influx of Ca^{++} during the action potential in the portal vein of the guinea pig.

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