대한생리학회지 : 제20권 제 1 호

Effect of Thyroid Hormone on the Electrical Activity of Rabbit Heart

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=국문초록=

토끼심장의 전기적 활동에 대한 갑상선 호르몬의 영향

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갑상선 호르몬의 표적기관(target organ) 중의 하나인 심장이 hyperthyroid 상태에서 심박동수의 증가, 부정맥 그리고 세포 수준에서 sodium, potassium pump 기능이 항진되는 것으로 보고되고 있다.

증진된 pump 기능과 더불어 positive chronotropic effect는 심장의 향도잡이로 알려진 동방결절과 심방근에 어떤 변화에 의하여 발현되는지 알아보기 위하여 3~6개월령의 토끼(체중 약 1.5 kg 내외)에 3,3′,5-*l*-triiodothyronine(T_3)을 투여하여 실험적으로 hyperthyroid 상태를 유도한 다음 심장세포 내에 유리미세전극을 삽입하여 기록한 결과 다음과 같은 성적을 얻었다.

1) 심박동수는 투여전(Day 1) 169.0±28.0 beat/min 에서 264.2±18.9beat/min(Day 7)으로 156% 가량 증가되었고 체중은 투여전 체중의 68.2±2.0%로 현저한 감소를 보였다.

2) T_3 투여군에서 활동전압기간은 148.0±29.1 msec 에서 107.0±13.6 msec 로 감소하여 심박동증가를 반영하였으나 그 외의 활동전압 parameter 에서 대조군과 유의한 차를 관찰할 수 없었다.

3) 세포막에 대한 potassium ion 투과성의 영향을 알아보기 위하여 10, 15, 20 mM-K^+ Tyrode 용액을 사용한 결과 SA node 에서 15mM K^+에서 활동전압 발사가 대조군에 비해 현저하게 감소하였고,

4) T_3 투여군에서 심방근의 안정막전압 탈분극 정도는 15 mM($p<0.05$), 20 mM K^+ Tyrode 용액($p<0.05$)에서 대조군보다 유의성있게 낮았다.

5) Sodium, potassium pump 기능은 대조군에 비해 동방결절(13.4±1.1 vs. 19.5±7.1 mV, $p<0.1$)과 심방근(15.1±5.5 vs. 25.8±10.0 mV, $p<0.025$)에서 모두 높은 값을 얻었다.

6) T_3에 의한 calcium ion의 영향을 알아보기 위하여 Ca^{++} channel blocker로 $MnCl_2$를 사용한 결과 T_3 투여군의 동방결절은 정상대조군의 것보다 낮은 농도의 $MnCl_2$ 용액에서 흥분성의 감소를 보였다.

INTRODUCTION

The relationship between thyroid and heart function was reported firstly by Parry in 1785. Since then, many investigations have been performed about the effect of thyroid hormone on heart function (Freedberg and Hamolsky, 1974).

In earlier studies there were many contro-

versies whether the effects of thyriod hormone on heart were developed through the direct and/or indirect ways (Freedberg and Hamolsky, 1974). Many workers who studied the action of thyroid hormone on heart suggested that thyroid hormone may regulate the number of cardiac alpha-adrenergic receptors (Sharma and Banerjee, 1978) and increase the number of beta-adrenergic receptors without a change in their affinity (Tse *et al.*, 1980; Furukawa *et al.*, 1982). However, recent studies on experimentally induced hyperthyroid animal suggested that increased cardiac intrinsic rate was not caused by adrenergic receptor supersensitivity but by a direct ways (Freedberg and Hamolsky, 1974). Sugiura *et al.* (1974) explored the effect of triiodothyronine (T_3) *in vitro* on cardiac rate of isolated rabbit atria; Triiodothyronine in media initiated beats of the ventricular single cell of the chick embryo (Wollenberger, 1964). Moreover, it was found that T_3 accelerated atrial-to-His' bundle (A-H) and His' bundle-to-ventricle (H-V) conduction time in thyrotoxic dog (El-Shahaway *et al.*, 1972). These affirmative results are likely to reveal as an evidence of direct action of T_3 on intrinsic heart rate.

The acceleration effect of T_3 on pacemaking of sinoatrial (SA) node, that is, the positive chronotropic effect, may explain vagal action inhibited (Heimbach and Crout, 1972) and that the related ionic currents due to ion transport on SA node are underlying.

Electrophysiological study on hyperthyroid rabbit atria (Arnsdorf and Childers, 1970) offered the suggestion that tachycardia was resulted from acceleration of repolarization, which was caused by the increased outward membrane current and rapid inactivation of inward sodium current. Potassium conductance was altered during action potential of SA node in hyperthyroid rabbits, that is, decreased potassium conductance in diastolic depolarization and increased potassium conductance in repolarization (Johnson *et al.*, 1973). And they suggested that thyroid hormone changes calcium ion binding to SA node cell membrane and thereby alters the parameters of the pacemaker transmembrane potential, although an increased sodium conductance relative to potassium conductance in diastolic depolarization is taken into consideration. Because the increased sodium conductance may not be a significant factor in diastolic depolarization in atrial pacemaker (Yamagishi and Sano, 1966).

Another effect of thyroid hormone is accelerated the sodium, potassium pump activity. Triiodothyronine increased sodium potassium pump activities significantly with a decreased sensitivity of cardiac sodium, potassium ATPase to digitalis (Curfuman *et al.*, 1977: Shimada and Yoshio, 1978).

There seems to be no report about the effect of T_3 on the pacemaker activity and resting membrane potential of atria. These problems will be remained obscure unless the ionic currents on pacemaker are systemically studied. Therefore, this study was undertaken to observe the effects of T_3 electrophysiologically on SA node and isolated rabbit atria.

MATERIALS and METHODS

1) Animals

Three to six month-old New Zealand White rabbits (900~2200 gram, av. 1450 gram) used in this experiments were devided into two groups, control and experimental group. These rabbits were kept to be adapted for 7 days.

The experimental animals were injected intramuscularily with thyroid hormone(T_3), 0.2 mg/kg/day for 7 days.

2) Preparation of Thyroid Hormone

The drug was prepared by dissolving sodium-l-triiothronine(Sigma chemical Co.) in physiological saline solution made alkaline by 0.1M NaOH and adjusted to pH 8.2 by adding 0.1 M HCl just before the injection. Body weight was measured and heartbeat was checked 3 times per day to estimate the thyroid state *in vivo*.

3) Experimental Procedure

The rabbit was stunned, bled by cutting the carotid artery. Being removed quickly, the heart was transferred to dissecting chamber filled with O_2 saturated Tyrode solution. In the dissecting chamber, sinoatrial (SA) node tissue and atria were seperated from the whole heart, and the wet weight of ventricular tissue was measured, expressed as grams per unit body weight (g/kg).

① **Electrophysiological recording:** SA node action potential and atrial membrane potential were obtained intracellularly by conventional microelectrode technique. Electrical activity was displayed on storage oscilloscope(Tektronix model 5113) and physiograph(Narco Biosystems MK-IV-P, 4 channel).

② **The general protocol for the study:** After recovery of tissue function in normal Tyrode solution for 30~60 minutes, impalements of electrode into the SA node cell or left atrial muscle cell were conducted. All experiments were proceeded after confirming the normal tissue status by uniform recording of electrical activity. After applying the experimental solution, time intervals for recovery of tissue under normal Tyrode solution were given for 20~30 minutes.

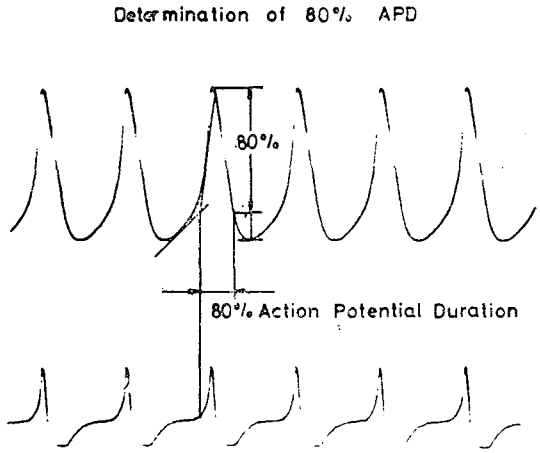


Fig. 1. Method of determination of 80% action potential duration(80% APD).

The electrical parameters measured were maximal diastolic potential(MDP), dV/dt, amplitude and duration of action potential. Fig. 1. shows the determination method of the action potential duration at 80% repolarization(80% APD). The action potential durationat 80% repolarization was determined from the threshold potential (start point of the maximal rate of rise of the action potential) to 80% repolarization potential (vertical length from MDP to the peak of amplitude was set to 100%). The firing rate of SA node cell was measured from the peak-to-peak time interval of the action potentials on the stored trace of oscilloscope screen. The atrial resting membrane potential was measured on the digital multimeter(Tektronix DM502A). The value of pump activities was expressed in mV (the difference between resting potential in potassium-free and hyperpolarized potential in 13 mM potassium Tyrode).

4) Experimental Solutions

The perfusate flowing through the horizontal tissue chamber at a rate of 2 ml/min was equilibrated with 100% O_2 and kept pH 7.4 at 36±0.5°C in all experiments.

The normal Tyrode solution contained NaCl 140, KCl 3, $MgCl_2$ 2, $CaCl_2$ 2, Tris-buffer 10, and added glucose 5.5mM. High potassium Tyrode solutions made as follows;

in order to make a high potassium Tyrode solution (such as 10, 15, 20 mM potassium), the precise amount of 1 M KCl was added to potassium-free Tyrode solution.

Manganese Chloride Tyrode solution was used as calcium channel blocker because of its reversible effect.

5) Statistical Analysis

The data are presented as means±standard error. To analysis the statistical significance, the Student t-test was used.

RESULTS

1) Effects of T_3 Administration

Chronic administration of 3,3',5-triiodo-l-thyronine, sodium salt (T_3) produced marked

Table 1. Changes of body weight and heartbeat in hyperthyroid group

	Rate of BW loss	Heartbeat(/min)
Day 1	1.0(36, 1463±353gram)	169.7±28.0(108)
Day 2	0.936±0.05(36)	190.1±26.3(108)
Day 3	0.864±0.06(34)	213.9±20.4(102)
Day 4	0.801±0.07(33)	228.0±19.7(99)
Day 5	0.762±0.06(29)	243.8±19.5(87)
Day 6	0.722±0.06(22)	245.8±19.8(52)
Day 7	0.685±0.06(18)	264.2±18.9(51)
Day 8	0.682±0.05(5)	

i) Numbers in parenthesis of rate of body weight(BW) loss column is the number of animals. Body weght of the first day(Day 1) is served reference for calculation.

$$\text{Rate of BW Loss} = \frac{\text{(Body weight of corresponding day after Day 1)}}{\text{(Body weight at Day 1)}}$$

ii) Numbers in parenthesis of Heartbeat column indicate the number of measurement. The measurement of heartbeat was repeated three times per one animal.

Table 2. Comparison of the cardiac parameters of control group with those of hyperthyroid group

	Control	Hyperthyroid	P value
Heartbeat(beat/min.)	169.7±28.0(108, Day 1)	264.2±18.9(51, Day 7)	<0.005
Ratio of body weight loss(%)	100(36, Day 1)	68.2±5.0(18, Day 7)	<0.005
Ventricular wet weight per unit body weight(g/kg)	1.79±0.16(8)	1.96±0.35(13)	—
APD 80% (msec.)	148±29.1(23)	107±13.6(30)	<0.005

i) Ventricular weight was measured with wet condition just after seperation.

ii) The parameters measured in euthyroid state were conducted as control values.

iii) APD 80% means the action potential duration from the point of threshold to the pointoı 80% repolarization. See Fig. 1.

physiological changes of the rabbits. At Day 1, T_3 was injected after measuring both the initial body weight and heartbeat *in vivo*. Table 1 and Fig. 2 show that the body weight was decreased to 68% of the initial body weight (D1) while the beat was increased from 169.6±28.0 to 264.2±18.9 beats per minute, by ca. 156%. The above result offered the evidence that tachycardia was induced by the injection of T_3.

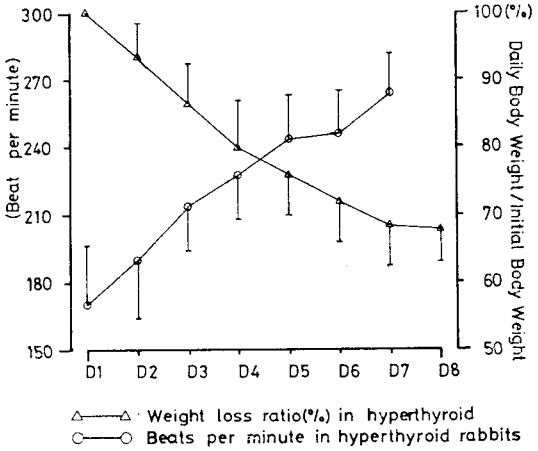


Fig. 2. Changes in body weight and heartbeat in T_3 treated group.

Eighty percent of action potential duration (80%APD) on SA node *in vitro* was matched with the increase of heartbeat(Table 2).

2) Response to External High Potassium Tyrode

In this study, the changes in action potential, beat in SA node, and resting membrane potentials in atrial muscle cells were observed through the intracellular electrical recordings obtained in high potassium Tyrode such as 10, 15, 20 mM potassium. Spontaneous firing

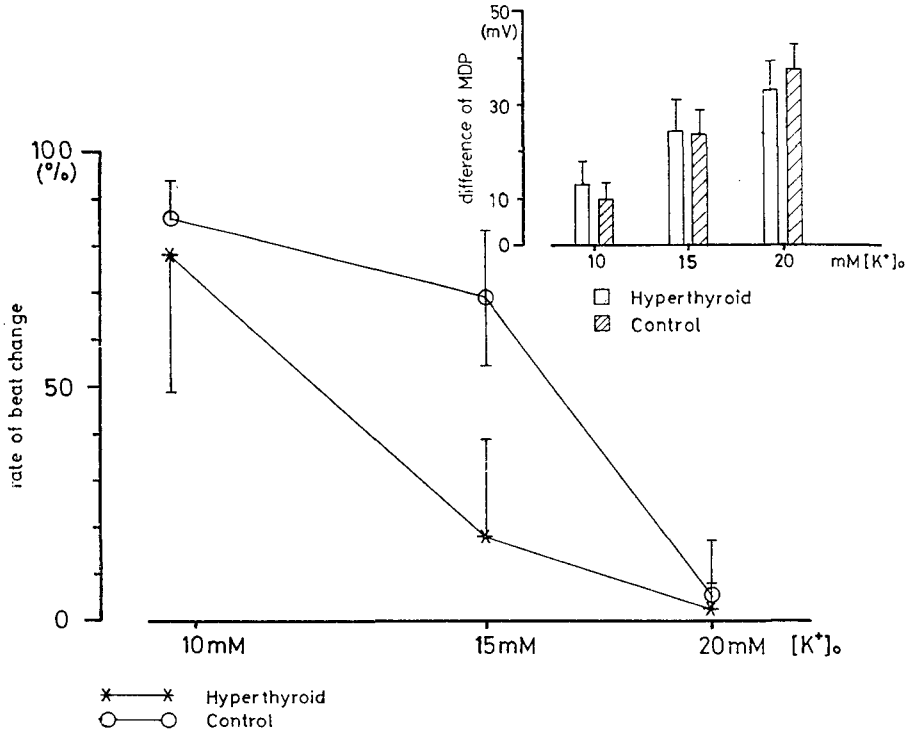
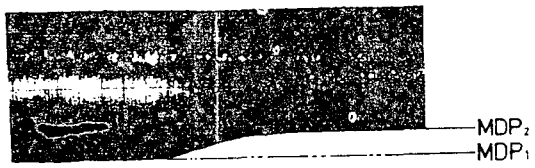


Fig. 3. Differences of maximal diastolic potential and rate of beat changes at various high potassium Tyrode solution.

Table 3. Changes in parameters of action potential in SA node at various high K^+ Tyrode solution

	Concentration in mM	Control	Hyperthyroid	P value
MDP Difference(mV)	10-K Tyrode	11.3±3.5(8)	13.1±4.7(13)	—
	15-K Tyrode	23.0±5.3(5)	23.5±6.6(11)	—
	20-K Tyrode	37.7±5.2(6)	33.3±5.6(9)	—
Rate of beat(%)	10-K Tyrode	86.2±8.4(7)	78.2±28.7(11)	—
	15-K Tyrode	69.2±14.5(4)	17.9±20.7(8)	<0.005
	20-K Tyrode	4.8±11.7(6)	2.3±5.9(9)	—
MDP(mV)	10-K Tyrode	58.5±10.5(8)	58.6±13.2(13)	—
	15-K Tyrode	51.6±4.7(5)	47.7±10.3(11)	—
	20-K Tyrode	36.0±3.5(6)	35.4±7.4(9)	—

BPM: beats per minute, MDP: maximal diastolic potential
— : no significant, K: potassium ion
i) MDP Difference=(MDP in normal Tyrode)—(MDP in high K^+ Tyrode).
ii) Rate of Beat=(BPM in high K^+ Tyrode/BPM in normal Tyrode)×100.
iii) Numbers in parenthesis indicate the number of observation.



MDP difference = $MDP_1 - MDP_2$

Fig. 4. Measurement of maximal diastolic potential(MDP) difference induced by the change of solution containing 10mM potassium.
MDP_1: normal Tyrode
MDP_2: 10 mM potassium Tyrode

of action potential was disappeared at 20mM potassium in control group while it was disappeared at 15 mM potassium in hyperthyroid group (Fig. 3).

Maximal diastolic potential of both control and hyperthyroid animals were more depolarized as the concentration of external potassium increased, but it was difficult to discriminate a difference between control and hyperthyroid group(Fig. 3 and 4, Table 3). Cardiac positive chonotropic action by T_3 suggested the presence of the factors that T_3 effect might be produced through acceleration of SA node firing or decreased conduction time from SA node to ventricular muscle. Taking into consideration of these factors, the resting membrane potential in the small pieces of left atria was measured.

Fig. 5 shows that depolarized resting potential difference between 10 mM potassium Tyrode and normal Tyrode solution in atrial cell was 15.3±8.1 mV in control and 10.4±4.0 mV in T_3-treated group. At 15 mM potassium Tyrode, differences of resting potential in control and experimental group were 25.8±6.8 mV and 15.4±8.3 mV respectively. The difference values at 20 mM potassium Tyrode were 32.2±8.5 mV in control and 19.7±8.3 mV in hyperthyroid group(Table 4). Resting membrane potential of euthyroid atrial muscle cells was more depolarized than those of hyperthyroid state without significant difference of resting potential in both groups(Fig. 6).

Sodium, potassium pump activity on altered thyroid state pump activity was measured by the following method; the tissue (SA node or atrial muscle) was immersed

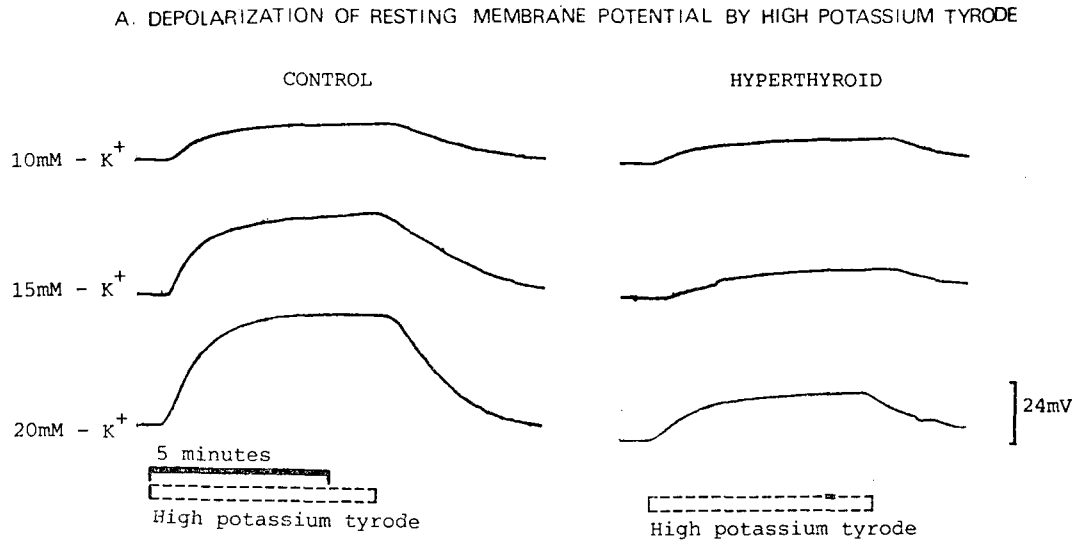


Fig. 5. Depolarization of membrane potential of atrial muscle cell in high potassium Tyrode. Dashed line represents the application of experimental high potassium Tyrode.

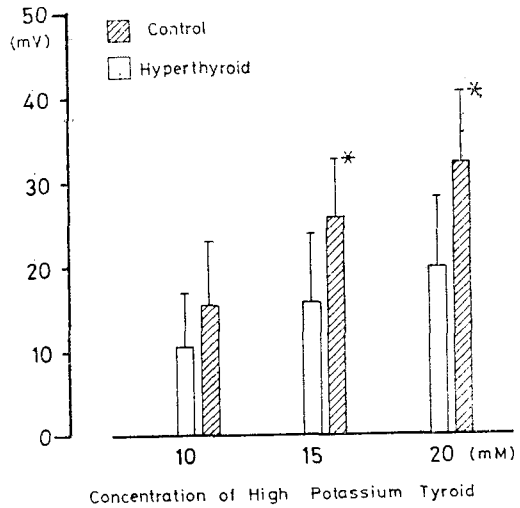


Fig. 6. Comparison of resting membrane potential difference between hyperthyroid and normal control group in atrial muscle cells.
* : Statistically significant.

into potassium free Tyrode solution for 10~15 minutes, then 13 mM potassium Tyrode solution was put into tissue bath. While recording on SA node, 13 mM potassium Tyrode was applied after the beats were stopped. The representitive intracellular records of pump activity was appeared by switching potassium free Tyrode into 13 mM potassium Tyrode solution (Fig. 7). The long time application of extracellular potassium free solution block pump activity. The next perfusion of high potassium Tyrode caused the resting potential of SA node and atrial muscle cell hyperpolarized by the activated pump.

The pump activity values obtained at control and hyperthyroid state in SA node were 13.4±1.4 mV, 16.8±6.9 mV respectively (Fig. 8). In atrial muscle cell, 16.8±7.2 mV in control, 25.8±10.0 mV in hyperthyroid group and those activity value of hyperthyroid group was obtained significantly increased in both tissues.

3) Effect of Manganese Chloride on SA Node

Manganese chloride was used as calcium channel blocker to examine the change in calcium transport by T_3 on SA node. These

Table 4. Response of atria to external high potassium Tyrode

	Concentration in mM	Control	Hyperthyroid	p value
Difference of	10-K^+ Tyrode	15.3±8.1(7)	0.14±6.3(9)	
Resting membrane potential	15-K^+ Tyrode	25.8±6.8(6)	15.4±8.3(7)	<0.05
in atria(mV)	20-K^+ Tyrode	32.2±10.0(6)	19.7±8.4(7)	<0.025
Em(mV)	K^+-Free Tyrode	70.4±17.5(9)	71.8±10.1(11)	—
	10-K^+ Tyrode	63.6±10.4(7)	66.1±6.9(9)	
	15-K^+ Tyrode	51.2±10.0(6)	57.6±7.1(7)	—
	20-K^+ Tyrode	46.7±10.1(6)	54.7±4.9(7)	<0.01
Calculation	10-K^+ Tyrode	68.8 mV		
Value of Em	15-K^+ Tyrode	59.2 mV		
(Nernst Eq.)	20-K^+ Tyrode	51.6 mV		

Em: membrane potential

i) Membrane potential was calculated by using Nernst equation.
$E_m = -61\,mV \log$(external K^+ concentration in mM/140 mM)

ii) Difference of resting membrane potential was measured by using the method method in Fig. 5.

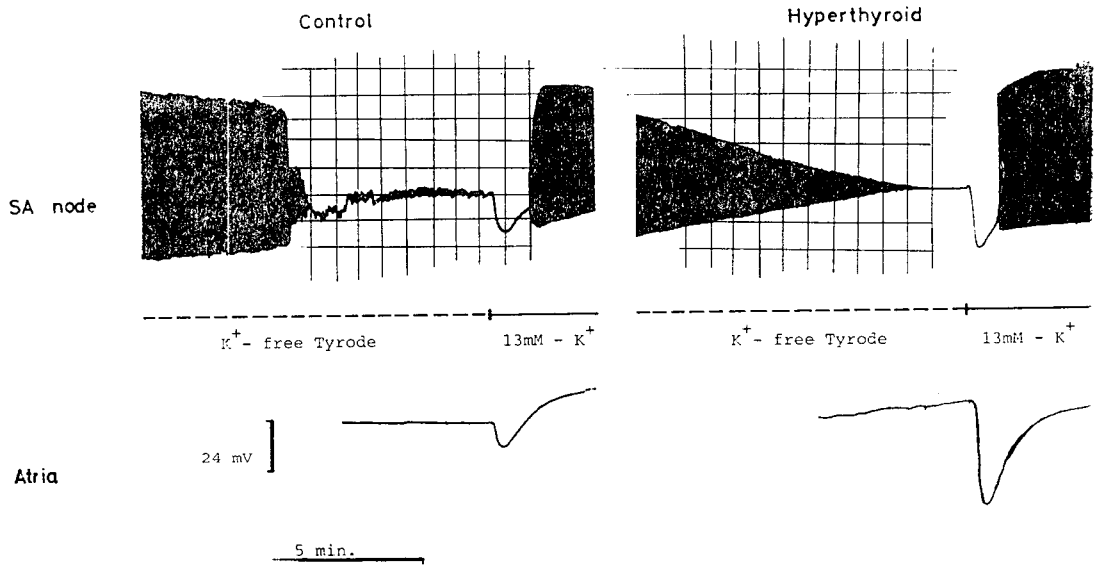


Fig. 7. Electrical recording of pump activity on SA node and atrial muscle cell.

suggested that the T_3-indiuced hyperthyroid SA node were more sensitive to $MnCl_2$ than SA node in euthyroid state.

Spontaneous firing of hyperthyrioid SA nodes were blocked mostly below 0.8 mM $MnCl_2$ while beats of control SA nodes were stopped above 1 mM $MnCl_2$. Beating of SA node immersed in $MnCl_2$-Tyrode solution was decreased in a dose-dependent manner. The data was plotted against the $MnCl_2$ concentration. ID_{50}(50% inhibitory dose) obtained from the first order regression curves in Fig. 9 was about 0.6 mM in hyperthyroid state, 1.1 mM in control group, respectively.

DISCUSSION

Thyroid hormone-induced cardiac hypertrophy reported by others(Gemill, 1958; Golber and Kandror, 1969; Skelton *et al.*, 1972) could not be found in this experiment as determined by the ratio of ventricular weight (gram) to body weight(kg). This fact might be explained by the shorter duration of T_3-treatment. In the hyperthyroid rabbits, this study shows that the increase of sodium, potassium pump activity in sinoatrial node ($p<0.1$) and left atrial muscle cell ($p<0.025$) was considerably matched with the report which description about the functional pump sites on the cell membrane were increased by t hyroid hormone (Curfman *et al.*, 1977; Folke, M. and L. Sestoft, 1977; Haber and Loeb, 1982; Kim and Smith, 1984, 1985). The higher pump activity was caused by the induction of additional pump site from inactive form rather than by the production of new pump protein (Kim and Smith, 1984). Although not being able to confirm whether these results by T_3 were originated from primary effect, or from secondary effecct

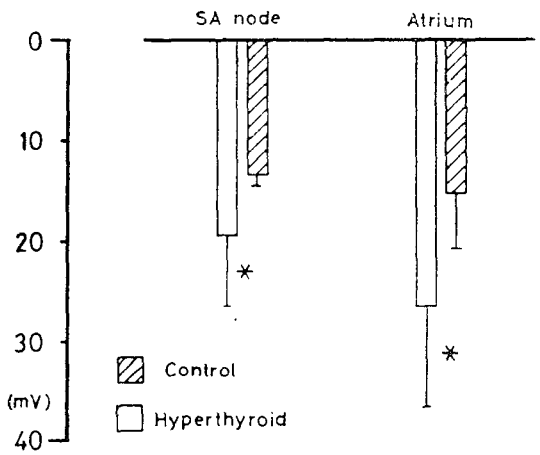


Fig. 8. Comparison of pump activity(from Fig. 7).
* : Statistically significant.

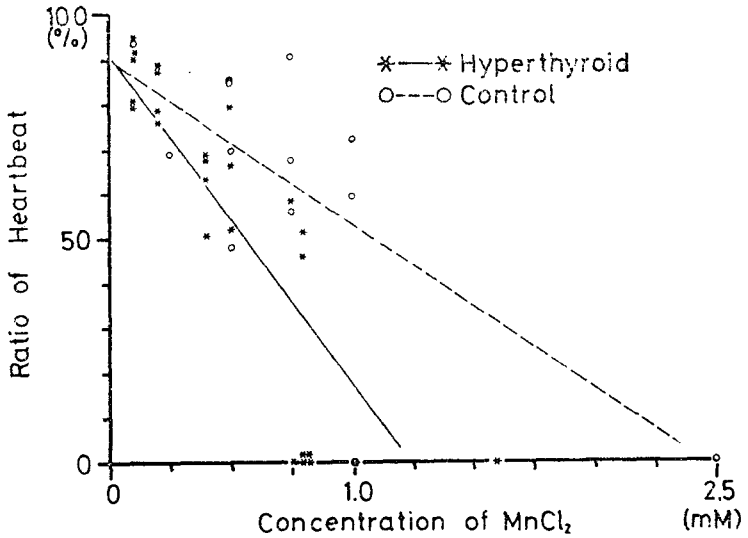


Fig. 9. Effect of manganese ion on SA node.

via prior changes in intracellular ion concentration (Haber and Loeb, 1982) through this experiment, however there were many differences on action potential parameters and atrial resting membrane potential between hyperthyroid and euthyroid state.

Another T_3-dependent effect on heart was elevation of spontaneous rate (Arnsdorf and Childers, 1970; Johnson *et al.*, 1973; Folke, M. and L. Sestoft, 1977; Phillipson and Edelman, 1977; Kim and Smith, 1985). The explanation which increase of the intrinsic rate by T_3 was originated from background sympathetic drive (Freedberg *et al.*, 1970; Sharm a an Banerjee, 1978; Tse *et al.*, 1980) or from direct effect (Heimbach, D.M. *et al.*, 1972; Guanieri, T. *et al.*, 1980; Jaeger, J.M. *et al.* 1981) requires further studies, but it is possible to infer the changes upon the pacemaker-related ionic currents so that cardiac chronotropic effect was caused. If augmentation of spontaneous rate and conduction velocity (or coordination of both) lead to elevate heartbeat, it is reasonable to think that the frequency of pacemaker action potential might play a major role in the absence of the difference on conduction velocity according to the thyroid state (Folke and Sestoft, 1977).

The changes of the membrane potential responsible to potassum ion in atrial muscle cell according to the altered thyroid state (Fig, 5). The resting membrane potential was less depolarized than that of normal control group at 15 mM potassium Tyrode ($p<0.05$) and 20 mM potassium Tyrode ($p<0.025$). And depolarized resting potental (response to high potassium Tyrode) in T_3-treated group had a tendency to be at more negative potential relatively (Table 4). These facts which response of resting membrane potential to external potassium concentration is not acted like a potassium electrode suggested the presence of additional factor concerning the determination of resting membrane potential by T_3.

Also it is likely to account for the augmentation of electrogenic pump activity due to an evidences such as increase of ouabine binding sites (Kim and Smith, 1985) or increase of ouabain resistance (Crie, J.S. *et al.*, 1983) because the net pump current carries outward current, and then hyperpolarized the resting potential at any potential level (Biron, R. *et al.*, 1979).

The response of action potential parameters to high potassium ion was not distinguishable except the reduced 80% APD ($p<0.005$) between the two groups. It could be observed the general decrease on dV/dt and magnitude of action potential in SA node of T_3 treated group, but data analysis was not conducted because of functional and geometrical characteristics of rabbit SA node (Isenberg, G. et al., 1974).

However excitability of SA node to 15 mM potassium was significantly reduced to 17.9% ($p<0.005$) in hyperthyroid rabbits.

The suppression of the sinoatrial node automaticity to high extracellular potassium ion was due to the decrease in magnitude of both transient inward current and outward current tail. Therefore the threshold of action potential might shift in a positive direction then the diastolic interval was prolonged (Bleeker W.K. *et al.*, 1980). At 20 mM potassium Tyrode, excitability on sinoatrial node of euthyroid rabbit was also suppressed. So significant difference in excitability of both group was not observed.

It is well known that potassium, calcium and sodium ion may be involved in a diastolic

depolarization which causes automatic excitability in action potential on SA node (Noma, A., 1976; Noma and Irisawa, 1976a). If the beat of SA node was affected by the sequency of time-and voltage-dependent currents due to these ions, a relative reduction for potassium-sensitive excitability may be suggested that unknown changes are occurred in the ionic currents. To examine another ionic activity in hyperthyroid state, the result by using the reversible calcium channel blocker, $MnCl_2$, showed that excitability on SA node was disappeared at lower concentration of $MnCl_2$ than the case of control group like Fig. 9. Severe increase of spontaneous rate in heart is accompanied by the decreased dV/dt in contrast to effect of adrenaline. The conductances for soidum and calcium will be subjected to a high degree of inactivation while potassium conductance involved in repolarization will still be higher than potassium conductance at rest because second action potential will be occurred prior to the complete repolarization of the previous one. Consequently, the result is a short action potential with a reduced peak due to low sodium and potassium conductance (Noma and Irisawa, 1976a,b).

Therefore thyroid hormone is likely to regulate the ionic channels of the membrane, or bring the conformational change on calcium channel so that the relatively higher antagonistic effect of manganese for calcium ion can be caused by lowering the calcium conductance.

SUMMARY

The present study was carried out to observe the effect of triiodothyronine on heart, one of the target organ of thyroid hormone.

There are many reports that tachycardia, arrythmia, and augmentation of sodium, potassium pump activity are caused in hyperthyroid animal. To examine these cardiac positive chronotropic effects on sinoatrial (SA) node and atrial muscle, hyperthyroid state was induced experimentally by the injecion of 3, 3', 5-1-triiodothyronine (T_3) in 3~6 month-old rabbits. Then, intracellular recordings by inserting glass microelectrode into cell were obtained in SA node and atrial muscle.

The results can be summarized as follows:

1) Heartbeat was increased from 169.6±28.0 to 264.2±18.0 beats per minute, while body weight was decreased to 68% of the initial body weight (Day 1).

2) In experimental group, the duration of action potential at 80% repolarization was decreased from 148.0±29.1 to 107±13.6msec. This suggested the increase heartbeat.

3) The firing rate in hyperthyroid group markedly reduced under the 15 mM potassium Tyrode ($p<0.005$).

4) In hyperthyroid group, depolarization of atrial muscle cell was lowered significantly in 15 mM ($p<0.05$), 20 mM ($p<0.05$) potassium Tyrode solution.

5) Sodium-potassium pump activities in experimental group were higher than those in control group in both SA node ($p<0.1$) and atrial muscle ($p<0.025$).

6) In lower concentration of $MnCl_2$, the excitability of SA node in hyperthyroid group was decreased more than that in control group. Effective inhibitory dose (ID_{50}) was 0.6 mM in hyperthyroid statd and 1.1 mM in control group.

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