Decreasing Potassium Conductance

— A Possible Mechanism of Phasic Coronary Vasospasm —

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IN vasospastic angina pectoris, the attacks and/or ST elevation frequently occur cyclically.¹ Despite a number of studies, however, the underlying mechanisms for coronary vasospasm and those for cyclical occurrence of the spasm are not well known. Recently, a new substance, nicorandil² was found to increase K conductance (g_K) of the myocardium³ and coronary smooth muscles⁴ and to alleviate anginal attacks in patients with variant angina pectoris.⁵ These findings led us to examine whether the sub-

stances which decrease g_K could induce cyclic (phasic) coronary vasospasm.

METHODS

Experiments were carried out using mongrel or beagle dogs of either sex weighing 7.0–15.4 kg. Animals were anesthetized with sodium pentobarbital 35 mg/kg, i.v. The chest was opened by fourth intercostal thoracotomy of the left side, and the heart was isolated. The left

Fig.1. Phasic contractions induced by 10^{-2} M 3, 4-DAP. The agent was administered at arrow.

Key Words:

Phasic spasm
3, 4-diaminopyridine
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Fig.2. Upper one thirds: Relationship between resting tension and peak tension of phasic contraction and that between resting tension and peak tension achieved during K⁺ contracture. Middle one thirds: Relationship between resting tension and transmural pressure calculated by Laplace's formula. Lower one thirds: Relationship between resting tension and peak tension expressed as g/ wet weight of the rings (ww) or g/area of the inner surface (mm²).

anterior descending or circumflex coronary artery was carefully removed and cut into rings 3 mm in width, which were suspended in 20 ml muscle bath filled with Krebs-Henseleit solution of the following millimolar composition: NaCl

TABLE I PHASIC CONTRACTION OF CANINE CORONARY ARTERY INDUCED BY AMINOPYRIDINES AND TETRAETHYLAMMONIUM CHLORIDE

Substances	Concentration (M)	Tonic contraction	Phasic contraction
2-AP	10^{-3}	1/2	0/2*
	3×10^{-3}	1/2	0/2
	10^{-2}	5/7	0/7
3-AP	10^{-3}	0/1	0/1
	3×10^{-3}	1/3	0/3
	10^{-2}	5/6	0/6
4-AP	10^{-3}	3/3	0/3
	3×10^{-3}	3/3	0/3
	10^{-2}	7/9	2/9
2, 3-DAP	10^{-2}	0/3	0/3
2, 5-DAP	10^{-3}	3/5	0/5
	10^{-2}	2/5	0/5
2, 6-DAP	10^{-2}	3/3	0/3
3, 4-DAP	10^{-4}	1/3	0/3
	3×10^{-4}	2/2	0/2
	10^{-3}	8/12	1/12
	6×10^{-3}	1/6	2/6
	10^{-2}	0/11	11/11
TEA	10^{-3}	0/6	0/6
	10^{-2}	6/8	1/8**
	1.5×10^{-2}	1/7	4/7**

* = number of preparations in which phasic contractions was induced/total number of the preparations tested; ** = transient

118, KCl 4.0, CaCl₂ 1.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 5. The solution in the bath was maintained at 37°C and aerated with a gas mixture of 95% O₂ and 5% CO₂. The pH of the solution was 7.37–7.49. The coronary rings were connected to an isometric transducer (Nihonkoden TB-611T) and tension developments were recorded on an ink-wrighting recti-recorder (Sanei 8S). In the first series of experiments, resting tension was changed stepwise from 0.5 to 3g in order to examine the relationship between the resting tension and tension developments. In the second series in which membrane potential was recorded, 4g resting tension was applied to avoid dislocation of the micro-

TABLE II PHASIC CONTRACTIONS INDUCED BY 3, 4-DAP

	Concentration (M)			
	10^{-3}	3×10^{-3}	6×10^{-3}	10^{-2}
<i>Dog</i>				
Femoral artery	0/3	0/3	0/3	2/3
Carotid artery	0/3	0/3	1/3	2/3
Basilar artery	0/3	0/3	0/3	3/3
Renal artery	0/3	0/3	1/3	2/3
<i>Pig</i>				
Coronary artery	0/3	0/3	1/3	2/3
<i>Monkey</i>				
Coronary artery	0/3	0/3	0/3	3/3
<i>Human</i>				
Coronary artery	0/4	0/4	1/4*	3/4

* = Phasic contractions appeared but were replaced by tonic ones.

electrode. In the third series in which chemical substances were applied, the resting tension was adjusted to 2g. The level of the tension achieved after application of 10^{-4} M papaverine HCl was called zero g. After observing the response of the coronary rings to 2.5×10^{-2} M KCl, the substances which decrease g_K were applied to the bath. The substances used were 2-aminopyridine (2-AP), 3-AP, 4-AP, 2, 5-diaminopyridine (2, 5-DAP), 2, 3-DAP, 2, 6-DAP, 3, 4-DAP and tetraethylammonium chloride (TEA)⁶⁻⁹

Similarly, epicardial coronary arteries were removed from anesthetized pigs and rhesus monkeys and cut into rings 3 mm in width. Also, coronary arteries were removed from cadavers (40–63 years old, 2 female and 2 male) within 7 hours after death, and cut into rings 3 mm in width. These coronary rings were suspended into the bath.

Recording Membrane Potential

In one series of experiments, canine coronary rings were placed in a box-like muscle bath filled

Fig.3. Changes in membrane potential of a canine coronary smooth muscle during phasic contraction. O = oscillation

TABLE III

A. Relationship between Membrane Potential and Developed Tension after Administration of 3, 4-DAP

Concentration of 3, 4-DAP (M)	Membrane potential (mV) <i>n</i> = 3	Developed tension (g) <i>n</i> = 3
0	48.5 ± 6.3	0
10 ⁻³	45.0 ± 7.0	0.2 ± 0.1
3 × 10 ⁻²	39.4 ± 8.3	0.4 ± 0.1
10 ⁻²	28.3 ± 10.0	0.7 ± 0.4
mean ± SD		

B. Changes in Membrane Potential during Phasic Contraction

	<i>n</i> = 6
Resting potential during relaxation phase	-39.1 ± 3.4 mV
Time lag between depolarization and contraction	1.23 ± 0.58 sec
Spike-like wave	5/6
Plateau level	-21.6 ± 8.0 mV
Oscillation	6/6
Potential before hyperpolarization	-30.5 ± 6.5 mV
Time lag between hyperpolarization and relaxation	9.4 ± 4.3 sec
mean ± SD	

with Krebs-Henseleit solution. After connecting them to an isometric transducer, a glass micro-electrode was inserted through the outer surface into the smooth muscles to measure membrane potential. The membrane potential was magnified by an amplifier (Sanei OR05), displayed on an oscilloscope (Nihonkoden VC-7) and was recorded on an ink-wrighting rectirecorder (Sanei 8S) simultaneously with of tension, and the changes in the potential after application of 3, 4-DAP were examined.

Drug Administration

All drugs used in this study were dissolved in distilled water and were applied to the bath during contraction phase of phasic contraction induced by 3, 4-DAP. The effects on cycle length of contraction, peak tension of contraction phase, and on tension during relaxation phase were examined. The time required for the reappearance of phasic contraction after drug administration was called reappearance time. These parameters were compared with the average values of 3 cycles before administration. When *P* value was below 0.05 (Student's *t* test), the change was considered to be significant.

Morphological Examination

According to Takeuchi, et al., the morphological characteristic of vasospasm is the appearance of perinuclear vacuolization in the vascular smooth muscles.^{10,11} Therefore, we examined whether perinuclear vacuolization appears during phasic contractions caused by 3, 4-DAP. During either the contraction or relaxation phase of the phasic contractions, the solution in the bath was rapidly removed and the mixture of 1% osmium acid and 1% potassium pyroantimonate was dripped onto the coronary rings. The morphological changes and the distribution of Ca²⁺ were examined electronmicroscopically.

RESULTS

1. Phasic Contraction of Coronary Artery

Following the application of 10⁻²M 3, 4-DAP, phasic contraction developed in all canine coronary rings tested (Fig. 1). Below this concentration, the appearance of phasic contraction was less frequent although tonic contraction did appear frequently (Table I). The phasic contractions were also induced by 4-AP, but less frequently. The cycle length of the contractions induced by 3, 4-DAP and 4-AP ranged from 30 sec to 1 hour, while the cycle length of those caused by TEA was within 1 min. When a

Fig.4. Electronmicroscopic changes associated with phasic contraction. Upper one thirds: before 3, 4-DAP. Middle one thirds: during contraction induced by 3, 4-DAP. Lower one thirds: during relaxation phase. The arrows indicate perinuclear vacuolization.

resting tension of 3g was applied, the peak tension achieved by canine coronary rings exceeded 4g. This value was 2.4 folds larger than that of K contracture (Fig. 2). The transluminal

pressure calculated by Laplace's formula (transluminal pressure = tension/ radius) was over 200 mmHg (Fig. 2).

Phasic contraction was not specific to the

Fig.5. Upper half: Effect of nicorandil (SG 75) on phasic contractions.
Lower half: Effect of nifedipine on phasic contractions.

Fig.6. Effects of changing pH on phasic contractions. The pH was lowered with HCl and was then elevated with NaOH.

canine coronary artery. The contractions were also induced by 3, 4-DAP in canine renal, basilar, carotid and femoral arteries and in swine, monkey and human coronary arteries (Table II).

2. Changes in Membrane Potential during Phasic Contractions

Following the application of 10^{-3} M 3, 4-DAP to canine coronary rings, the smooth muscles slightly depolarized and contracted slightly. With increasing concentration, the muscles depolarized and contracted further (Table III-A). Five to 30 min after application of 10^{-2} M, 3, 4-DAP, an abrupt lowering of membrane potential followed by relaxation appeared in 3 while a further elevation of the potential followed by an abrupt contraction appeared in the remaining one preparation. Thereafter phasic contractions developed. The relationship between the changes in membrane potential and tension was examined

in 6 cycles of 4 preparations. During relaxation phase, the average value of membrane potential was -39.1 mV and was elevated abruptly 1.2 sec prior to contraction. Following the abrupt elevation, a spike like wave appeared in 5 cycles, and which was followed by plateau formation. During contraction phase, the potential was gradually lowered despite a further increase in tension. Thereafter, oscillation appeared, which was followed by an abrupt hyperpolarization. Relaxation occurred 9.4 sec after hyperpolarization (Fig. 3 and Table III-B).

3. Morphological Changes Associated with Phasic Contraction

Perinuclear vacuolization was not observed in the smooth muscle fibers to which 3, 4-DAP had not been applied. On the other hand, the perinuclear vacuolization was observed in the majority of smooth muscle fibers in both contraction

TABLE IV EFFECTS OF VARIOUS SUBSTANCES ON PHASIC CONTRACTION OF CANINE AND HUMAN CORONARY ARTERIES

Substances	Concentration (M)	Phasic contractions	
		canine	human
SG 75	10^{-4}	↓	↓
Nitroglycerin	10^{-6}	—	↓
Nifedipine	10^{-6}	↓	↓
Verapamil	10^{-5}	↓	
Isoproterenol	10^{-7}	↓	↓
Prostacyclin	10^{-7}	↓	↓
W-7	10^{-4}	↓	↓
Chlorpromazine	10^{-4}	↓	
Ca ²⁺	6×10^{-3}	↓	
Saponin	10^{-5}	↓	
Ouabain	10^{-4}	tonic contraction	
Quinine	10^{-5}	tonic contraction	
A23187	10^{-5}	↑	
Caffeine	10^{-4}	↓	
Procaine	10^{-4}	↑	
Dantolorene	10^{-4}	↑	
Oligomycin	10^{-4}	—	
Tetrodotoxine	10^{-5}	—	
Prazosin	10^{-6}	—	
Phentolamine	10^{-6}	—	
Atropine	10^{-6}	—	
pH	< 7.2	↓	↓
pH	> 7.7	tonic contraction	

↑ = augmented; ↓ = eliminated; — = unchanged

and relaxation phases. No obvious difference in Ca distribution was observed between contraction and relaxation phases in this study (Fig. 4).

4. Effects of Various Drugs on Phasic Contractions

The phasic contraction was eliminated by 10^{-4} M or over of nicorandil (SG 75) which increases g_K .⁴ Although tension development was reduced, the phasic contraction was not eliminated by nitroglycerin. The phasic contraction was also eliminated by 10^{-6} M nifedipine and 10^{-5} M verapamil which blocks voltage dependent Ca channels, 10^{-7} M isoproterenol and 10^{-7} M prostacyclin which increases cAMP,¹³ 10^{-4} M W-7 and 10^{-4} M chlorpromazine which inhibit calmodulin,¹⁴ a high concentration of Ca²⁺ (6×10^{-3} M) which increases g_K ,¹⁵ 10^{-5} M

saponin which damages cell membrane,¹⁶ and by lowering pH below 7.2 with HCl or CO₂ which blocks Ca²⁺ influx¹⁷ (Fig. 6 and Table IV). The phasic contractions were replaced by tonic ones after application of ouabain which blocks Na-K ATPase,¹⁸ quinine which blocks Na-Ca exchange channel,¹⁹ and by elevating pH with NaOH (Fig. 7). The tonic contraction induced by quinine was replaced by phasic ones after application of Ca²⁺. Application of a high concentration of Ca²⁺ during tonic contraction induced by ouabain caused a slight decrease in developed tension but could not induce phasic contractions. A further increase in tension was induced by applying Ca²⁺ during tonic contraction induced by ouabain and quinine (Fig. 8). A Ca ionophore with A23187²⁰ increased the duration of contraction but not the peak tension. The phasic

Fig.7. Replacement of phasic contractions by tonic ones after application of ouabain or quinine, and the effect of Ca^{2+} on the tonic contractions.

Fig.8. Effects of ergonovine maleate on canine coronary smooth muscle activity before and after application of 3, 4-DAP.

contraction was slightly suppressed by caffeine which suppresses Ca uptake by SR and accelerates Ca efflux from SR²¹ augmented by procaine which suppresses Ca^{2+} -induced Ca^{2+} release from SR²² and dantrolene which suppresses Ca^{2+} efflux from SR²³ and was not affected by

oligomycin which inhibits Ca^{2+} uptake by mitochondria²⁴. The phasic contractions were not influenced by tetrodotoxin which inhibits Na channel, prazosin which blocks α_1 adrenergic receptors, atropine which blocks muscarinic receptors or by phentolamine which blocks both

Fig.9. Ergonovine-induced tension development after application of 3, 4-DAP or 3, 4-DAP and NaOH.

alpha₁ and alpha₂ adrenergic receptors (Table IV).

5. Effect of Pretreatment with 3, 4-DAP on Ergonovine-Induced Contraction

Effects of ergonovine on canine coronary smooth muscle activity was examined before and after the administration of low concentrations of 3, 4-DAP which did not cause phasic contraction. After the administration of 10⁻⁴ M 3, 4-DAP, ergonovine caused larger increase in tension development than before administration. The magnitude of tonic contraction caused by the same doses of ergonovine was increased by elevating the concentration of 3, 4-DAP. In addition, phasic contractions were frequently induced by 10⁻⁶ M ergonovine after application of 10⁻³ M 3, 4-DAP (Fig. 8). Ergonovine-induced contraction was further augmented by administration of NaOH (Figs. 8 and 9).

DISCUSSION

The results in this study indicates that the

substances which decrease g_K can induce phasic contractions not only in the coronary artery but also in the other arteries. Among the substances used, 4-AP, 3, 4-DAP and TEA could induce the phasic contractions. It is likely that these substances are more potent in decreasing g_K than the others. Tonic contraction was induced by low concentrations, and phasic contractions were induced by high concentrations of these substances. In addition, the magnitude of the depolarization of the smooth muscles increased by increasing concentration. This fact indicates that small decrease in g_K results in tonic contraction, and large decrease is required for phasic contractions.

Morphologically, perinuclear vacuolization, which is a characteristics of vasospasm, was observed in the preparation in which phasic contractions occurred. The transmural pressure calculated by Laplace's formula was over 200 mmHg. This fact indicates that the coronary artery can contract against 200 mmHg of blood pressure. Therefore, the phasic contractions

Fig.10. Schematic representation of possible mechanisms of phasic contractions induced by 3, 4-DAP and that of supersensitivity of coronary artery to ergonovine.

observed in this study can be called vasospasm in vitro.

The canine coronary artery contracted and then relaxed synchronous to the changes in

membrane potential. This fact indicates that electromechanical coupling plays the major role in the phasic contractions. A spike-like wave was frequently observed preceding contraction.

However, it was not observed in one cycle although the magnitude of contraction was not smaller, indicating that spike formation is not essential for contraction. Although there is no direct evidence, an abrupt decrease in g_K may have resulted in depolarization causing abrupt contraction and an abrupt increase in g_K in hyperpolarization, resulting in relaxation.

In this study, the phasic contractions were eliminated by nicorandil which increases g_K ⁴ and by nifedipine and verapamil which blocks voltage dependent Ca channel. Therefore, it is likely that 3, 4-DAP caused reduction in g_K , which in turn depolarized the cell membrane resulting in the opening of a voltage dependent Ca channel, and accordingly in contraction. In such a partially depolarized state, as has been shown in this study, a decrease in g_K may result in further opening of voltage dependent Ca channels and accordingly in influx of large amount of Ca^{2+} , leading to strong contractions which does not occur in physiological condition. The phasic contraction was replaced by tonic ones following administration of either ouabain which blocks Na-K ATPase,¹⁸ or quinine that blocks Na-Ca exchange channel.¹⁹ This fact may suggest that both Na-K pump and Na-Ca exchange channels are essential for phasic relaxation. Probably, the increased intracellular Ca^{2+} was exchanged by extracellular Na^+ through the Na-Ca exchange channel, and Na^+ which flowed in was pumped out by the Na-K pump and caused K^+ influx. In addition to this mechanism, the increased intracellular Ca^{2+} directly increased g_K ,¹⁵ which led to the relaxation of the smooth muscles. Also, an increase in intracellular H^+ , and accordingly a decrease in pH due to contraction, may have also reduced the Ca^{2+} influx,¹⁷ resulting in relaxation. When intracellular Ca^{2+} was thus decreased, g_K of the cell membrane decreased again, leading to another contraction. Although definite evidence is lacking, repetition of these ionic movements may be the fundamental mechanism of phasic coronary vasospasm (Fig. 10).

The canine coronary artery became supersensitive to ergonovine after application of low concentrations of 3, 4-DAP. Also, the arteries became hypersensitive to prostaglandin $F_{2\alpha}$, alpha adrenergic receptor stimulants, and to thromboxane A_2 analogues (unpublished observation). Under a partially depolarized state, the ergonovine may have caused a further opening of Ca channels directly or through

decreasing g_K , or may have stimulated receptor-operated Ca channels resulting in influx of large amount of Ca^{2+} .

Clinically, it is not known why coronary vasospasm occurs in a certain group of patients with angina pectoris, why the spasm is prone to occur at night and why the attacks frequently occur cyclically. Is an unidentified neurohumoral factor increased, or are the coronary smooth muscles supersensitive to a certain factor due to the changes in cell membrane caused by atherosclerosis or is there another unknown mechanism in such patients? The results of this study may suggest that an essential mechanism of vasospasm is a reduction in g_K . A slight lowering of pH was enough to eliminate the phasic contractions and a slight elevation was enough to augment the spasm. This fact suggests that during the day time, the pH of the environment of the smooth muscles is lowered due to exercise, inhibiting Ca^{2+} influx and accordingly vasospasm, and inversely while sleeping, the spasm appears due to elevation of pH.

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