Electrophysiological Study on the Effect of Several Drugs on the Free Wall of the Right Ventricle in Dogs with Pulmonary Stenosis

TSUNETAKA MATOBA[†]

Eight mongrel dogs were operated to produce pulmonary stenosis. Eight weeks after the operation, the free wall of the right ventricle with myocardial lesions was excised and a strip of the muscle was prepared for this study. The preparations immersed in oxygenated Tyrode's solution were given several drugs, such as Thiamine pyrophosphate (Cocarboxylase), Thiamine Tetrahydrofurfuryl Disulfide (TTFD), Thiamine Propyl Disulfide (TPD), Thiamine hydrochloride, Adenosine Triphosphate (ATP) and Desacetyl-lanatoside C (Lanatoside C). The effect of these drugs on specimens were observed electrophysiologically regarding transmembrane action potential, its duration and contractile tension as compared with the normal heart muscle.

SINCE THE YEAR 1936 the synthesis of thiamine has become a fascinating experience for physicians. As various derivatives of thiamine have been synthesized, KIMURA¹⁻³ tried to apply a very large dose of them to treatment of the heart disease and found their clinical effects on patients with myocardial lesions, especially with electrocardiographic changes of ST-T waves.

It seems biochemically established that thiamine participates in the intermediary carbohydrate metabolism in the liver, the brain and the nerves. And although an exact information on the mechanism of their action is not yet obtained, the thiamine, its derivatives and ATP are available for the treatment of heart diseases and others.

A considerable number of publications⁴⁻⁸ have appeared in recent years regarding the electrophysiological effect of thiamine and its derivatives on the normal heart muscle studied by using the ultramicroelectrode method developed by LING and GERARD⁹. However, little is known concerning the effect of them on the

heart muscle with pathologic changes.

The purpose of the present paper is to investigate and compare the effect of thiamine and its derivatives, ATP and Lanatoside C on the heart muscle of normal and pulmonary stenosis concerning the transmembrane action potentials, the duration of them and contractile tensions.

MATERIALS AND METHODS

All experiments were performed on mongrel dogs of either sex, weighing 9 to 20kg. They were anesthetized with Thiopental Sodium, 30 mg/kg intravenously, with supplements given as required and tied out on a board in a supine position. The right venous cardiac catheterization was carried out. The Cournand catheter, No. 7 or 8 F, was inserted from the right jugular vein into the pulmonary artery and retracted from its artery into the right ventricle while the pressure was continuously being recorded. Under the positive pressure respiration thoracotomy was conducted at the level of the third or fourth intercostal space, and the pericardium was excised. The pulmonary artery was dissected free from the aorta and constricted on the site 10 mm away from the root with a teflon tape. Thus, the lumen of this

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[†] The Third Department of Internal Medicine (Director: Prof. N. Kimura), Kurume University School of Medicine, KURUME

site was reduced to half of the original size calculated from the diameter; this led to the development of pulmonary stenosis and subsequently, to that of right ventricular lesions. Penicillin was administered in the whole area of the thorax before closing and at the same time injected intramuscularly. Of thirteen dogs, eight surviving pulmonary stenosis (PS group) were then left alone for the next 8 weeks. No exercise was forced upon them. Electrocardiograms and scalar-vectorcardiograms using McFEE system¹⁰ were followed up once a week under the general anesthesia.

About eight weeks after operation, the right venous cardiac catheterization was performed again and the retracted pressure between the right ventricle and the pulmonary artery was recorded. Under the same anesthesia and artificial respiration the heart was removed and immersed immediately in an oxygenated Tyrode's solution (25°C) until studied in the muscle chamber.

In the same way the free walls of the right ventricles of ten normal dogs (control group) were removed.

The composition of the Tyrode's solution in millimoles/liter was: NaCl 136.8 mM; KCl 2.68 mM; CaCl₂ 1.8 mM; Mg Cl₂ 0.49 mM; NaH₂PO₄ 3.3 mM; glucose 1 g and distilled, de-ionized water to make 1,000 ml, and was added the buffer solution, 60 ml, of 0.1 M phosphoric sodium (pH=7.3).

The muscle chamber had a final volume of 45 ml Tyrode's solution which was bubbled with 100 per cent oxygen gas and maintained at 36°C ($\pm 1^\circ$). The level of the solution in the chamber was kept constant by an over-flow tube. The muscle preparation, measuring about 5×12 mm in size, was mounted with the endocardial surface upwards on gum plate and held lightly by a small pin. Transmembrane resting and action potentials were recorded from the same cell in the endocardial layer throughout one experiment. The terminal Purkinje fiber was avoided. The resistances of the recording pyrex

glass ultramicroelectrodes filled with 3 M-KCl ranged from 5 to 16 megohms. The ultramicroelectrodes were used flexibly mounted ones.¹¹ The preparation was stimulated at a constant rate of 96 per minute through a thin copper wire (Nihon Kohden MSE-3R). The recording system employed consisted of a cathode follower input DC amplifier (Nihon Kohden MZ-3B), a cathode ray oscilloscope (Toshiba ST-1795A) and a camera (Nikon). The contractile tension was also recorded from the same preparation utilizing a force-displacement transducer (Nihon Kohden SBIT), a electronic manometer (Nihon Kohden MP3B) and a visigraph (San'ei Instrument Ltd. FR-201).

After experiments the preparations fixed in 10 per cent formalin were embedded in paraffin and stained with hematoxylin and eosin. Microscopic study was made on them.

The following drugs were applied directly to the muscle chamber: Thiamine pyrophosphate (Cocarboxylase), Thiamine Tetrahydrofurfuryl Disulfide (TTFD), Thiamine Propyl Disulfide (TPD), Thiamine hydrochloride (B₁-HCl), Adenosine triphosphate (ATP) and Desacetyl-lanatoside C (Lanatoside C). The dose of these drugs were divided into two kinds of concentration as follows: Cocarboxylase and ATP were 0.011 and 0.033 mg/ml, TTFD, TPD and B₁-HCl were 0.055 and 0.165 mg/ml, and Lanatoside C was 1.7×10^{-4} and 4.4×10^{-4} mg/ml, respectively. For each dose, five to eight experiments were carried out and the preparations once used were thrown away.

RESULTS

1) Evidence of myocardial lesions of the right ventricle

a) Cardiac catheterization

The Cournand catheter, No. 7 or 8F, was inserted from the right jugular vein into the pulmonary artery. The pressure in the pulmonary artery was greatly reduced. When the catheter was retracted from the pulmonary artery into the right ventricle, a characteristic pressure was recorded (Fig. 2). The mean gradient of the retracted pressure in PS group was increased as compared with that before operation ($P < 0.05$) (Table I). The mean gradient of preoperation was 11.2 ± 2.9 mmHg.

b) Histological findings

Micropathological examinations of the free wall of the right ventricle in all the PS and normal groups were done concerning myocardial lesions; hypertrophy, degeneration, inter-

Fig. 1. The experimental apparatus.

Fig. 2. Retracted pressure from right ventricle to pulmonary artery. PAP: Pulmonary artery pressure. RVP: Right ventricular pressure. Paper speed: 50 mm/sec.

TABLE I PRESSURE OF RIGHT VENTRICLE AND PULMONARY ARTERY IN PULMONARY STENOTIC DOGS

Number of Dog	RVP*	PAP*	RVP-PAP*
1	46 mmHg	22 mmHg	24 mmHg
5	50	24	26
6	62	26	36
7	38	26	12
8	68	22	46
10	58	22	36
12	—	—	—
13	46	26	20
Mean $\pm$ S. D.	52.5 $\pm$ 10.7	24.3 $\pm$ 2.3	25.4 $\pm$ 13.1

*RVP=Right Ventricular Pressure, PAP=Pulmonary Artery Pressure in Systolic.

stitial edema and fibrosis.

As indicated in Fig. 3, hypertrophy and degeneration in PS group were shown in all the preparations examined and tended especially to extend from the endocardial layer to

the pericardial one. Degenerative changes consisted mainly in the vacuolization and swelling of myocardial fibers, and partly in the disappearance of striates. The interstitial edema was prone to be slight in comparison to the above-mentioned changes. No fibrosis was seen in the preparations. In the dog No. 10 hypertrophy was observed in the endocardial layer of the left ventricle (Table II). No pathologic changes of myocardial fibers were also detected in all of control.

c) Electrocardiograms and scalar-vectorcardiograms

After the operation electrocardiogram and scalar-vectorcardiogram made by employing McFEE system were followed up once a week.

Electrocardiogram revealed no hypertrophy of the right ventricle, while the amplitude of R waves in all precordial leads and of T waves in $V_{2,3,5,6}$ were reduced to three-fourths or two-thirds of the control value.

TABLE II MICROPATHOLOGIC FINDINGS OF RIGHT VENTRICLE IN PULMONARY STENOTIC DOGS

Number of Dog	Hypertrophy			Degeneration			Interstitial Edema		
	End.	Myo.	Epi.	End.	Myo.	Epi.	End.	Myo.	Epi.
1	++	+	—	+	—	—	+	—	—
5	+	—	++	+	—	++	—	—	++
6	++	+	—	++	—	—	+	—	—
7	+	—	—	—	—	—	—	—	—
8	++	+	+	+	—	—	—	—	—
10	++	+	++	++	+	++	—	+	+
12	+	—	—	+	—	—	+	—	—
13	+	—	—	—	—	—	—	—	—

End: Endocardial layer, Myo.: Myocardial layer, Epi.: Epicardial layer. —, +, ++ and ++: Degrees of Pathologic Lesions; —: less than 10 per cent, +: 10 to 30 per cent, ++: 30 to 60 per cent, ++: more than 60 per cent.

Fig. 3. Histological findings of free wall of the right ventricle in pulmonary stenotic dog. Left showed normal myocardium. Right illustrated hypertrophy, degeneration and interstitial edema of myocardial fiber. (Mag.: above $\times 100$, under $\times 400$)

Vectorcardiogram showed that the QRS-T angle was opened from 4 to 8 degrees in the horizontal plane and other changes were not remarkable.

2) Transmembrane action potential

Transmembrane action potential obtained from the free wall of the right ventricle of the PS group did not varied significantly from that of controls: before administration, the action potential of PS group and controls had a mean value of $113.7 \pm 14.8 \text{ mV}$ and $114.1 \pm 14.6 \text{ mV}$, respectively. There was also no significant difference between before and after the administration of drugs (Fig. 4).

3) Duration of transmembrane action potential

A duration of transmembrane action potential (AP duration) was observed in all experiments as shown in figure 4. The AP duration was gradually shortened in all the experiments and revealed no prominent difference between

Fig. 4. Effect of drugs on transmembrane action potential and its duration. Action potential was not changed, however action potential duration was shortened. Above was control, and under 30 minutes after the administration.

the PS group and control. However, in case of

Fig. 5 A. Effect of Cocarboxylase on 90 per cent AP duration.

Fig. 5 D. Effect of Thiamine-HCl on 90 per cent AP duration.

Fig. 5 B. Effect of TTFD on 90 per cent AP duration.

Fig. 5 E. Effect of ATP on 90 per cent AP duration.

Fig. 5 C. Effect of TPD on 90 per cent AP duration.

Fig. 5 F. Effect of Lanatoside C on 90 per cent AP duration.

Fig. 6 A. Effect of Cocarboxylase on tension.

Fig. 6 D. Effect of Thiamine-HCl on tension.

Fig. 6 B. Effect of TTFD on tension.

Fig. 6 E. Effect of ATP on tension.

Fig. 6 C. Effect of TPD on tension.

Fig. 6 F. Effect of Lanatoside C on tension.

Cocarboxylase, TTFD and ATP, it tended to be prolonged by degrees 20 minutes after administration (Fig. 5).

4) Contractile tension

The preparation was stretched with an initial load of 607 ± 26.3 mg by using a pin and a force-displacement transducer and contractile tension was recorded. As illustrated in Fig. 6, observations were made for 30 minutes after administration of drugs.

The administrations of Cocarboxylase and TTFD in both doses (the former was 0.011 and 0.033 mg/ml, the latter 0.055 and 0.165 mg/ml) caused a marked increase in PS group as compared with controls. In case of Cocarboxylase the difference of PS group and control was statistically significant ($P < 0.05$ at 25 minutes and $P < 0.02$ at 30 minutes): in the PS group they had a mean value of 167 ± 27.2 per cent in the smaller dose and 170.6 ± 28.9 per cent in the larger one at 30 minutes, while in controls they had 123.6 ± 11.1 per cent and 122.6 ± 19.3 per cent, respectively. TTFD showed a significant difference between the PS group and control 30 minutes after administration ($P < 0.05$). However there were not any significant difference between the smaller and the larger one. It was noteworthy that the response of the heart muscle in the PS group to drugs, Cocarboxylase and TTFD was more intense.

In other drugs (TPD, B_1 -HCl, ATP and Lanatoside C), the contractile tension was gradually enhanced. However no significant difference between the PS group and the control were confirmed statistically. In Lanatoside C of the concentration of 1.7×10^{-4} mg/ml, the contractile tension of PS group was prominently increased at 30 minutes as compared with other. In all the cases of B_1 -HCl, the contractile tension showed a slight increase.

DISCUSSION

Many reports have been made as to the effect of thiamine and its derivatives on the transmembrane action potential, its duration and the contractile tension in the normal heart muscle of animals.⁴⁻⁸ However there are no reports on the heart muscle with pathologic changes. Our experiments were carried out

about the effect of drugs on the heart muscle of dogs with pulmonary stenosis as compared with the normal. Thiamine and its derivatives, ATP and Lanatoside C were administered both to a strip of muscle from the PS group which proved pathologically to be myocardial lesions and to the normal heart muscle in the chamber, and then action potential, its duration and contractile tension were observed. Our results obtained were that the duration of action potential was shortened by TPD, Thiamine-HCl and Lanatoside C and had a tendency of prolongation 20 minutes after administration of Cocarboxylase, TTFD and ATP. The contractile tension displayed a gradual increase in all the experiments except Cocarboxylase, TTFD and a part of Lanatoside C which showed a marked increase especially in the PS group.

Some investigations were reported⁴⁻⁸ regarding the effect of the above-mentioned drugs on the action potential, its duration and contractile tension: the results obtained were the prolongation of action potential duration, positive contractile tension and an unchanged action potential on the atrium of normal guinea pigs, rabbits, dogs and cats.

In our experiment action potential was not significantly different from that obtained from the PS group and that of controls. UHLEY¹² stated that in the experiment on the rats with cardiac hypertrophy no differences were noted in the action potentials recorded from the hypertrophied and normal groups. The same results were reported by KONISHI¹³ in his experiments of hypertrophied heart muscle of rabbits with pulmonary and aortic stenosis. It is clear from these data that transmembrane action potentials do not vary significantly between cardiac hypertrophied and the normal rabbits.

What is the relation between the action duration and contractile tension? NIEDERGERKE¹⁴ described that the experiments were performed with the results of a progressive increase in the contractile tension without showing any corresponding increase in the duration of action potential. KONNO and et al.⁴⁻⁸ reported concerning the data of the prolongation of both action potential duration and contractile tension in normal animals. And

SCHMIDT and CHANG¹⁵ found the prolongation of the duration of action potential and the transient prolongation of contractile tension. It may be that the factor of prolonging the duration of action potential is not identical with that of enhancing the contractile tension, while KAVALER¹⁶ assumed the same concept.

Our results obtained from the effects of thiamine and its derivatives, ATP and Lanatoside C were mentioned above. Why did the duration of action potential after administration of thiamine and its derivatives and ATP, especially TPD? The results obtained were interpreted as being given rise to under the following experimental conditions: 1) each solution of drugs is acid to litmus (i. e. pH = 5.2 in TTFD, less than 4 in TPD and 6.0 in B₁-HCl), especially the solution in large dose inclined to its acidity. 2) whether the compound is stable in the Tyrode's solution or not. 3) the time of observation is 30 minutes. 4) the preparations are injured by pulmonary stenosis. In this experiment the solution was not adjusted for pH with buffer. Therefore it may have so much potent depressant activity due to changes in pH on the heart muscle that the positive action of drugs themselves did not occur. The effective action of TPD may seem retarded so that its action was not observable within 30 minutes of the administration.

The mechanism of action of digitalis have been discussed by many investigators.¹⁷⁻²⁰ The ions of sodium, potassium and calcium activated the action of membrane transport which caused an increase of the excitation-contraction coupling. The role of the intercellular Ca⁺⁺ concentration is very important which in some manner is linked to the Na-K system. It is thought that thiamine, its derivatives, and ATP are responsible for the increase of the excitation-contraction coupling.

The enzymic mechanism of thiamine, its derivatives and ATP was previously described biochemically in details.²¹⁻²²

KIMURA et al.¹⁻³ reported that the administration of thiamine derivatives and ATP in large dose exert a potent effect on the cardiac insufficiency and myocardial damage and improved the electrocardiographic findings of ST-T waves in approximately 70 per cent of

patients with myocardial diseases. They assumed that these drugs activate the intermediary metabolism in the myocardium and thus produce the effective energy of a TCA cycle.

As mentioned above, thiamine derivatives and ATP may be valuable in the treatment of various heart diseases.

SUMMARY

1. Main pulmonary arteries in mongrel dogs were constricted in order to produce pulmonary stenosis. Eight weeks after operation myocardial fibers of the right ventricle were histologically detected and showed hypertrophy, degeneration and interstitial edema.
2. Electrocardiograms and vectorcardiograms revealed no hypertrophy of the right ventricle, but T waves and T loops were changed to some degree.
3. Observations were made concerning effect of drugs on the transmembrane action potential, its duration and contractile tension. The drugs employed were Cocarboxylase, TTFD, TPD, Thiamine hydrochloride, ATP and Lanatoside C.
4. Transmembrane action potentials obtained from the ventricular muscle cells of the PS dogs were not significantly different from that obtained from the normal cells. The same results were obtained from before and after administration.
5. The duration of the transmembrane action potential was shortened in general, while in case of Cocarboxylase, TTFD and ATP, it tended to be prolonged gradually 20 minutes after administration.
6. All the experiments were done to show the positive contractile tension. In cases of Cocarboxylase, TTFD and a part of Lanatoside C the results obtained were statistically significant between the PS and control groups. The heart muscle cells of the PS group responded to Cocarboxylase and TTFD more effectively than the control.

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