EFFECTS OF DILTIAZEM AND NITROGLYCERIN ON PROSTAGLANDIN F\textsubscript{2α} -INDUCED PERIODIC CONTRACTIONS OF ISOLATED HUMAN CORONARY ARTERIES

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This study characterizes the inhibiting effect of diltiazem and nitroglycerin on periodic contractions of isolated human coronary arteries. Isometric force of coronary ring segments from sixty-nine cadavers was recorded in a muscle bath. To quantify the experimental results, we used $3 \times 10^{-6}$ M prostaglandin F\textsubscript{2α} to induce the periodic contractions of a certain force. When diltiazem was added during the periodic contractions, the amplitude of oscillations gradually decreased until eventually oscillations ceased completely. The process prior to the cessation of the oscillations was characterized mainly by the inhibition of the contraction phase. The inhibition rate at the time of the complete cessation of oscillations was $49.3 \pm 6.3\%$ at $5 \times 10^{-7}$M. The time required for complete disappearance of oscillations was dependent on the diltiazem concentration. When nitroglycerin was added during periodic contractions, the oscillations did not disappear. Compared to the contraction phase, the relaxation phase was appreciably inhibited. With only $10^{-7}$M diltiazem, the rate of inhibition of the contraction phase was $22.0 \pm 7.7\%$, whereas the preliminary treatment with $5 \times 10^{-8}$M nitroglycerin led to a complete cessation of the oscillations, and suppression of the level of the contractions to a significantly greater extent, viz. $58.7 \pm 5.8\%$ (p < 0.001). Therefore, it is considered more effective in the treatment and prevention of coronary spasm to use diltiazem and nitroglycerin simultaneously rather than individually.

Calcium channel-blocking agents and nitroglycerin are now widely used in the management of patients with coronary spasm. Calcium channel-blocking agents are used mainly for the prevention of anginal attacks\textsuperscript{1─3} while nitroglycerin is employed in providing immediate relief from such attacks\textsuperscript{4─6} However, even upon the administration of calcium channel-blocking agents at adequate dosage, it is not possible to inhibit the attacks completely, but only to reduce the incidence of their occurrence\textsuperscript{7─8} In contrast, the administration of nitroglycerin during an attack of coronary spasm has a dramatic effect within a few minutes, but the inhibiting action is maintained only temporarily\textsuperscript{9} The mechanisms of these actions have yet to be clarified.

Kawasaki et al\textsuperscript{10} and Godfraind et al\textsuperscript{11} found that isolated postmortem human coronary arter-

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Fig.1. A typical example in which PGF$_{2\alpha}$ has been cumulatively added to an isolated human coronary artery (left panel). At a concentration of $10^{-6}$ M, incipient periodic contractions occurred, while at a concentration of $3 \times 10^{-6}$ M, the contraction reaction was reduced in both the contraction and the relaxation phases. In the right panel of the figure, the contraction rate in both phases corresponding to maximum contractions of the contraction phase is shown ($n=10$). From a concentration of $10^{-5}$ M, the contraction rate both in the contraction phase and the relaxation phase increased in a dose responsive manner.

ies developed periodic rhythmic contractions in a period of several minutes in a nutrient solution without exposure to vasoactive agents. Ginsburg et al. and Ross et al. also reported that coronary arteries obtained from recipient hearts of patients receiving cardiac transplant developed spontaneous periodic contractions in the same manner as our cadavers' coronary arteries.

The present study was thus undertaken to evaluate the different mechanisms accounting for the inhibiting behavior of coronary spasm exhibited by diltiazem and nitroglycerin using isolated postmortem human coronary arteries.

**METHODS**

Sixty-nine cadavers with no history of heart disease were used as the subjects of the study. They included 46 males and 23 females aged from 15 to 85, averaging 57.1 years. Immediately after death, the cadavers were placed in a room kept at a constant temperature of 8–9°C. The time from death to the isolation of the coronary arteries was 4.5 ± 3.3 hours (mean ± SD), the maximum interval being eight hours. The heart was removed and segments of the coronary arteries, each measuring approximately 1.5 cm in length, were collected from the proximal portion of the left anterior descending coronary arteries. The time that elapsed from the excision of the artery segments to the start of the experiments was no more than one hour. During this period, the specimens were preserved in a nutrient solution saturated with a mixture of 95% O$_2$ and 5% CO$_2$ at a temperature of 4°C. The coronary artery was cleaned of most adventitia and sectioned into 5 mm ring segments. The specimens were placed in a water bath within two minutes after removal from the cold nutrient solution. They were mounted over two parallel stainless steel rods, one of which was stationary and the other attached to an isometric strain gauge (TB 6111 T Nihonkoden Kogyo Co., Japan). The resting tension was adjusted to 1.5 g. The bathing solution was bubbled with a mixture of 95% O$_2$ and 5% CO$_2$ and was maintained at $37 \pm 0.3°C$. The pH of the solution was 7.4. The composition of the solution was as follows (in mM concentration): NaCl, 118; KCl, 4.7; CaCl$_2$, 2.5; MgCl$_2$, 1.2; NaH$_2$PO$_4$, 1.2; NaHCO$_3$, 25; and glucose, 11. The outer diameter of the coronary artery was 4.05 ± 0.5 mm ($n=123$).

To quantify the experimental results, we used prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) at a concentration of $3 \times 10^{-6}$ M to induce periodic contractions of a certain force. Before the start of the experi-

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![Graph showing the effect of calcium ion presence on periodic contractions induced by PGF$_2\alpha$.](image)

**Fig. 2.** The effect of calcium ion presence in the extracellular solution on the periodic contractions induced by PGF$_2\alpha$. The application of $3 \times 10^{-6}$ M PGF$_2\alpha$ in calcium-free solution was not found to produce periodic contraction. When the calcium ion level of the solution was raised to 2.5 mM, however, periodic contractions did occur. K-H: Krebs-Henseleit solution including 2.5 mM Ca$^{++}$.

ments, the preparations were allowed to equilibrate for 40–60 minutes in bathing media, during which time the fluid was replaced every 10–15 minutes. When spontaneous periodic contractions occurred, PGF$_2\alpha$ was not applied until after these contractions had ceased completely. At the end-of-the series of experiments, paraverine in a concentration of $10^{-4}$ M was added to produce maximum relaxation.

We have defined the level in which the maximum force occurs in the changes of the developed tension during spontaneous periodic contractions as the contraction phase and the level in which the minimum force occurs as the relaxation phase. These represent the values indicating the peaks and valleys of the oscillations. The results shown in the text and figures were expressed as mean values ± SEM. Statistical analysis was made by Student’s t-test.

Drugs used were diltiazem hydrochloride (Tanabe Pharmaceutical Co., Osaka, Japan), nitroglycerin (Nippon-Kayaku Co., Tokyo, Japan), prostaglandin F$_2\alpha$ (Ono Pharmaceutical Co., Osaka, Japan), and papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan).

**RESULTS**

Induction of Periodic Contractions of Human Coronary Arteries by PGF$_2\alpha$

Periodic contractions of ring preparations of human coronary arteries were induced by PGF$_2\alpha$ at a concentration of $10^{-6}$ M (Fig. 1). The developed tension increased dose-responsively within the range $10^{-6}$ to $3 \times 10^{-5}$ M for both the contraction phase and the relaxation phase. The amplitude of oscillation reached its maximum within the range $3 \times 10^{-6}$ to $10^{-5}$ M and dropped once a concentration of $3 \times 10^{-5}$ had been exceeded. The frequency tended to shorten as the concentration rose. With the application of $3 \times 10^{-6}$ M PGF$_2\alpha$, periodic contractions were induced in 80.5% of cases (99 out of 123 segments). The developed tension was $3893 \pm 199$ mg for the contraction phase and $2156 \pm 299$ mg for the relaxation phase.

The periodic contractions induced with $3 \times 10^{-6}$ M PGF$_2\alpha$ showed a complete cessation of the oscillations as a result of substitution of the PGF$_2\alpha$ by a calcium-free medium with 2 mM EGTA. When $3 \times 10^{-6}$ M PGF$_2\alpha$ was added to the medium, tonic contractions appeared; oscillations reappeared once the calcium ion concentration recovered 2.5 mM (Fig. 2).

Effect of Diltiazem on Periodic Contractions of Human Coronary Arteries Induced by PGF$_2\alpha$

When diltiazem was added during the periodic contractions of human coronary arteries induced by $3 \times 10^{-6}$ M PGF$_2\alpha$, the amplitude of oscillations gradually decreased until eventually oscillations ceased completely (Fig. 3, upper panel). The time required for complete disappearance of oscillations was dependent on the diltiazem concentration; the time was $90 \pm 20$ minutes for a concentration of $5 \times 10^{-7}$ M and $60 \pm 15$ minutes for a concentration of $10^{-6}$ M.

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(p < 0.001). At a concentration of 10^-7M, the amplitude decreased slightly, but it was not possible to observe a complete cessation of the oscillations within the examination period (Fig. 3, lower panel). The inhibition rates in the contraction phase for the concentration of 5 x 10^-7M after 30 minutes and 10^-6M after 10 minutes were increased significantly compared with those for 10^-7M. No significant difference was found during the entire observation time between inhibition rates in the relaxation phase in the range between 10^-7, 5 x 10^-7 and 10^-6M. The inhibition rate at the time of the complete cessation of oscillations was 49.3 ± 6.3% at a concentration of 5 x 10^-7M and 54.0 ± 6.9% at a concentration of 10^-6M. There was no statistically significant difference between the two inhibition rates. The process leading to cessation of the oscillations was characterized mainly by the inhibition of the tension during contraction phase, but in contrast, the tension during relaxation phase increased. Eventually these con-

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relaxation phase first rises to higher than its initial level within the range $5 \times 10^{-7}$M to $5 \times 10^{-6}$M. At higher concentrations, in the range $5 \times 10^{-5}$ to $10^{-4}$M, it is definitely inhibited. In the range $5 \times 10^{-7}$ to $10^{-6}$M, it was possible to observe a significant difference in the inhibition rates between contraction and relaxation phases ($p < 0.001$).

Effect of Nitroglycerin on the Periodic Contractions of Human Coronary Arteries Induced by PGF$_2\alpha$

The periodic contractions showed a disappearance in their oscillations by the application of diltiazem and the level of tension of contraction phase was maintained constant. However, when using nitroglycerin at that time, the level of tension of contraction phase was further suppressed (Fig. 5).

When nitroglycerin was added during periodic contractions, the amplitude of oscillations increased, but the oscillations did not disappear. On the upper panel of Fig. 6 an actual example of the addition of $5 \times 10^{-6}$M nitroglycerin is shown. Though the tension of relaxation phase after eight minutes showed an inhibition equivalent to 77% of the maximum developed tension, the inhibition rate for the contraction phase was only 9%. After a lapse of eight minutes, the inhibition rate remained unchanged both for the contraction phase and the relaxation phase. The lower panel is a time plot of the rates of inhibition of the contraction and relaxation phases obtained when varying the nitroglycerin concentration. The relaxation phase showed maximum

Figure 4 shows the difference between the rates of inhibition of contraction and relaxation phases of periodic contractions induced by PGF$_2\alpha$ during cumulative application of diltiazem. In the range $10^{-9}$M to $10^{-7}$M, there is no significant difference between the inhibition rates in either phase. From $5 \times 10^{-7}$M, the contraction phase is clearly inhibited. The contractions changed to tonic contractions.

Fig.4. The difference in the rates of inhibition of contraction and relaxation phases by diltiazem cumulatively added coronary artery induced by $3 \times 10^{-6}$M PGF$_2\alpha$. The respective inhibition rates were obtained by the method shown in the illustration. PPV = $10^{-4}$M papaverine; * $p < 0.001$.

Fig.5. The reaction obtained with the addition of $10^{-4}$M diltiazem and subsequent cumulative application of nitroglycerin after the induction of periodic contraction with $3 \times 10^{-6}$M PGF$_2\alpha$. The level of the sustained contraction after treatment with diltiazem was further suppressed by nitroglycerin.

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relaxation within 10 minutes at all concentrations, while the value for the inhibition rate after 10 minutes remained unchanged. It was found that the inhibition rate tended to be greater the higher the concentration. Thus, at a concentration of $10^{-8} \text{M}$, it was 83%, at $10^{-7} \text{M}$, 88% and at $10^{-6} \text{M}$, 94%. Similarly, it was found that the contraction phase also tended to reach a constant value after 10 minutes, but the inhibition rate after 10 minutes tended to be dose-dependent.

Nitroglycerin was used cumulatively to determine the rate of inhibition of the contraction and relaxation phases (Fig. 7). Compared to the tension level of contraction phase associated with a nitroglycerin concentration of $5 \times 10^{-9} \text{M}$, the tension level of relaxation phase was appreciably inhibited. The concentration range $10^{-7}$ to $10^{-5} \text{M}$, produced a significant difference ($p < 0.01$) (Fig. 8). The inhibition rate of the relaxation phase showed no statistically significant difference from the inhibition rate obtained when adding nitroglycerin during contractions produced by the administration of $3 \times 10^{-6} \text{M}$ PGE$_2$ in a calcium-free solution including 2 mM EGTA.

Synergistic Effects of Diltiazem and Nitroglycerin in Relation to the Periodic Contractions of Human Coronary Arteries Induced by PGE$_2$

We investigated the rates of inhibition of the contraction phase obtained when pretreating with $5 \times 10^{-9} \text{M}$ nitroglycerin for 60 minutes after induction of periodic contractions using $3 \times 10^{-6} \text{M}$ PGE$_2$, and by subsequently adding $10^{-7} \text{M}$ diltiazem (Fig. 9). With only $10^{-7} \text{M}$ diltiazem, the rate of inhibition of the contraction phase was $22.0 \pm 7.7\%$, whereas the preliminary treatment with $5 \times 10^{-8} \text{M}$ nitroglycerin led to a complete cessation of the oscillations, and suppression of the level of the contractions to a significantly greater extent, viz. $58.7 \pm 5.8\%$ ($p < 0.001$).

**DISCUSSION**

The results of this investigation have clearly demonstrated the difference in the action of diltiazem and nitroglycerin on the PGE$_2$-induced periodic contractions of isolated human coronary arteries. Kawasaki et al.$^{10}$ and Ginsburg et al.$^{12}$ have shown that the incidence of spontaneous periodic contractions in isolated human coronary arteries, the magnitude of developed tension, and the period of oscillations vary depending upon the time elapsed from death, the degree of atherosclerosis, and age. To carry out quantitative pharmacological investigations concerning the spontaneous periodic contractions occurring in the human coronary artery, it was necessary to induce this phenomenon at a high incidence under conditions kept as constant as

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Fig. 7. Response to cumulative addition of nitroglycerin during periodic contractions of human coronary artery induced by $3 \times 10^{-6}$ M PGF$_2\alpha$. PPV = $10^{-4}$ M papaverine.

Fig. 8. The difference in the rates of inhibition of contraction and relaxation phases following the addition of nitroglycerin during periodic contractions of the human coronary artery induced by $3 \times 10^{-6}$ M PGF$_2\alpha$. Both inhibition rates were obtained by the method shown in the illustration. The relaxation phase was clearly inhibited to a greater extent than the contraction phase. The symbol (*) indicates the statistical difference with respect to the contraction phase. Comparison showed no significant difference between the rate of inhibition of the relaxation rate of periodic contractions and the inhibition rate obtained after the cumulative addition of nitroglycerin in ionic contractions resulting from the administration of $3 \times 10^{-6}$ M PGF$_2\alpha$ in a calcium-free solution. * $p < 0.001$, ns = no significance; PPV = $10^{-4}$ M papaverine.

Fig. 9. The synergistic effect obtained by the combined use of diltiazem and nitroglycerin. The reaction was obtained by adding a low nitroglycerin concentration of only $5 \times 10^{-8}$ M prior to the addition of $10^{-7}$ M diltiazem during the occurrence of periodic contractions induced by $3 \times 10^{-6}$ M PGF$_2\alpha$. Compared with the reaction obtained when using diltiazem alone, the combined use of both drugs caused oscillations to cases gradually and helped to achieve a greater inhibiting effect. NTG = nitroglycerin.

Possible. Apart from PGF$_2\alpha$, it is also possible to use serotonin, noradrenaline, ergonovine and acetylcholine to induce periodic contractions. However, only PGF$_2\alpha$ has enabled us to obtain a high incidence (80.5%) and dose-responsive contraction curves for both the contraction phase and the relaxation phase.

Most spontaneous periodic contractions are
observed in the arteriolar smooth muscle cells\textsuperscript{15} and the portal-mesenteric veins\textsuperscript{16}; such contractions are rare in the larger arteries and in other veins. In the rat portal vein, periodic contractions are initiated by bursts of action potential\textsuperscript{16} and they might have a causal relation to the Ca\textsuperscript{2+}-spike\textsuperscript{17}. Spontaneous activities, whether tonic or periodic, depend on the availability of extracellular calcium\textsuperscript{18,19}. Johanson et al\textsuperscript{20} and Van Neuten et al\textsuperscript{21} attribute the genesis of myogenic tone to the spontaneous depolarization of the cell membranes of the vascular smooth muscles, and to increased calcium ion permeability caused by the opening of the specific potential-operated channels. It is tempting to suggest that the spontaneous periodic contractions of human coronary arteries are also dependent upon underlying electrical activity. Most of the calcium channel-blocking agents, including diltiazem, tend to inhibit the myogenic activity found in isolated rat portal veins\textsuperscript{22}. In the spontaneously active preparations of isolated guinea pig taenia coli, diltiazem suppressed both action potential and isometric contractions\textsuperscript{23}. As can also be clearly seen from Fig. 2, periodic contractions do not occur when PGF\textsubscript{2\alpha} is added to a nutrient solution from which the calcium ions have been removed. In this medium, only tonic contractions occur, and these tonic contractions are suppressed by the action of nitroglycerin. Thus, the action of diltiazem in the development of periodic contractions in human coronary arteries leads to the disappearance of the oscillations resulting from depolarization and is thought to inhibit only the tension produced by the calcium ion level of the extracellular fluid.

Nitroglycerin does not suppress the influx of calcium ions, and the inhibition of the relaxation phase — which cannot be achieved with diltiazem — may be possible as a result of inhibiting the calcium-ion-releasing mechanism of the storage sites. Ito et al\textsuperscript{24} have studied the smooth muscles of isolated pig coronary arteries, and established that nitroglycerin produces a non-selective suppression of the calcium-mobilization from the store sites with no noticeable change in membrane potential and membrane resistance, and suppresses the contractile response. Where the periodic contractions of human coronary arteries are concerned, nitroglycerin does not lead to the cessation of oscillations, but its action is attributed to inhibition in the relaxation phase. The initial rapid and marked suppression of relaxation phase after application of nitroglycerin might be due to hyperpolarization of the membrane which suppressed the activation of the voltage-dependent calcium-channel of the membrane, and thereby abolished or decreased spike activity\textsuperscript{25}.

Goldberg et al\textsuperscript{26} and Rich et al\textsuperscript{27} have demonstrated that calcium channel-blocking agents do not inhibit the diffuse mild vasospasm. For this reason, an explanation can be given on the basis of our experimental findings, which have led us to assert that the coronary artery tonus is not reduced by more than about 50\%, even when diltiazem is applied in concentrations exceeding those usually used in clinical practice, viz. at least 50 ng/ml\textsuperscript{28–30}. Even when diltiazem is administered at the maximum possible dose to remain within the effective concentration in the blood in order to prevent spasm, it appears impossible to achieve a significant increase in the effect of inhibition of spasm, although it may well be possible to reduce the time required until the maximum degree of dilatation of the coronary artery is reached.

The administration of diltiazem during an anginal attack caused by coronary spasm does not relieve the attack, whereas the application of nitroglycerin during an attack eliminates the attack completely within a period of no longer than a few minutes. The results obtained from our current investigations are in vitro results and do not exactly coincide with the above data, but it is legitimate to assert that the pharmacological behavior of these two agents in suppressing vascular contractions is very similar to the process of clinically observed relief of anginal attacks. From the results of our investigations, it can be seen that the use of diltiazem at clinically relevant and effective concentrations leads to a suppression of the maximum contractions in the isolated human coronary artery to an extent of no more than about 50\%. Thus, although diltiazem has only a limited or incomplete relaxation effect, it does have a preventive action on coronary spasm. In the event of intense or unrelenting coronary spasm of a kind and degree unsuppressible by diltiazem, the only alternative is to resort to the relieving effects of nitroglycerin. Clinical results are being reported that suggest there is a greater effectiveness in alleviating coronary spasms with combined therapy of the simultaneous administration of a calcium channel-blocking agent and nitroglycerin\textsuperscript{31–33}. The simultaneous suppression of the extracellular...
calcium ion influx and of the release of calcium ions from the storage sites leads to the inhibition of vascular contractions; thus, it is necessary to use diltiazem and nitroglycerin simultaneously in order to produce a vasodilatory effect.

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