Kor J Physiol (1992) 26(2), 99-111

Calcium Current and Background Current Activation in L-triiodothyronine Loaded Ventricular Myocytes of the Rabbit

Jin Han*, Eui Yong Kim*, Jae Hee Han, Choon Ok Park**, Seong Geun Hong****
Chae Hun Leem, Insuk So, Won-Kyung Ho, Yung E Earm and Ho Kyung Sung

Department of Physiology, College of Medicine, Seoul National University, Inje University, Gyeongsang National University**, Korea*

=ABSTRACT=

Permissive action of thyroid hormone at the level of Ca channel and responsible mechanisms underlying thyroid hormone-induced change in myocardial contractile state and T_3-induced arrhythmias were investigated in rabbit ventricular or atrial myocytes using whole cell patch clamp technique. Single cells were isolated by Langendorff perfusion with collagenase. Cardiac myocytes were incubated in low-Cl^-, high-K^+ medium containing 1μM L-triiodothyronine (T_3) at 4°C for 2-10 hours.

The calcium currrent (I_{Ca}) was increased in T_3 loaded cells, however, the shape of current-voltage curve and reverse potential did not altered. Cyclic AMP, cyclic GMP, isoprenaline and 3-isobutyl-1-methyl-xanthine increased I_{Ca} in euthyroid and hyperthyroid conditions, and acetylcholine blocked the increase of I_{Ca} in T_3 loaded cells. The amplitude of I_{Ca} was much larger after perfusing cGMP than cAMP in both conditions, whereas the degree of increase of I_{Ca} was greater after perfusing cAMP than cGMP in T_3 loaded cells. The degree of increase of I_{Ca} after perfusing isoprenaline or IBMX also was greater in T_3 loaded cells than in control cells. Background current induced by isoprenaline also increased in T_3 loaded cells. The Ca-release-dependent inward current was increased in amplitude but its activation and inactivation time course was not changed in T_3 loaded cells. Activation of Na-pump current was not changed in T_3 loaded cells. From the above results it is suggested that thyroid hormone-induced increase in the contractile state of cardiac myocytes are accompanied by augmented I_{Ca} and the increase of Ca release from sarcoplasmic reticulum and the permissive action of thyroid hormone to catecholamines could induce arrhythmias through the increase of I_{Ca} and background current.

Key Words: Thyroid hormone, rabbit cardiac myocytes, whole cell voltage clamp techinique, calcium current, background current, cyclic nucleotides.

INTRODUCTION

Hyperthyroidism has been known to increase the rate of tension development (Buccino et al, 1967) and the peak developed tension (Taylor et al, 1969). As for the mechanism of the increased cardiac contractility in the hyperthyroid, some suggested that there may be alterations in the regulation of Ca movements within the cell (Nayler et al, 1971; Suko, 1973; Limas, 1978; Guarnieri et al, 1980) and also across the sarcolemmal membrane (Kim et al, 1987) while others proposed that increased cardiac contractility in the hyperthyroid is due to alterations in the myosin isoenzymes (Morkin et al, 1983). Besides, thyroid hormone excess shortens the cardiac refractory period, reduces electrical threshold, increases the rate of diastolic repolarization, and decreases the duration of the action potential (Dillmann, 1983).

This study was supported by a 1990 RESEARCH GRANT from the MINISTRY OF EDUCATION and the 1992 Research Promoting Fund of Seoul National University College of Medicine

The enhanced reperfusion arrhythmia in hyperthyroid rats were significantly reduced by verapamil (3×10^{-8} M) (Miyazawa et al, 1989) and the number of premature ventricular extrasystoles per hour was significantly decreased during diltiazem therapy (Roti et al, 1988). Thus Ca channel might be closely related to the increased cardiac contractility and the generation of arrhythmia.

On the other hand, many symptoms of thyrotoxicosis such as tachycardia, increased cardiac output, tremor, sweating and nervousness are suggestive of increased sympathetic activity. However, there is no evidence that hyperthyroid patients have increased catecholamine secretion (Coulombe et al, 1976; Coulombe et al, 1977). Instead, the existence of an enhanced responsiveness to endogenous catecholamines has been postulated since the number and the affinity of ß-adrenergic receptors are increased in many tissues. Thyroxine increases myocardial sensitivity to catecholamines and other biogenic amines (Coville & Telford, 1970). Through this permissive action, thyroxine might be able to increase the cardiac contractility and induce arrhythmia.

We performed this investigation in order to see the mechanisms that thyroxine increases the myocardial sensitivity to catecholamine at the level of Ca channel and the effects of thyroxine on ionic currents related to cardiac contractility, i.e., Ca current, Ca-release-dependent inward current and Na-pump. Part of this work has been presented in abstract form (Han et al, 1992).

METHODS

Preparations

Single atrial or ventricular cells of the rabbit in the euthyroid or in the hyperthyroid were isolated by a method similar to that described by Earm et al (1990). Briefly the heart was perfused with low Ca^{2+}- Tyrode solution (30-50 μM Ca^{2+}) containing collagenase (20 mg per 50 ml, Worthington, type II) for 10-15 min by using a Langendorff perfusion system. Atrial and ventricular tissues were dissected out seperately and mechanically agitated to disperse the cells and then stored in low-Cl^-, high-K^+ medium in the refrigerator.

Solution used to superfuse cells contained (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; NaH_2PO_4, 0.33; glucose, 5; HEPES, 5; adjusted to pH = 7.4 with NaOH. The internal solution of the patch electrode to record Ca current contained (in mM): Cs-aspartate, 110; Mg-ATP, 5; di-Tris creatine phosphate, 2.5; disodium creatine phosphate, 2.5; $MgCl_2$, 1; HEPES, 5; TEA-Cl (tetraethylammonium chloride), 20; EGTA (ethyleneglycol-bis-(ß-aminoethyl ether) N, N, N′, N′-tetraacetic acid), 5; adjusted to pH = 7.4 with CsOH. For a solution to record an inward tail current Cs-aspartate was replaced by equimolar K-aspartate and TEA-Cl by KCl, EGTA was decreased to 0.1 mM and the pH was adjusted to 7.4 with KOH. For a solution to record a background current activated by isoprenaline, Cs-aspartate was replaced by 100 mM KCl and 20 mM taurine. In rabbit ventricular cells isoprenaline activated the background current similar to that in guinea pig ventricular cells in the presence of internal taurine (see Leem et al (1992)). We added Ni, Cd, Ba, ouabain and ditiazem to the external solution to block Ca current, Na-Ca exchange current, K current and Na pump etc. The internal solution to record Na pump current contained (in mM): NaCl, 30; HEPES, 5; EGTA, 40; $MgCl_2$, 10; TEA-Cl, 20; disodium creatine phosphate, 5; Na_2-ATP, 5; cGMP, 0.1; GTP 1; adjusted to pH = 7.4 with KOH.

Induction of hyperthyroidism

To induce hyperthyroidism, we used L-triiodothyronine (T_3). Chronic hyperthyroidism was induced by daily peritoneal injections of L-triiodothyronine (Sigma), 0.1 mg/Kg/day for 7 days. Free T_3 levels were determined in the serum using commercial kit (T_3 RIA kit, Callestad, USA). Free T_3 levels were 0.42 ± 0.33 pg/ml (n = 5) in the euthyroid and 5.82 ± 2.42 pg/ml (n = 4) in the hyperthyroid. The heart

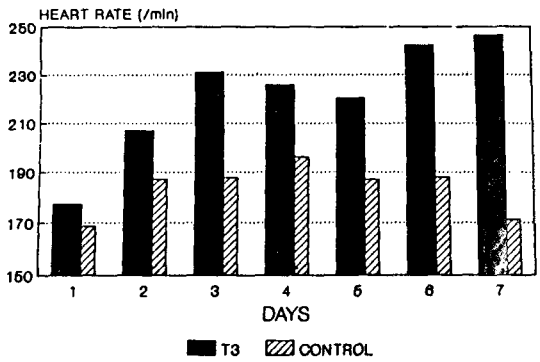

Fig. 1. Comparison of the change in heart rate in hyperthyroid and euthyroid hearts. The heart rate increased day by day in hyperthyroid rabbits. The heart rates in the hyperthyroid were 177 ± 10/min(n = 9), 207 ± 17/min(n = 9), 231 ± 48/min(n = 9), 226 ± 22/min(n = 7), 220 ± 23/min(n = 6) 242 ± 22/min(n = 7) and 246 ± 30/min(n = 7) for 1,2,3,4,5,6 and 7 day, respectively. The heart rates in the euthyroid were at the ranges from 168/min to 196/min.

rate increased day by day from 177 ± 10/min (n = 9) to 246 ± 30/min (n = 7) (Fig. 1) and body weight decreased from 1.12 ± 0.15 Kg (n = 5) to 0.98 ± 0.09 Kg (n = 5) in the hyperthyroid.

In vitro hyperthyroidism was induced by loading T_3 into isolated single cells in KB solution containing 1 μM T_3 at 4°C for 2-10 hours (Felzen et al, 1989). The results about Ca currents obtained from 2 methods were similar, so we mainly performed experiments in the condition of *in vitro* hyperthyroidism.

Recordings of ionic currents

The cells were voltage-clamped by using a whole-cell patch-clamp apparatus (Axopatch 2C or List, EPC-7) according to the original technique developed by Hamill et al (1981). Glass electrode with resistance of 2-3 MΩ were used. The data were recorded on a pulse code modulator (PCM) data recorder (NF, BR-6400) for future analysis. Data were also displayed on a digital oscilloscope (Hitachi, 6041) and pen recorder (Gould, Recorder 220) and could then be directly reproduced onto an X-Y recorder (Graphtec, MP4100). All values are expressed as mean ± standard deviation (S.D).

RESULTS

The effects of T_3 on Ca current

Cardiac contractility and the generation of arrhythmia are closely related to Ca influx through Ca channel (Reuter,1983; Tsien, 1983), so we performed experiments on the effects of T_3 on Ca current. T_3 increased Ca current at the range from −20 mV to +60 mV (Fig. 2A) but did not change reversal potential and the bell-shaped I-V curve (Fig. 2B). T_3 increased the peak amplitude at 10 mV from 0.14 ± 0.057 nA/50pF (n = 10) to 0.22 ± 0.074 nA/50pF (n = 12).

One μM isoprenaline known as ß-agonist increased Ca current by 171 ± 35% (n = 5) in the euthyroid and by 203 ± 51% (n = 5) in the hyperthyroid(Fig. 3A). The increase of Ca current by IBMX (195 ± 48%, n = 5) or isoprenaline and IBMX (206 ± 50%, n = 5) were similar to the increase of Ca current by isoprenaline in the hyperthyroid. Acetylcholine binds muscarinic receptor through which it decreases cardiac contractility, Ca current and the plateau of action potential (Biegon & Pappano, 1980; Josepson & Sperelakis, 1983; Carmeliet & Mubagwa, 1986). Acetylcholine had no effect on Ca current in the euthyroid, while acetylcholine decreased Ca current in the hyperthyroid from 0.23 ± 0.054 nA/50pF (n = 12) to 0.13 ± 0.048 nA/50pF (n = 6) (Fig 3B).

Isoprenaline increases intracellular cAMP via GTP-binding proteins. To see whether T_3 increases Ca current in the presence of cAMP through the direct phosphorylation of Ca channel, we studied the effect of cAMP on the increase of Ca current by T_3. When we used internal solution containing 100 μM cAMP, peak amplitude of Ca current at 10 mV was 0.30 ± 0.120 nA/50pF (n = 13) in the euthyroid

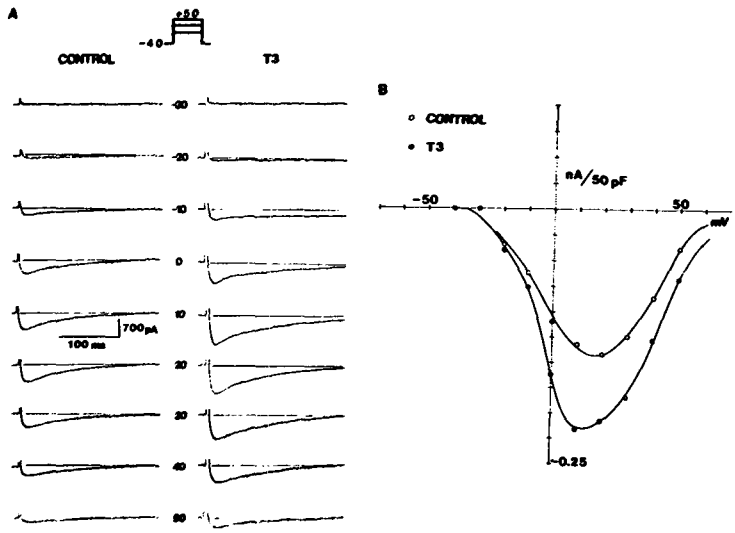

Fig. 2. Effect of thyroid hormone on the current-voltage relationship of the calcium current in rabbit ventricular myocyte. The holding potential was −40 mV. In A, the calcium currents for depolarizing voltage-clamp steps elicited from the holding potential to test potentials ranging from −30 to +50 mV for 200 msec. Traces of control and hyperthyroid myocytes are superimposed for each voltage. In B, the current-voltage relation (I-V curve) was plotted and showed that thyroid hormone (filled circle) increased the calcium current in the whole range of membrane potential and the peak activation of the calcium current was activated at +10 mV and its amplitude was 0.14 ± 0.057nA/50pF in control and 0.22 ± 0.074nA/50pF in hyperthyroid myocytes.

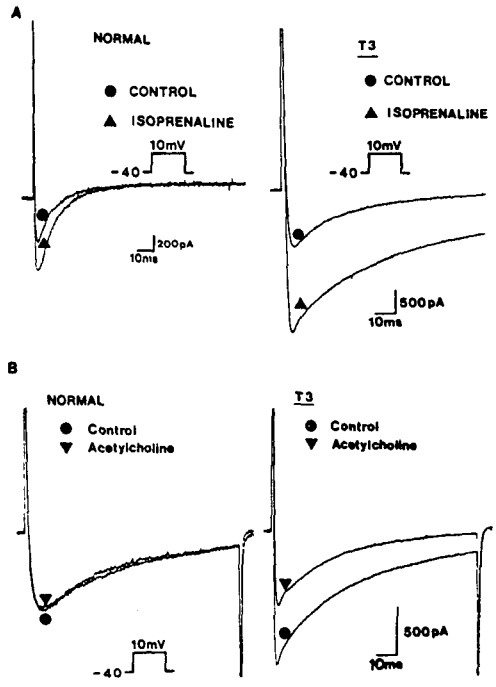

Fig. 3. Effect of isoprenaline and acetylcholine on the calcium current. In euthyroid state (left record of A), peak current was evoked by depolarization to +10 mV from holding potential −40 mV. The duration of stimulation was 100 ms. Same current recorded after exposure to 1 μM isoprenaline. In hyperthyroid state (right record of A), current was recorded by the same experimental protocols as above. The degree of increase of the calcium current after perfusing isoprenaline was greater in hyperthyroid state than in euthyroid state. Circles, control; triangles, 1 μM isoprenaline. In (B), the holding potential was −40 mV and the amplitude of the clamp pulse was +10 mV for 100 ms. 1 μM acetylcholine (triangle) reduced the thyroid hormone-induced increase of the calcium current.

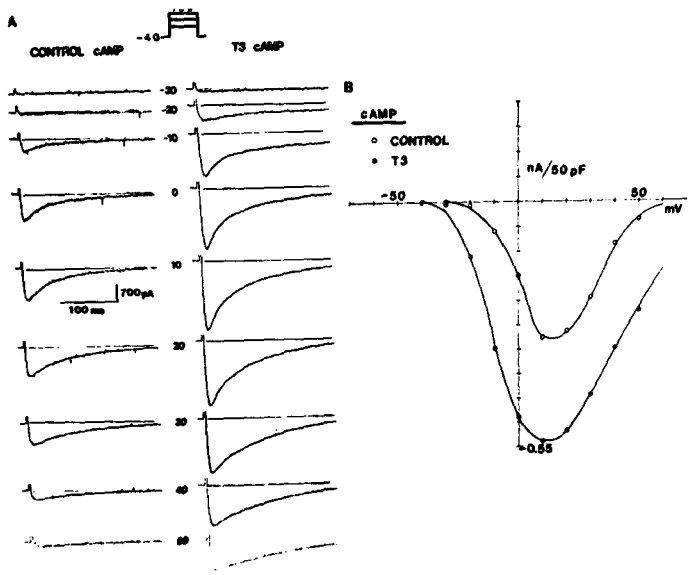

Fig. 4. Effect of internal application of 100 μM cAMP on the activation and the current-voltage relationship of the calcium current in hyperthyroid myocytes and control. In A, the calcium current traces activated by cAMP at the membrane potential from −30 mV to +50 mV were shown in the two groups of myocytes. In B, the current-voltage relationship was plotted and showed that in the presence of cAMP the calcium current was greater in hyperthyroid myocytes (filled circle) than in control (open circle). The peak current level was activated at +10 mV in both cases and their amplitude were 0.43 ± 0.118 nA/50 pF (n = 9) and 0.30 ± 0.120 nA/50 pF (n = 13) in hyperthyroid cell and control, respectively.

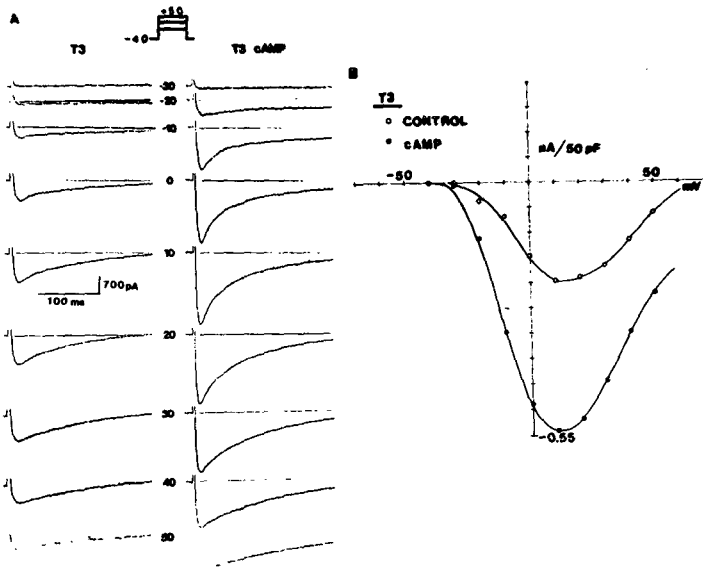

Fig. 5. In hyperthyroid state, the activation (in A) and the current-voltage relationship (in B) before and after internal application of cAMP. The calcium currents were increased by 100 μM cAMP (filled circle) in the whole range of membrane potential.

(Fig. 4) and was 0.43 ± 0.118 nA/50pF (n = 9) in the presence of T_3 (Fig. 5A, right column and 5B, filled circle) but cAMP did not change the reversal potential and the bell-shaped I-V curve both in the euthyroid and in hyperthyroid. Acetylcholine increases intracellular cGMP and decreases Ca current (Hartzell & Fischmeister, 1986). To see the effect of acetylcholine on Ca current in hyperthyroid, we used internal solution containing 100 μM cGMP. Ca current increased even in the case of 100 μM cGMP and peak amplitude at 10 mV was 0.52 ± 0.200 nA/50pF (n = 15) (Fig. 6). Thus cGMP alone can increase Ca current contrary to the results of other investigators (Hartzell & Fischmeister, 1986; Levi et al, 1989; Ono & Trautwein, 1991). Ca current increased further in the presence of T_3 and the peak amplitude at 10 mV was 0.63 ± 0.055 nA/50pF (n = 11) (Fig. 7A, right column and 7B, filled triangle). Effects of cAMP and cGMP on the current-voltage relationship of the calcium current were compared in the euthyroid and hyperthyroid (Fig. 8). The degree of increase of the calcium current was greater in case of cAMP than cGMP in hyperthyroid state; T_3 enhanced the response of the calcium current to cAMP.

The effect of T_3 on background current activated by isoprenaline

Catecholamines activated a background current via cAMP pathways and the background current might induce arrhythmia especially in ischemic hearts in which blood and urinary concentrations of noradrenaline, adrenaline or their

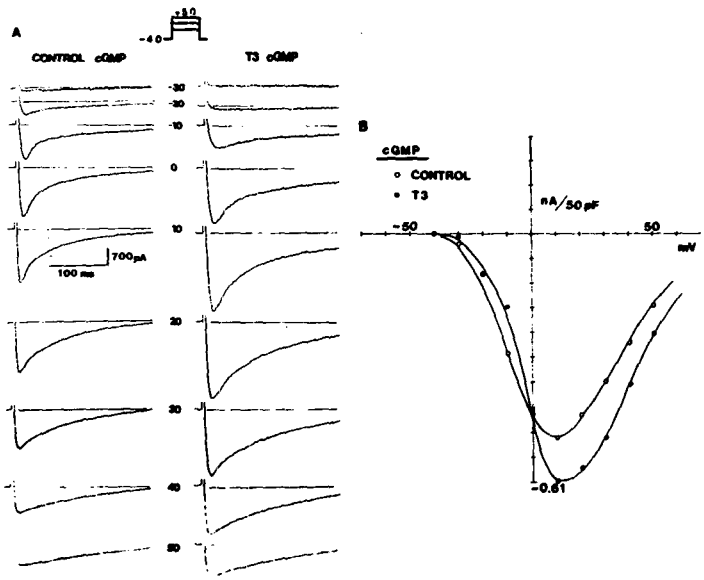

Fig. 6. Effect of cGMP on the activation and the current-voltage relationship of the calcium current in hyperthyroid state and control. 100 μM cGMP was applied from internal solution of the patch pipette. In A, the calcium current traces activated by cGMP at the membrane potential from −30 mV to +50 mV were shown in the two groups of myocytes. In B, the current-voltage relationship in control (open circle) and hyperthyroid state (filled circle) were plotted on the normalized current scale with membrane capacitance of 50 pF. The peak current level was activated at 10 mV in two groups of cells and their amplitude were 0.63 ± 0.055 nA/50 pF (n = 11) and 0.52 ± 0.200 nA/50 pF (n = 15) in hyperthyroid cells and control, respectively.

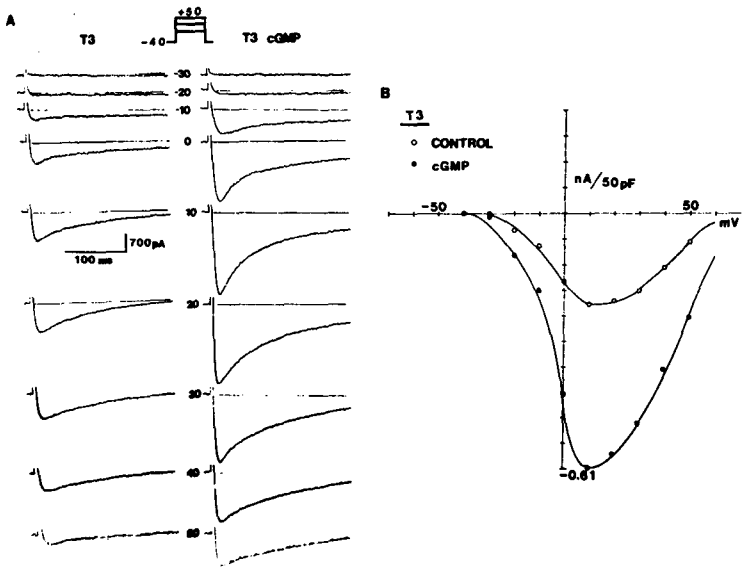

Fig. 7. In hyperthyroid state, the activation (in A) and the current-voltage relationship (in B) before and after internal application of cGMP. The calcium currents were increased by 100 μM cGMP (filled circle) in the whole range of membrane potential.

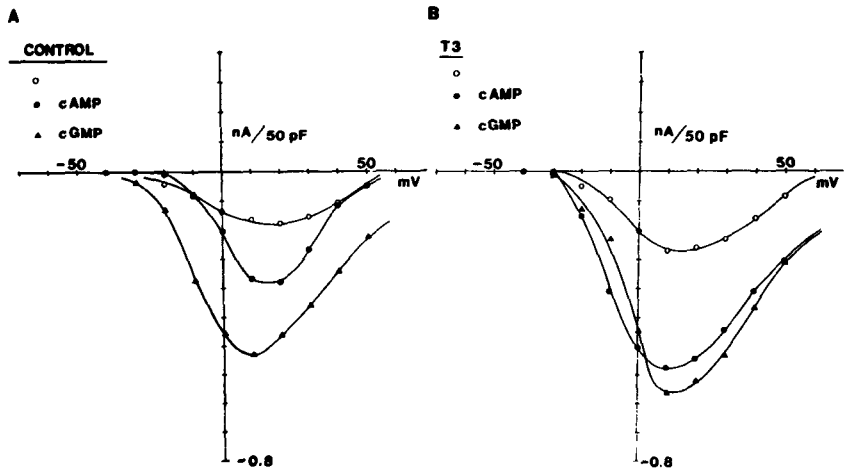

Fig. 8. Effects of cAMP and cGMP on the current-voltage relationship of the calcium current. Open circles, control; filled circles, 100 μM cAMP; filled triangles, 100 μM cGMP. In A and B, effect of cAMP and cGMP on the current-voltage relationship of the calcium currents were shown in normal control and in hyperthyroid state, respectively. cAMP or cGMP alone increased the calcium current in both control and hyperthyroid myocytes. The amplitude of the calcium current was greater after perfusing cGMP than cAMP. In hyperthyroid state, the degree of increase of the calcium current was greater in case of cAMP than cGMP.

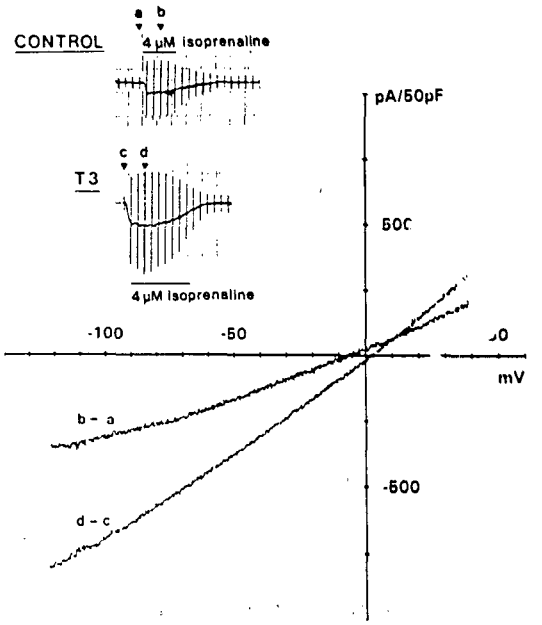

Fig. 9. Difference of the effect of isoprenaline on background current between in euthyroid and in hyperthyroid state in rabbit ventricular myocyte. Pipette solution contained mainly KCl. In euthyroid state, isoprenaline activated background current and holding current shifted to inward direction at holding potential of −40 mV and current amplitude was increased to inward and outward direction. In hyperthyroid state, the current amplitude was much larger than that in euthyroid state. The difference current (b-a or d-c in euthyroid or hyperthyroid state, respectively) was obtained by subtracting the current traces of before (a or c in euthyroid and hyperthyroid state) from after (b or d in euthyroid or hyperthyroid state) perfusing 4 μM isoprenaline and normalized as cell capacity was 50 pF.

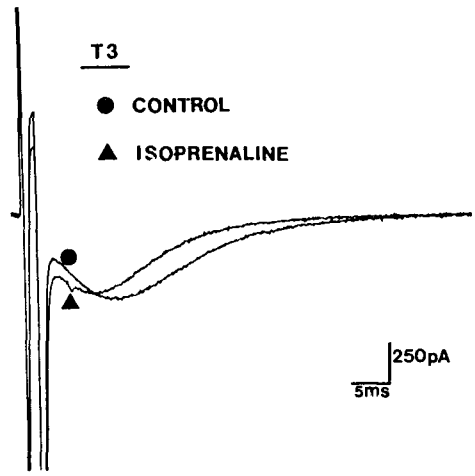

Fig. 10. Effect of isoprenaline cAMP on the inward tail current in atrial myocyte in hyperthyroid rabbit. The inward tail current was recorded at −70 mV after depolarizing the membrane to +40 mV for 2 ms. In hyperthyroid state, the inactivation time course of the inward current was accelerated by superfusion of 1 μM isoprenaline though its amplitude was not so much changed.

metabolic products increase (Egan et al, 1987, 1988; Noble et al, 1989; Noble, 1990). We tested the possibility that T_3 might also increase the myocardial sensitivity to catecholamine in this background current. The background current was recorded by using ramp pulse (0.8 V/sec) from a holding potential of −40 mV. T_3 increased the inward shift of holding current by isoprenaline and the amplitude of the background current further in both the inward and outward directions (Fig. 9, n = 5).

The effects of T_3 on Ca-release-dependent inward current

The inward tail current recorded by Earm et al (1990, 1991) was closely related to intracellular Ca transient, especially released from SR and the current was inward current of Na-Ca exchange (Earm & Noble, 1990). To see the effect of T_3 on the intracellular Ca concentration and Na-Ca exchange, we performed experiments on the effects of T_3 on the inward tail current. While MacKinnon & Morgan (1986) reported that T_3 did not change the peak average cytoplasmic calcium concentration during contraction but enhanced the time course

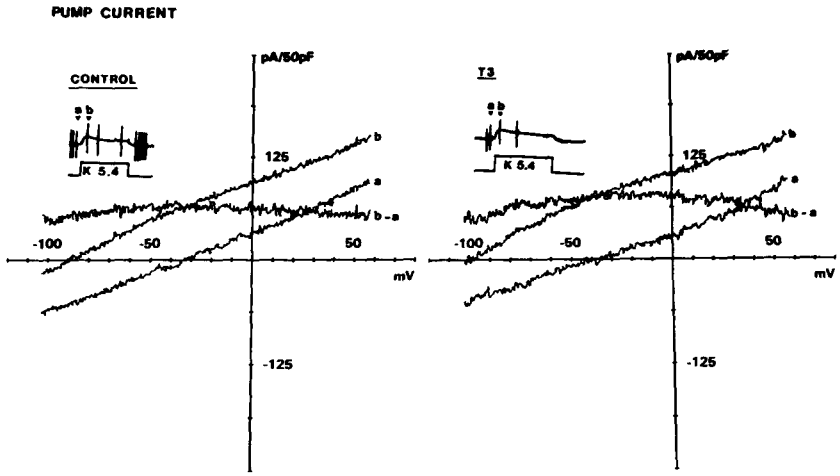

Fig. 11. Effect of thyroid hormone on the Na pump current in rabbit ventricular myocyte. The Na pump current was measured as K-activated current. Following an equilibration period of 10-30 min in the standard superfusion medium, a K-free solution was applied to the ventricular myocytes. When the membrane current at a holding potential of −20 mV had reached a constant value, short pulses of K-containing (5 mM) media were repeatedly delivered to the cell. Since the evoked outward current was almost completely inhibited by ouabain, a specific blocker of the Na-K pump, we conclude that the K-induced outward current is the Na pump current. The characteristics of Na pump current were not significantly different in euthyroid and hyperthyroid atrial myocytes.

of the calcium transient especially decay of the peak calcium transient, our results showed that in the hyperthyroid peak amplitude increased from 200-400 pA to 300-700 pA but there was no changes in the time to peak and inactivation time course. Isoprenaline (1 μM) did not change the peak amplitude but inactivation time course in the presence of T_3 became faster (Fig. 10).

The effect of T_3 on Na-pump current

Na-pump regulates $[Na]_i$ which is closely related to cardiac contractility and triggered activity such as afterdepolarization. Recently Harrison et al (1992) reported the close relationship between contraction and Na_i. In general T_3 are known to enhance the activity of Na-K pump. T_3 enhanced enzyme activity of Na-K ATPase (Ismael-Beigi & Edelman, 1971; Lo et al, 1976; Ismael-Beigi et al, 1979; McDermott & Klein, 1983), the number of Na pump which binds to ouabain (Edelman, 1974; Lo et al, 1976; Curfman et al, 1977; Lin & Akera, 1978; Kim & Smith, 1985) and the number of functional Na pump (Kim & Smith, 1984). As a result T_3 could decrease Na_i, cardiac contractility and sensitivity to cardiac glycoside, e.g., ouabain. Thus to see the effect of T_3 on Na-pump current, we used a similar method that described by Gadsby et al (1985). We activated Na-pump current by changing external K from 0 mM to 5 mM in the condition of 30 mM Na_i (Fig. 11). This current was also sensitive to ouabain. T_3 had no effect on Na-pump current in T_3-loaded cells for 2-10 hours (n = 5).

DISCUSSION

In the present study we investigated the mechanisms that thyroxine increased myocardial sensitivity to catecholamine at the level of Ca channel and the effects of thyroid hormone on ionic currents related to cardiac contractili-

ty and arrhythmia. The major findings were: (1) Thyroid hormone increased calcium current in the absence or presence of cAMP and the degree of increase of Ca current by T_3 in the presence of cAMP was greater than that in cGMP. (2) The background current activated by catecholamines was also increased by T_3. (3) The inward tail current, related to intracellular Ca and Na-Ca exchange, was also increased by thyroid hormone but Na-pump current was not changed by thyroid hormone.

Ca channel plays a major role in regulating cardiac contractility and heart rate (Reuter, 1983; Tsien, 1983). Ca channel itself is also regulated by phosphorylation via cAMP cascade. T_3 increased calcium current (Fig. 2) and enhanced the response of calcium current to isoprenaline (Fig. 3). These results suggest that T_3 increases the number of the ß-adrenergic receptor or enhances the function of the calcium channel. The response of calcium current to T_3 in the presence of cAMP suggest that T_3 can increase Ca current without the increase of the number of ß-adrenergic receptors. The degree of increase of Ca current by T_3 was greater in the presence of cAMP than cGMP (Fig. 8), although cGMP itself increased Ca current much more than cAMP, that is, the absolute value of Ca current at 10 mV was slightly larger in the presence of cGMP than cAMP. Thus T_3 has permissive action on the process through which cAMP increases Ca current; thyroxine can enhance Ca channel activities through the increase of Ca channel densities or sensitivity to phosphorylation. The results that cAMP incresing agents, i.e., isoprenaline, IBMX, increased Ca current further in the hyperthyroid and acetylcholine reduced only the increase of Ca current by T_3(see also Heschler et al, 1986) also suggest that T_3 act through cAMP-cascade. Acetylcholine does not appear to increase intracellular cGMP and decrease calcium current through cGMP-stimulated phosphodiesterase (Hartzell & Fischmeister, 1986) but may have an effect on Ca channel via GTP-binding proteins. Since cGMP increased calcium current in the euthyroid(Fig. 6A), the mechanism that cGMP increases calcium current is different from cGMP-inhibited phosphodiesterase (Ono & Trautwein, 1991). This mechanism was slightly enhanced by T_3 and involved in the modulation of calcium channel independently of T_3-sensitive cAMP mechanism.

Acute myocardial infarction releases catecholamines and the higher the level of catecholamine the greater the risk of severe arrhythmia(Jewitt et al, 1969). Isoprenaline activated a background current more larger in the presence of T_3. These results together with the effects of T_3 on Ca currents indicate that arrhythmia induced in the hyperthyroid reflects the increase of the susceptibility to arrhythmogenic currents and increased responsiveness to isoprenaline effect on Ca current. Results from experiments on inward tail currents also suggest that arrhythmogenic Na-Ca exchange current might induce arrhythmia in the condition of digitalis toxicity (Noble 1986; Fedida et al, 1987a, b).

In our experiments T_3 increased not only Ca current but inward tail current related to intracellular Ca. These results show that T_3 could increase the cardiac contractility and maximal amplitude of contraction. While there was no change in the inactivation time course and the time to peak of inward tail current in our results, other investigators(Buccino et al, 1967; Taylor et al, 1969; Johnson et al, 1973; Sharp et al, 1985) reported that thyroid hormone enhanced the relaxation rate of contraction and shortened the time to peak amplitude of contraction. However since thyroid hormone also increased the activity of ATPase(Hoh et al, 1978; Flink et al, 1979; Morkin et al, 1983), the effects of thyroid on $[Ca]_i$ transients together with the effect on myosin ATPase might enhance the relaxation of contraction and shortened the time to peak amplitude of contraction. The result that T_3 increased the peak amplitude of inward tail current but did not change the time course of activation and inactivation suggests that T_3 enhanced the release and reuptake function of sarcoplasmic reticulum (SR). Alternatively it suggests that the function of SR can handle such an intracellular Ca transient increase. Since isoprenaline

enhanced the time course of activation and inactivation of Ca-release-dependent inward current, it is more possible that T_3 enhanced the release and reuptake function of SR.

Because T_3 did not enhance the activity of Na-K pump, the other possibility to be considered would be related with the control of $[Ca]_i$. Recently Gao et al,(1992) reported that ß-stimulation can increase pump current(Ip), but only if $[Ca]_i$ is above about 150nM in rat and guinea-pig ventricular myocytes. Therefore further studies need whether or not T_3 also would increase Ip in case of high $[Ca]_i$, above about 150nM. Secondly if T_3 influence activity of Na-K pump by enhancing protein synthesis, loading T_3 into cells for less than 12 hrs at 4°C would not be enough for such process to occur. Felzen et al(1989), however, performed experiment on *in vitro* effects of triiodothyronine and reported that the presence of cycloheximide abolished action potential duration(APD) shortening by T_3 after superfusion of cycloheximide for 2hr at 36°C. Because the effect of thyroxine on Ca current was same both in vivo hyperthyroid and in vitro hyperthyroid in our result, the possibility that thyroxine enhanced the activities of Ca channel and Na-pump through different routes cannot be excluded.

In conclusin, our results demonstrate that T_3 increases Ca influx, Ca released from sarcoplasmic reticulum and isoprenaline-activated background current, to change cardiac contractility and induce arrhythmia and could interact with cAMP-cascade.

REFERENCES

Biegon R & Pappano A(1980) Dual mechanism for inhibition of calcium-dependent action potentials in avian ventricular muscle. *Circ Res* **46,** 353-362

Buccino RA, Spann JF Jr, Pool PE, Sonnenblick EH & Braunwald E(1967) Influence of the thyroid state on the intrinsic contractaile properties and energy stores of the myocardium. *J Clin Invest* **46,** 1669-1682

Carmeliet E & Mubagwa K(1986) Changes by acetylcholine of membrane currents in rabbit cardiac Purkinje fibres. *J Physiol* **371,** 201-217

Coulombe P, Dussault JH, Letarte J & Simard SJ(1976) Catecholamine metabolism in thyroid diseases: I. Epinephrine secretion rate in hyperthyroidism and hypothyroidism. *J Clin Endocrinol Metab* **42,** 125-131

Coulombe P, Dussault JH & Walker(1977) Catecholamine metabolism in thyroid diseases: II. Epinephrine secretion rate in hyperthyroidism and hypothyroidism. *J Clin Endocrinol Metab* **44,** 1185-1189

Coville PE & Telford JM(1970) Influence of thyroid hormones on the sensitivity of cardiac and smooth muscle to biogenic amines and other drugs. Br J Pharmacol **39,** 49-68

Curfman GD, Crowley TJ & Smith TW(1977) Thyroid-induced alternations in myocardial sodium- and potassium-activated adenosine triphosphatase, monovalent cation active transport, and cardiac glycoside binding. *J Clin Invest* **59,** 586-590

Dillmann WH(1983) Thyroid hormones and the heart. In:Openheimer JH(ed) *Thyroid Today.* vol 6, No 2. Boots-Flynn, Deerfield, Illinois. Earm YE, Ho WK & So IS(1990) Inward current generated by Na-Ca exchange during the action potential in single atrial cells of the rabbit. *Proc R Soc Lond* **B240,** 61-81

Earm YE, Ho WK & So IS(1991) Effect of Ca-buffer concentration and stimulus interval on the voltage dependency and timecourse of calcium-release-dependent inward current in rabbit atrial myocytes. *Proc R Soc Lond* **B264,** 285-292

Earm YE & Noble D(1990) A model of the single atrial cell:relation between calcium current and calcium release. *Proc R Soc Lond* **B240,** 83-96

Edelman IS(1974) Thyroid thermogenesis. *N Engl J Med* **290,** 1303-1308

Egan TM, Noble D, Noble SJ, Powell T & Twist VW(1987) An isoprenaline activated sodium-dependent inward current in ventricular myocytes. *Nature* **1987,** 634-637

Egan TM, Noble D, Noble SJ, Powell T, Twist VW & Yamaoka K(1988) On the mechanism of isoprenaline- and forskolin-induced depolarization of single guinea-pig ventricular myocytes. *J Physiol* **400,** 299-320

Fedida D, Noble D, Rankin AC & Spindler AJ(1987a) The transient inward current, i_{TI}, and related contraction in guinea-pig ventricular myocytes. *J Physiol* **392,** 523-542

Fedida D, Shimoni Y, Noble D & Spindler AJ(1987b) Inward currents related to contraction in guinea-pig ventricular myocytes. *J Phyiol* **385,** 565-589

Felzen B, Sweed Y & Binah O(1989) Electrophysiological effects of thyroid hormons in guinea-pig ventricular muscle: Time course and relationships to blood levels. *J Mol Cell Cardiol* **21,** 1151-1161

Flink IL, Rader JH & Morkin E(1979) Thyroid hormone stimulates synthesis of a cardiac myosin isozyme. Comparison of the two-dimensional electrophoretic patterns of the cyanogen bromide peptides of cardiac myosine heavy chains from euthyroid and thyrotoxic rabbits. *J Biol Chem* **254,** 3105-3110

Gadsby DC, Kimura J & Noma A(1985) Voltage dependence of Na/Ca pump current in isolated heart cells. Nature **315,** 63-65

Gao J, Mathias RT, Cohen IS & Baldo GJ(1992) Isoprenaline, Ca^{2+} and the $Na+$-K^+ pump in guinea-pig ventricular myocytes. *J Physiol* **449,** 689-704

Guarnieri T, Filburn CR, Beard ES, & Lakatta EG(1980) Enhanced contractile response and protein kinase activation to threshold levels of ß-adrenergic stimulation in hyperthyroid rat heart. *J Clin Invest* **65,** 861-868

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ(1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* **391,** 85-100

Han J, Kim EY, Leem CH, So IS, Ho WK & Earm YE(1992) Calcium current and background current activation in L-triiodothyronine(T_3) loaded ventricular myocytes of the rabbit. *J Mol Cell Cardiol* **24** (Supplement IV), 40P

Harrison SM, McCall E, Boyett MR(1992) The relationship between contraction and intracellular sodium in rat and guinea-pig ventricular myocytes. *J Physiol* **449,** 517-550

Hartzell HC & Fischmeister R(1986) Opposite effects of cyclic GMP and cyclic AMP on Ca^{2+} current in single heart cells. Nature **323,** 273-275

Hescheler J, Kameyama M & Trautwein W(1986) On the mechanism of muscarinic inhibition of the cardiac calcium current. *Pflügers Arch* **407,** 182-189

Hoh JFY, McGrath PA & Hale PT(1978) Electrophoretic analysis of multiple forms of rat cardiac myosin:effects of hypophysectomy and thyroid replacement. *J Mol Cell Cardiol* **10,** 1053-1076

Ismael-Beigi F, Bissel DM & Edelman IS(1979) Thyroid thermogenesis in adult rat hepatocytes in primary monolayer culture. *J Gen Physiol* **73,** 369-383

Ismael-Beigi F & Edelman IS(1971) The mechanism of the calorigenic action of thyroid hormone:stimulation of Na and K-activated adenosine triphosphatase activity. *J Gen Physiol* **57,** 710-722

Jewitt DE, Mercer CJ, Reid D, Valori C, Thomas M & Shillingford JP(1969) Free noradrenaline and adrenaline secretions in relation to the development of cardiac arrhythmias and heart failure in patients with acute myocardial infarction. *Lancet* **1,** 635-641.

Johnson PN, Freedberg AS & Marshall JM(1973) Action of thyroid hormone on the transmembrane potentials from sinoatrial node cells and atrial muscle cells in isolated atria of rabbits. *Cardiology* **58,** 273-289

Josephson I & Sperelakis N(1982) On the ionic mechanism underlying adrenergic-cholinergic antagonism in ventricular muscle. *J Gen Physiol* **79,** 69-86

Kim DH & Smith TW(1984) Effects of thyroid hormone on sodium pump sites, sodium content, and contractile response to cardiac glycoside in cultured chick ventricular cells. *J Clin Invest* **74,** 1481-1488

Kim D & Smith TW(1985) Effects of thyroid hormone on calcium handling in cultured chick ventricular cells. *J Physiol* **364,** 131-149

Kim D, Smith TW & Marsh JD(1987) Effect of thyroid hormone on slow calcium channel function in cultured chick ventricular cells. *J Clin Invest* **80,** 88-94

Leem CH, So IS, Ho WK & Earm YE(1992) Taurine modulates the inward background current induced by isoprenaline in isolated ventricular cells

of the rabbit. *J Physiol* **446,** 146P.

Levi RC, Alloatti G & Fischmeister R(9189) Cyclic GMP regulates the Ca-channel current in guinea pig ventricular myocytes. *Pflügers Arch* **413,** 685-687

Limas CJ(1978) Enhanced phosphorylatin of myocardial sarcoplasmic reticulum in experimental hyperthyroidism. Am *J Physiol* **234,** H426-H432

Lin MH & Akera T(1978) Increased Na,K-ATPase concentration in various tissues of rats caused by thyroid hormone treatment. *J Biol Chem* **253,** 723-726

Lo CS, August TR, Liberman VA & Edelman IS(1976) Dependence of renal Na,K-adenosine triphospatase activity on thyroid status. *J Biol Chem* **251,** 7826-7833

MacKinnon R & Morgan JP(1986) Influence of the thyroid state on the calcium transient in ventricular muscle. *Pflügers Arch* **407,** 142-144

Matsuoka S, Ehara T & Noma A(1990) Chloride-sensitive nature of the adrenaline-induced current in guinea-pig cardiac myocytes. *J Physiol* **425,** 579-598

McDermott P & Klein I(1983) Effectof thyroid hormone on cultured rat heart cells. *Clin Res* **31,** 205A

Miyazawa K, Hashimoto H, Uematsu T & Nakashima M(1989) Electrophysiological abnormalities and enhanced reperfusion arrhythmias in isolated hearts of hyperthyroid rats. *Br J Pharmacol* **97,** 1093-1100

Morkin E, Flink IL & Goldman S(1983) Biochemical and physiological effects of thyroid hormone on cardiac performance. *Prog Cardiovasc Dis* **25,** 435-464

Nayler WG, Merrillees NCR, Chipperfield D & Kurtz JB(1971) Influence of hyperthyroidism on the uptake and binding of calcium by cardiac microsomal fractions and on mitochondrial structure. *Cardiovasc Res* **5,** 469-482

Noble D(1986) Sodium-calcium exchange and its role in generating electric current. In:Nathan R(ed) *Cardiac muscle: the regulation of excitation and contraction.* Chapter 6. Academic Press, New York, p171-200

Noble D, DiFrancesco D & Denyer J(1989) Ionic mechanism in normal and abnormal cardiac pacemaker activity. In:Jacklet JW(ed) *Neuronal and cellular oscillators.* Marcel Deker, New York, p59-85

Ono K & Trautwein W(1991) Potentiation by cyclic GMP of ß-adrenergic effect on Ca^{2+} current in guinea-pig ventricular cells. *J Physiol* **443,** 387-404

Reuter H(1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* **301,** 569-574

Roti E, Montermini M, Roti S, Gardini E, Robushi G, Minelli R, Salvi M, Bentivoglio M, Guiducci U & Braverman LE(1988) The effect of ditiazem, a calcium channel-blocking drug, on cardiac rate and rhythm in hyperthyroid patients. *Arch Intern Med* **148,** 1919-1921

Sharp NA, Neel DS & Parson RL(1985) Influence of thyroid hormone levels on the electrical and mechanical properties of rabbit papillary muscle. *J Mol Cell Cardiol* **17,** 119-132

Suko J(1973) The calcium pump of cardiac sarcoplasmic reticulum. Functional alterations at different levels of thyroid state in rabbits. *J Physiol* **228,** 563-582

Taylor RR, Covell JW & Ross J Jr(1969) Influence of the thyroid state on left ventricular tension-velosity relations in the intact, sedated dog. *J Clin Invest* **48,** 775-784

Trautwein W & Kameyama M(1987) The mechanism of ß-adrenergic regulation of calcium channels: intracellular injections and patch clamp studies. In: Noble D & Powell T(ed) *Electrophysiology of single cardiac cells.* Chapter 5. Academic Press, New York, p105-123

Tsien RW(1983) Calcium channels *in excitable cell membranes. Ann Rev Physiol* **45,** 341-358