コイ摘出心臓の心機能と心電図に対する前負荷圧,後負荷圧および温度の影響

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Effects of Preload, Afterload and Temperature on the Cardiac Function and ECG in the Isolated Perfused Heart of Carp

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The isolated perfused heart of carp was examined to study the effects of input pressure (preload), ventral aortic pressure (afterload) and temperature on the intrinsic mechanical properties and ECG of the heart. Increase in both cardiac output ($\dot{V}_b$) and stroke volume (SV_h) and no marked change in heart rate (HR) with increasing preload indicates that the cardiac function is determined basically by preload. No marked response was observed in any of parameters of cardiac function to changing afterload.

SV_h, HR and ECG intervals were highly temperature-sensitive, but $\dot{V}_b$ was rather constant in a range of 15 to 35°C. At temperatures lower than 10°C, marked decrease in both $\dot{V}_b$, bradycardia and abnormal extension of ECG intervals were observed. At temperatures higher than 37.5°C, although atrial pulsation was still high, $\dot{V}_b$ began to rapidly decrease and the arrest of ventricular pulsation was often observed. At 40°C, a complete cardiac arrest was observed in all preparations.

The factors affecting cardiac function of teleost heart has been summarized in several papers.[1–5] Studies on the effect of venous pressure (preload) or vascular resistance (afterload) on cardiac function of teleost heart has been carried out in a few species, rainbow trout and some marine teleosts.[5–7]

With regard to effects of temperature on the cardiac parameters of teleost, it has been reported that increase in the ambient temperature generated the positive inotropy and negative chronotropy to the *in situ* heart of rainbow trout, sea raven and ocean pout.[4–7] Acute drop of ambient temperature produced reduction in heart rate, extension in ECG intervals[8] of intact carp. Little is known concerning the relation of cardiac function and ECG to environmental factors. In the present work, the isolated perfused heart of carp was examined to study the changes in the cardiac function (heart rate, cardiac output, stroke volume) and ECG elements according to changes in preload, afterload or temperature.

Material and Methods

Isolated Perfused Heart Preparation

Fifteen carp weighing 500–800 g obtained from a fish farm were acclimated at 25°C in a laboratory tank for over 2 weeks. A fish was anesthetized in 400 ppm solution of 2-Phenoxyethanol and instantly killed by a sharp cranial blow. The heart (part of the sinus venosus, the atrium, the ventricle and the bulbus arteriosus) was isolated taking care especially not to puncture the atrium adhering to the pericardium.

The isolated heart was immediately submerged in oxygenated saline (Na^+; 140, Cl^-; 132, K^+; 5.0, HCO_3^-; 12.0, $H_2PO_4^-$; 2.6, Mg^{2+}; 1.0, SO_4^{2-}; 1.0, Ca^{2+}; 1.3, glucose; 5.5 mmol·l^{-1}, heparin 500 units·l^{-1}). Teflon tubing of 2.0 mm in outer diameter was planted to the atrium at the junction with the sinus venosus as the input cannula. Teflon tubing of 1.7 mm in outer diameter was planted to the ventral aorta as the output cannula. The cannulae were secured with silk suture not to leak the perfusate.

The cannulated heart preparation was set on an acrylic plate and immersed in an organ bath of saline within a water jacket to keep the organ at a constant temperature of 25°C (Fig. 1-A). The acrylic plate had 3 pairs of Ag-AgCl electrodes arranged to make right angle each other (Fig. 1-B). Every pair of electrodes were set not to contact directly with the heart surface.

The isolated heart was perfused with the oxygenated saline of 25°C for initial 20 min ('acclimation period'). The input cannula was connected with a syringe barrel (20 m*l*, I in Fig. 1-A) to produce an input pressure. This pressure is referred to as 'preload.' The output cannula was connected

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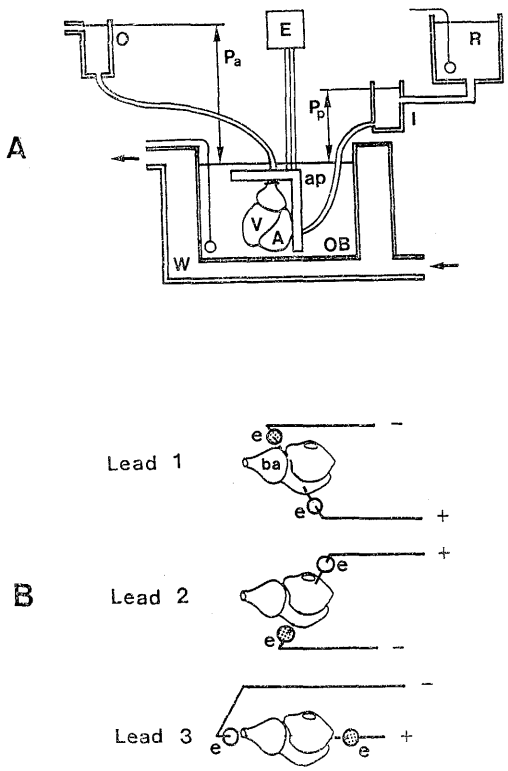

Fig. 1. A: Diagram of the apparatus used for perfusing the isolated heart. B: positions of the electrodes for recording ECG. R: perfusate reservoir; I: input pressure head; P_p: preload; *O*: output pressure head; P_a: afterload; E: electrocardiograph; **ap**: acrylic plate, OB: organ bath; W: water jacket for temperature control; **e**: Ag-AgCl electrode; *A*: the atrium; V: the ventricle; **ba**: the bulbus arteriosus.

with another syringe barrel (5 m*l*, O in Fig. 1-A) to produce an output pressure. This pressure is referred to as 'afterload'. The preload and afterload were kept at 2.0 cmH_2O and 10 cmH_2O, respectively, during the acclimation period. This value of afterload was to simulate the vascular resistance.

Minute volume of cardiac output ($\dot{V}_b$), stroke volume of cardiac output (SV_h), heart rate (HR) and ECG were recorded during the acclimation period. $\dot{V}_b$ ($ml \cdot min^{-1}$) was determined by measuring the total volume of saline ejected from the heart preparation in 1 min.

SV_h (m*l*) was calculated as follows;

$$SV_h\ (ml) = \dot{V}_b\ (ml \cdot min^{-1}) / HR\ (min^{-1})$$

HR (min^{-1}) was determined by counting QRS complexes in a minute. ECG was recorded with an electrocardiograph (Fukuda Denshi, FD-12A). Analysis of ECG elements was the same as described in the previous paper.[8]

The temperature of water flowing through the water jacket was controlled by a thermoregulator (Taiyo Kagaku Kogyo: CL-19).

These values are referred to as 'initial values.' Preparations which showed arrythmia during acclimation period were omitted.

Exp. 1 Effect of Preload and Afterload

The experiment was performed under a constant temperature of 25°C. The experimentation started in 15–20 min of the acclimation period, with a preload of 2.0 cmH_2O and an afterload of 10 cmH_2O. The effect of changing preload ranging from 1.0 to 5.0 cmH_2O was examined at a fixed afterload of 10 cmH_2O. The effect of changing afterload ranging from 5 to 30 cmH_2O was examined with a fixed preload of 2.0 cmH_2O.

Exp. 2 Effect of Temperature

The experiment was performed under a constant preload of 2.0 cmH_2O and a constant afterload of 10 cmH_2O. The experimentation started in 15–20 min of the acclimation period, with a saline temperature of 25°C. Temperature of saline in the organ bath and the perfusate was lowered from 25°C to 5°C at a rate of 0.2°C·min^{-1} and then raised from 5°C to 45°C at the same rate.

Results

Initial Conditions of Heart Preparations

The initial values of HR, $\dot{V}_b$, SV_h and ECG elements recorded during acclimation period are summarized in Table 1.

During acclimation period, no marked changes was observed in any parameters. In ECG elements during acclimation period, longer intervals and lower voltages than those of intact resting carp[8,11] were observed, but an abnormal profile was not observed in ECG of any lead.

Exp. 1

Effects of changing preload or afterload on $\dot{V}_b$, SV_b, HR and ECG elements are presented in Figs. 2–7.

Rising preload always produced an increase in $\dot{V}_b$ and SV_h, while there was little change in HR (Fig. 2-A~C). Time elements of ECG slightly extended with rising preload, but there was no significant difference in any element compared to

Table 1. Cardiac functions and ECG properties of the isolated perfused heart of carp during acclimation period. Values are expressed in $\bar{X}\pm SE$. Numbers in parentheses indicate the number of preparation examined

Heart mass	(g)		1.48 ±0.17	(11)
Preload	(cmH_2O)		2.0	
Afterload	(cmH_2O)		10.0	
Temperature	(°C)		25	
$\dot{V}_b$	($ml \cdot min^{-1}$)		5.34 ±0.84	(11)
SV_h	(ml)		0.087±0.014	(11)
HR	(min^{-1})		53.0 ±3.7	(11)
P_i	(s)		0.064±0.006	(7)
PQ_i	(s)		0.161±0.004	(7)
QRS_i	(s)		0.090±0.004	(7)
QT_i	(s)		0.489±0.032	(7)
P_v	(mV)	Lead-1	0.07 ±0.02	(7)
		Lead-2	0.15 ±0.04	(7)
		Lead-3	0.08 ±0.02	(7)
QRS_v	(mV)	Lead-1	0.23 ±0.05	(7)
		Lead-2	0.48 ±0.15	(7)
		Lead-3	0.17 ±0.02	(7)
T_v	(mV)	Lead-1	0.11 ±0.03	(7)
		Lead-2	0.14 ±0.04	(7)
		Lead-3	0.07 ±0.02	(7)

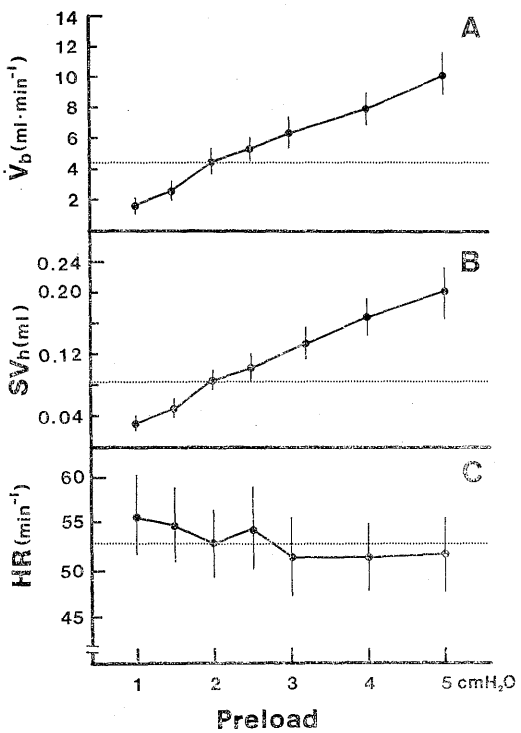

Fig. 2. The effect of prelad on $\dot{V}_b$, SV_h and HR of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. The dotted line indicates the level during acclimation period.

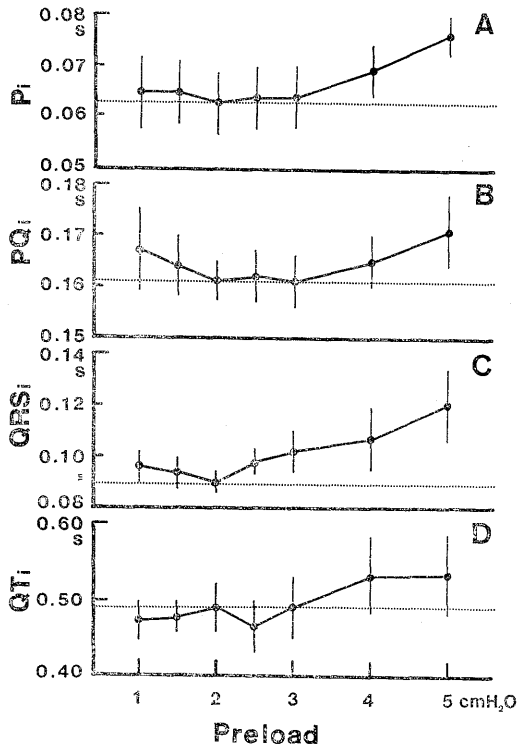

Fig. 3. The effect of prelad on the time elements of ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. The dotted line indicates the level during acclimation period.

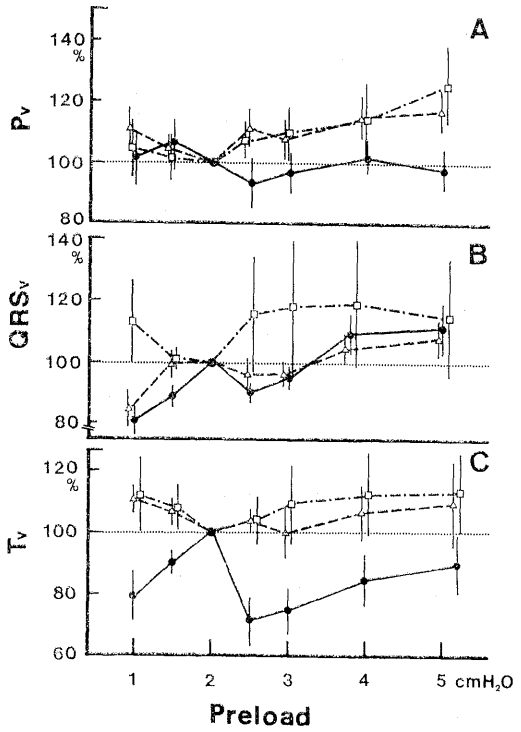

Fig. 4. The effect of preload on the voltage elements ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. ●—●: Lead-1, △---△: Lead-2, □·-·□: Lead-3. The dotted line indicates the level during acclimation period.

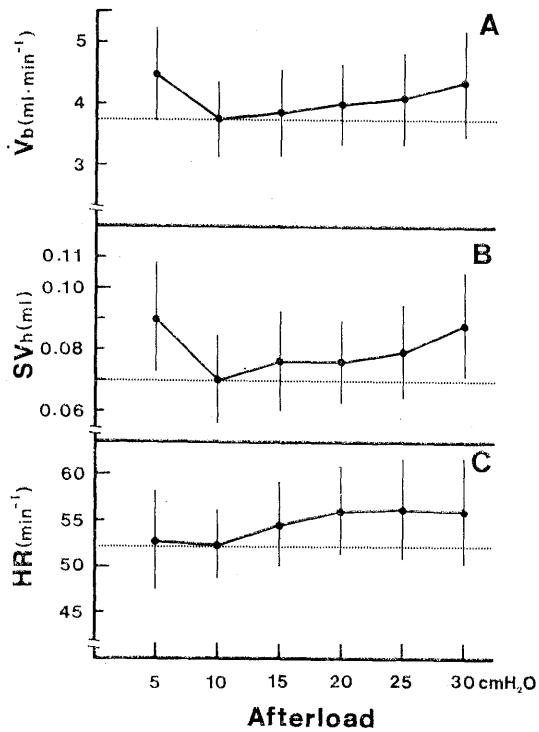

Fig. 5. The effect of afterload on $\dot{V}_b$, SV_h and HR of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. The dotted line indicates the level during acclimation period.

the initial value (Fig. 3-A~D). In relative values of voltage elements of ECG, rising preload produced a decrease in T_v at Lead-3 and a slight increase in P_v at Lead-2 and Lead-3, but a significant difference was not observed in any parameter compared to the initial value (Fig. 4-A~C).

Rising afterload from 10 to 30 cmH_2O produced a slight increase in the mean values of $\dot{V}_b$, SV_h and HR (Fig. 5-A~C). In time elements of ECG, rising afterload produced slight extension in P_i, QRS_i and QT_i but a significant difference compared to the initial value was not observed in any parameters (Fig. 6-A~D). In voltage elements of ECG, rising afterload produced an increase in P_v at Lead-2 and 3 and a slight increase in QRS_v at Lead-3, but a significant differences compared to the initial value was not observed in any of parameters (Fig. 7-A~C).

Exp. 2

An example of change in profile of ECG with changing temperature is presented in Fig. 8. Changes in cardiac functions with changing temperature are also presented in Figs. 9–11.

Minute volume of cardiac output ($\dot{V}_b$) was almost constant during 15~35°C. It rapidly decreased at temperatures of 5~10°C, and also at 37.5–40°C. It did not recover even when the temperature was restored from 40 to 25°C (Fig. 9-A).

Stroke volume of cardiac output (SV_h) showed an inversely proportionate change to heart rate with changing temperature in the range of 15 to 35°C. It rapidly decreased at temperatures of 5~10°C and to almost zero at 5°C. It also decreased to almost zero at temperature of 40°C (Fig. 9-B).

Heart rate (HR) changed proportionately to temperature in a range of 10 to 35°C. It decreased to almost zero at 5°C and also turned to rapidly decrease at temperatures of 37.5°C–40°C (Fig. 9-C).

Time elements of ECG showed a marked inversely proportionate changes with temperature. Especially, PQ_i and QRS_i at 10°C extended 5–7 times of the initial values (Fig. 10-A~D).

In voltage elements of ECG, an increase in both QRS_v and T_v in Lead-3 with decreasing temperatures was observed. While, there was no

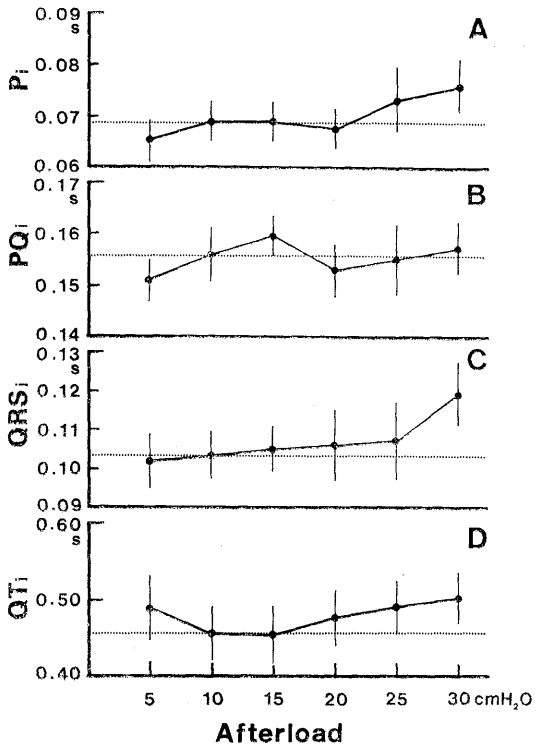

Fig. 6. The effect of afterload on the time elements of ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. The dotted line indicates the level during acclimation period.

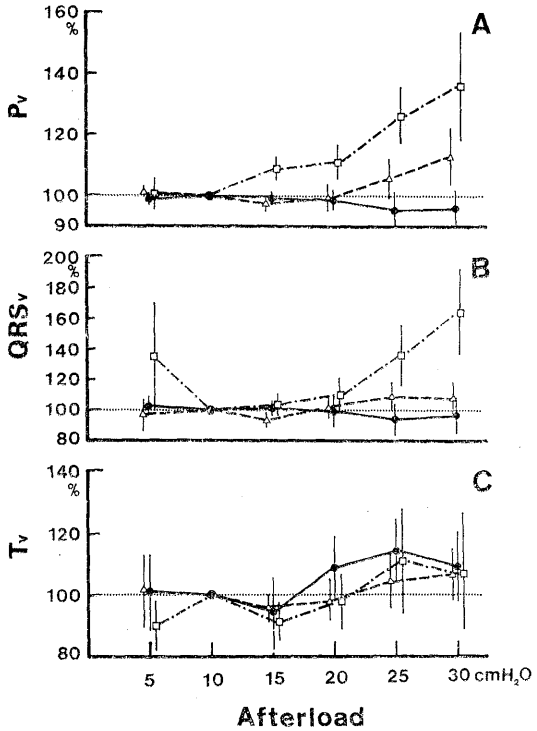

Fig. 7. The effect of afterload on the voltage elements of ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. ●—●: Lead-1, △---△: Lead-2, □-·-□: Lead-3. The dotted line indicates the level during acclimation period.

marked change in P_v in any lead (Fig. 11-A~C).

ECG profile was highly variable during the experiment. In the period when temperature dropped to lower than 10°C, QRS complex was impossible to detect because it became almost flat. And in the period when temperature was raised to 37.5°C, QRS complex often disappeared although P still remained (Fig. 8).

Discussion

Cardiac Functions and ECG during Acclimation Period

Minute volume of cardiac output ($\dot{V}_b$) of intact resting carp of which body mass and experimental temperature (554±43 g, 24.5±0.5°C) were close to those of the present study was estimated to be 20.2±5.3 $ml\cdot min^{-1}$ by an indirect method (Fick principle).[9] In the present study, $\dot{V}_b$ during acclimation period (5.34±0.84 $ml\cdot min^{-1}$) was considerably smaller than that of above-mentioned report. Minute volume of cardiac output is the product of heart rate (HR) and stroke volume of cardiac output (SV_h) or the differentce between

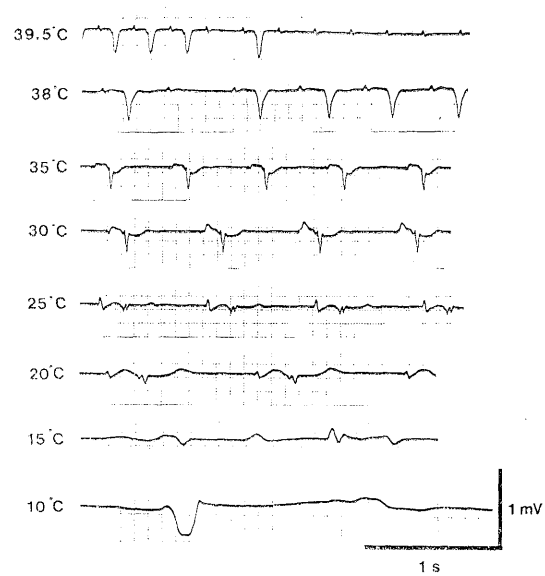

Fig. 8. A records of ECG (Lead-2) of the isolated perfused heart of carp with changing temperature. The duration of *QRS* complex showed extreme extension and $\dot{V}_b$ decreased to almosf zero at 10°C. The ventricular contraction was arrested by irregular atrioventricular block at 38°C. The record at 39.5°C showed the complete arrest of ventricular contraction.

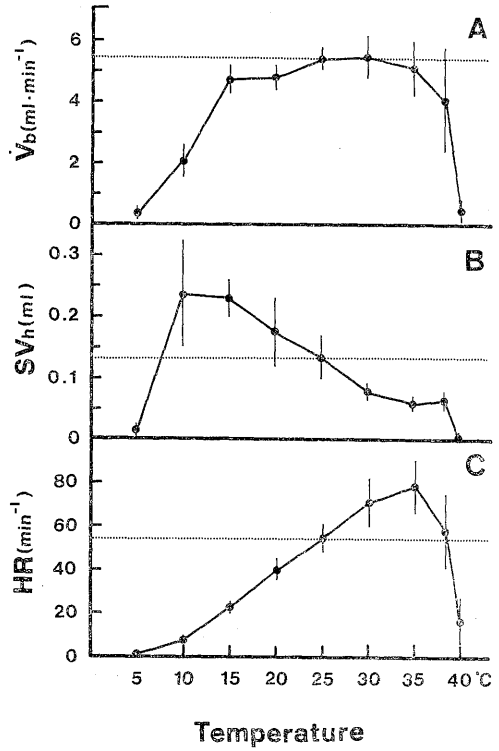

Fig. 9. The effect of temperature on $\dot{V}_b$, SV_h and HR of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm$SE. the dotted line indicates the level during acclimation period.

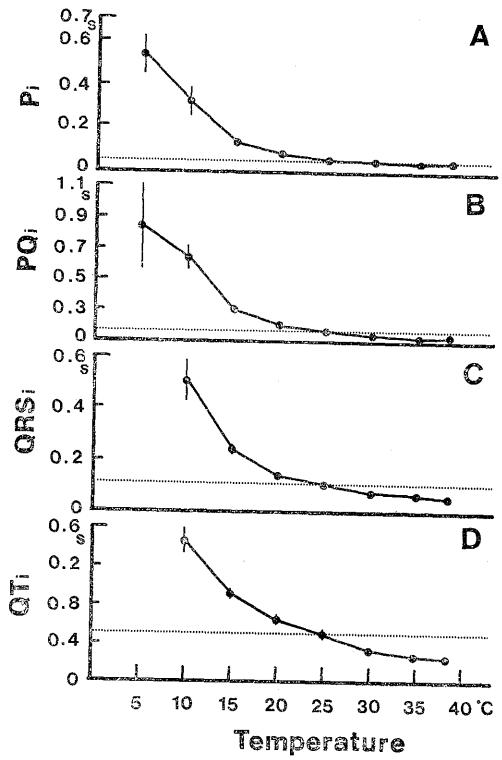

Fig. 10. The effect of temperature on the time elements of ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm$SE. The dotted line indicates the level during acclimation period.

the end-diastolic and the end-systolic volumes of the ventricle. As seen later, HR of an isolated heart during acclimation period was slightly higher than that of intact resting carp at temperatures close to the present study.[8,10] The small $\dot{V}_b$ of the isolated heart is therefore considered to be attributed to a small stroke volume.

It has been reported that the isolated perfused heart of rainbow trout was arrhythmic and its beating rate was lower than that of the heart in intact resting trout examined at the same temperatures[11,12] even if it was electrically stimulated. The reason of arrhythmic and inhibited beat of the isolated perfused heart has been explained by inhibition of the intrinsic rhythm generated by the sinoatrial pacemaker owing to incision of the sinus venosus.[11,12] In the present study, the sinus vensous was incised but the heart preparation did not show arrhythmia and its beating rate did not decrease during acclimation period. This suggests that the intrinsic rhythm of the isolated heart of carp was not inhibited by isolation. It may be explained by poor distribution of pacemaker in the sinus venosus and/or existence of another pacemaker. The pacemaker in the sinus venosus is reported to be located at the sinoatrial valves,[13] and another pacemaker has been found in the atrioventricular funnel.[13] The beating rate of our heart preparation did not decrease, probably because both pacemakers are left intact when isolated by dissection at the plane distant from the sinoatrial valves.

Heart rate (HR) of intact resting carp at temperatures close to the present study has been reported to be 27～52 min^{-1} (40 min^{-1} in the mean value).[8,10] In the present study, HR of the isolated heart during acclimation period (53 min^{-1} in the mean values) was slightly higher than that of above-mentioned reports. In the heart *in situ* of teleost, HR is controlled by firing frequency of pacemeakers,[1] inhibitory tone of the vagus nerve[2,13] and catecholamine levels in blood.[14,15] It is considered that higher HR of the isolated heart was owing to an absence of the vagal inhibition.

ECG during acclimation period showed longer time elements and lower voltage elements com-

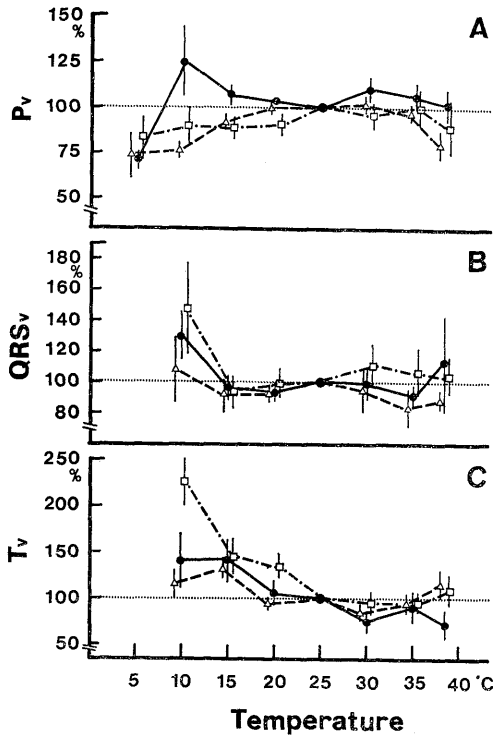

Fig. 11. The effect of temperature on the voltage elements of ECG of the isolated perfused heart of carp. Values are expressed in $\bar{X}\pm SE$. ●—●: Lead-1, △---△: Lead-2, □-·-□: Lead-3. The dotted line indicates the level during acclimation period.

pared to those obtained from the above mentioned intact resting carp.[8,10] It is considered that the difference in time elements was owing to the difference in composition between the perfusate and the blood and the difference in voltage elements was probably owing to the difference in positions of electrodes.

Effects of Preload and Afterload on Cardiac Functions and ECG

Rising preload clearly produced an increase in both $\dot{V}_b$ and SV_h without a marked change in HR, while increasing afterload had no marked or consistent effect on any parameter probably owing to constant preolad. These results are similar to those in isolated perfused heart of rainbow trout[11] and apparently indicate that the mechanical activities of isolated perfused heart of carp intrinsically depend on preload through the Frank-Starling mechanism.

In the isolated perfused hear tof rainbow trout, afterload representing the vascular resistance was raised from 30 cmH_2O to 70 cmH_2O but $\dot{V}_b$ did not decrease.[11,12] In the present study, change in afterload was limited in a range up to 30 cmH_2O because increasing in afterload up to 30 cmH_2O produced an extreme expansion of the bulbus arteriosus although there was no decrease in $\dot{V}_b$. This indicates that the capacity and elasticity of the bulbus arteriosus of carp differs from those of rainbow trout.

A slight extension in time elements of ECG with rising preload may indicate that electrical activity of the heart responds to increased stretch with an increase in contractile force, although the extension was not statistically significant. No consistent change in voltage elements of ECG with rising preload or afterload may be owing to a possible shift of excitation pathways. There has been no report concerning change in ECG elements with changing preload or afterload. The present study indicates that ECG elements are pressure-sensitive.

Effect of Temperature on Cardiac Functions and ECG

It was reported that an increase in ambient temperature from 5 to 20°C produced an increase in both $\dot{V}_b$ and HR and little change in SV_h in intact lingcod and flounder.[7,16] In the present study, $\dot{V}_b$ was kept in a constant level, while HR increased and SV_h decreased with increasing temperature, in a range of 15~35°C. The difference in the effect of temperature on $\dot{V}_b$ is considered to be owing to an inversely proportionate change in SV_h and HR in the present case.

When the temperature dropped to 10°C, $\dot{V}_b$ turned to decrease because of a substantial decrease in HR accompanied with a slight increase in SV_h. At 5°C, $\dot{V}_b$ showed a further decrease owing to decreases in both SV_h and HR.

When temperature was raised to 37.5°C, $\dot{V}_b$ decreased and an arrest of ventricular pulsation was observed although atrial beating remained constant. A decrease in $\dot{V}_b$ is considered to be owing to the arrest of ventricular pulsation.

A complete ventricular arrest was observed at 40°C and $\dot{V}_b$ did not recover even when the temperature was restored to 25°C. It is therefore considered that an arrest of ventricle at temperatures of 37.5°C and higher was owing to denaturation of the myocardium following decrement of atrioventricular conduction. Decrement of atrioventricular conduction is confirmed by escapes of QRS complex as seen in Fig. 8.

An increase in SV_h with decreasing temperature from 25 to 10°C is considered to be owing to an increase in the end-diastolic volume of ventricle and an extension in the duration of ventricular contraction. The former is explained by an extension of ventricular filling time with decreasing HR and constant preload. The latter is confirmed by an extension of QT_v.

When temperature dropped to 5°C, SV_h turned to decrease. The decrease in SV_h is probably owing to contracture of the ventricular muscle by an inactivation of the muscle cell.

A decrease in SV_h with increasing temperature has been observed in the heart in situ of rainbow trout and some marine teleosts.[4,6] In intact lingcod and flounder, an increase in ambient temperature ($3 \sim 5°C \cdot h^{-1}$) produced an increase in HR and little change in SV_h.[7,16] Little change in SV_h in these species was interpreted by an increase in venous return canceling the effect of an increase in HR.[1,4] Their viewpoint was partly supported by the fact that number of muscle capillaries in crusian carp was reduced by acclimatization from winter conditions to summer ones.[17] In the present study, SV_h decreased with increasing temperature. The decrease in SV_h is considered to be attributed to a reduction in ventricular filling time owing to increasing HR and constant preload.

Heart rate (HR) decreased with decreasing temperature. In the denervated heart, heart rate depends on the firing frequency of the pacemaker. The firing frequency depends on the time for membrane potential to reach the threshold level from the resting level. A decrease in HR with decreasing temperature observed in the present study may be caused by a longer time required for the membrane potential to reach the threshold level from the resting level, estimated from the observed extension of time elements of ECG.

Reduction in HR with decreeasing temperature was severer in the present study than that in intact carp.[8] The difference may be attributed to the difference in time required for thermal conduction from the ambient water to the heart.[18]

Time elements of ECG were highly temperature-sensitive. Extension of time elements of ECG with decreasing temperature from 25 to 10°C is similar to that of the heart *in situ* of intact carp[8] and indicates that conduction in myocardium is retarded by lower temperature. On the other hand, shortening of time elements of ECG with increasing temperature from 25 to 35°C indicates that conduction in myocardium is accelerated by higher temperature. However, a decrease in the degree of shortening of time elements of ECG at temperatures higher than 25°C may indicate that the acceleration of conduction in myocardium declines at the higher temperature.

When the temperature dropped to 10°C, increase in both QRS_v and T_v in Lead-3 were observed. It was reported that an acute drop of ambient temperature produced a slight increase in voltage elements of ECG in intact carp[8] and shift in cellular cation concentrations in goldfish.[19] In the clinical electrocardiography, profile of ECG is greatly affected by intracellular cation (Ca^{2+}, Na^+, K^+) concentrations.[20] In the present study, an increase in both QRS_v and T_v in Lead-3 at 10°C may be owing to a possible change in intracellular cation concentrations and/or a possible shift in positions of electrodes by expansion of the ventricle.

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