Electrophysiological abnormalities and enhanced reperfusion arrhythmias in the isolated hearts of hyperthyroid rats

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- 1 The influence of hyperthyroidism on electrophysiological characteristics and on reperfusion arrhythmias was examined in rat hearts.
- 2 Electrophysiological studies were performed with glass microelectrodes, and the experiments on reperfusion arrhythmias were done in isolated perfused hearts.
- 3 Ventricular muscle from hyperthyroid rats was more prone than that from euthyroid rats to develop triggered activity under conditions believed to cause myoplasmic Ca^{2+} overload.
- 4 The severity of reperfusion arrhythmias was significantly enhanced in hyperthyroid preparations as compared with euthyroid ones.
- 5 The enhanced reperfusion arrhythmias in hyperthyroid rats were significantly reduced by propranolol (3×10^{-7} M), lignocaine (1×10^{-5} M) and verapamil (3×10^{-8} M), but not by nadolol (3×10^{-7} M) or prazosin (3×10^{-7} M).
- 6 These results suggest that increased heart rate due to hyperthyroidism and responses mediated via either α - or β -adrenoceptors were not dominant causes of enhanced reperfusion arrhythmias in hyperthyroid hearts.
- 7 The increased tendency to develop triggered activity which was observed in the electrophysiological study, may be one possible explanation of enhanced reperfusion arrhythmias in hyperthyroid hearts.

Introduction

Myocardium from pathophysiological models in rats such as streptozotocin-induced diabetes (Nordin *et al.*, 1985) and cardiac hypertrophy induced by renal hypertension (Gulch *et al.*, 1979; Aronson, 1981) has a propensity to develop Ca^{2+} overload as reflected by the appearance of delayed afterdepolarizations and triggered activity. It has been reported that reperfusion of the myocardium after ischaemia elicits accumulation of intracellular Ca^{2+} (Whalen *et al.*, 1974) and malignant ventricular dysrhythmias (Penkoske *et al.*, 1978). Therefore, it might be expected that the myocardium which has such abnormal electrophysiological properties would develop altered reperfusion arrhythmias. From this

point of view, we examined first the electrophysiological abnormality in the ventricular muscles from hyperthyroid rats and then investigated the effects of hyperthyroidism on reperfusion arrhythmias in rat isolated perfused hearts. Furthermore, thyroxine increases myocardial sensitivity to catecholamines and other biogenic amines (Coville & Telford, 1970). Catecholamines are released from the myocardium during reperfusion after an ischaemic period *in vivo* (Riemersma, 1982) and *in vitro* (Rochette *et al.*, 1980), and it has been suggested that catecholamine release and α -receptor stimulation are involved in the development of reperfusion arrhythmias (Sheridan *et al.*, 1980). Hence, it is conceivable that reperfusion arrhythmias may also be affected by thyroid state-induced receptor alteration. In additional experiments, the effects of some drugs on reperfusion arrhythmias were also investigated to clarify the relationship of some mediators to the arrhythmias.

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Methods

Animals

Male Wistar rats weighing 220–240 g were randomly assigned to a euthyroid or hyperthyroid group. In the latter, a hyperthyroid state was induced by daily intraperitoneal injection of 1 mg kg^{-1} sodium L-thyroxine suspended in 0.5% carboxymethyl cellulose sodium salt for 8–12 days (Hashimoto & Nakashima, 1978). The euthyroid group received an equivalent volume of the vehicle. Animal care and feeding were identical in both groups.

Electrophysiological study

After rats were anaesthetized with ether, hearts were rapidly removed and the left ventricular papillary muscles were excised. The papillary muscle was mounted in a tissue chamber and superfused with modified Tyrode solution which was equilibrated with 95% O_2 :5% CO_2 and maintained at 35°C . Control modified Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, CaCl_2 2.4, MgCl_2 1.0, NaH_2PO_4 0.5, NaHCO_3 12.0 and glucose 5.5. Transmembrane action potentials were recorded by glass microelectrodes filled with 3 M KCl and having a tip resistance of 10 to 20 M Ω . The microelectrode was coupled with an Ag-AgCl electrode and connected to an amplifier. External electrical stimulation was delivered to the tissue through bipolar silver electrodes. Stimuli were rectangular pulses, 1 ms in duration and 2 times the threshold voltage. Action potential parameters were measured at a cycle length of 1000 ms after 60 min equilibration of muscle in control Tyrode solution. Continuous recordings of electrical activity were obtained on a recorder and analysis of action potentials was performed by a microcomputer interfaced with an analogue-digital converter for pulse analysis (Nihon Kohden, ATAC-450).

In the study of triggered activity, each muscle was equilibrated in control Tyrode solution ($\text{Ca}^{2+} = 2.4 \text{ mM}$) for about 60 min, and then exposed sequentially to Tyrode solution containing increased concentrations of Ca^{2+} ; 4.8 and 9.6 mM. The stimulation

protocol during superfusion with each solution was as follows: 20 stimuli at a cycle length of 2000 ms, followed by stimulation at a cycle length of 150 ms with trains of 10, 20 and 40 stimuli; a quiescent period of 20 s was allowed after each stimulation sequence (Nordin *et al.*, 1985). Continuous recordings were obtained on a chart recorder.

Reperfusion arrhythmias

Rats were pretreated with heparin sodium (200 iu, i.v.), anaesthetized with ether and the hearts were quickly excised and placed in chilled, heparinized and preoxygenated Krebs-Henseleit (K-H) solution. After a glass cannula filled with the solution had been inserted into the aorta, the heart was mounted in a water-jacketed chamber and perfused retrogradely by the Langendorff technique with K-H solution at a constant pressure (80 mmHg) at 37°C (Sakai & Shiraki, 1978). The K-H solution had the following composition (mM): NaCl 118, KCl 4.7, CaCl_2 1.2, MgSO_4 1.18, KH_2PO_4 1.18, NaHCO_3 24.88 and glucose 11.1. The solution was saturated with 95% O_2 :5% CO_2 , and the pH was 7.4 at 37°C . A polyethylene cannula was inserted via the left atrium into the left ventricle to record the left ventricular cavity pressure (LVP). Silver wire electrodes were attached at the origin of the aorta and the apex of the ventricle to record the electrocardiogram (ECG). In some euthyroid heart preparations, the heart rate was fixed by electrical pacing. The isolated heart was perfused for 20 min to stabilize cardiac function. Drug treatment was started after stabilization by changing the perfusate to one which contained drug and the drug level was then maintained throughout the remainder of the experiment. Fifteen minutes later, the left coronary artery was ligated at a position about 2–3 mm from its origin. Reperfusion arrhythmias were induced by untying the coronary ligature after 10 min of ischaemia. At the end of each experiment, the heart was weighed.

Statistical analysis

The Mann-Whitney U-test was used to compare the data for reperfusion arrhythmias between euthyroid

Table 1 Characteristics of experimental animals

	BW (g)	HW (mg)	HR (beats min^{-1})	LVP (mmHg)
Euthyroid	261 ± 5 (23)	712 ± 19 (21)	299 ± 8 (21)	93.3 ± 2.4 (20)
Hyperthyroid	226 ± 2 (56)	885 ± 10 (48)	366 ± 14 (48)	105.9 ± 4.1 (48)
	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS

Data are given as mean $\pm$ s.e.mean. Numbers in parentheses: number of preparations measured. BW: body weight; HW: heart weight; HR: heart rate; LVP: left ventricular pressure; NS: not significant at $P = 0.05$. Statistical significance was determined by Student's *t* test.

Table 2 Electrophysiological characteristics of papillary muscle cells

	RMP (mV)	AMP (mV)	$\dot{V}_{max}$ (V s ⁻¹)	APD ₅₀ (ms)	APD ₉₀ (ms)	ERP (ms)
Euthyroid (n = 8)	-77.0 ± 1.0	99.9 ± 1.3	156 ± 6	11.1 ± 0.8	44.6 ± 3.1	31.7 ± 1.3
Hyperthyroid (n = 8)	-74.1 ± 1.4 NS	98.5 ± 1.3 NS	153 ± 9 NS	15.3 ± 0.6 P < 0.01	71.9 ± 2.6 P < 0.01	58.1 ± 5.2 P < 0.01

Results are expressed as mean ± s.e.mean.

RMP: resting membrane potential; AMP: amplitude of action potential; $\dot{V}_{max}$: maximum rate of depolarization; APD_x: duration of action potentials to x% of complete repolarization; ERP: effective refractory period; NS: not significant at P = 0.05. Statistical significance was determined by Student's *t* test.

and hyperthyroid groups. The Chi-squared test was used to analyze the data on the tendency of the myocardium to develop triggered activity. Student's *t* test was used to compare various other characteristics between each group. A probability value of less than 0.05 was considered to be statistically significant.

Results

Characteristics of experimental animals

The characteristics of experimental animals are summarized in Table 1. Hyperthyroid rats failed to gain their body weight. In the hyperthyroid group, the hearts were significantly hypertrophied and the heart rate was significantly greater than that observed in the euthyroid group.

Electrophysiological characteristics

The electrophysiological characteristics of papillary muscle cells obtained from euthyroid and hyperthyroid rat hearts are detailed in Table 2. There were no significant differences in resting membrane potential (RMP), amplitude of action potential (AMP) and maximum rate of depolarization ($\dot{V}_{max}$) between euthyroid and hyperthyroid groups. The most obvious effects of hyperthyroidism on action potential parameters were marked prolongation of the action potential duration (APD) and of the effective refractory period (ERP).

Triggered activity

Figure 1 shows typical recordings of action potentials from papillary muscle cells from euthyroid and hyperthyroid rats superfused with modified Tyrode solution containing 2.4 and 9.6 mM Ca²⁺. In the

Figure 1 Typical recordings of action potentials after stimulation with trains of pulses in rat papillary muscle cells. In each record, the first two action potentials were the last of 20 action potentials elicited at the control cycle length of 2000 ms. The rapid train stimulation developed 40 action potentials at a cycle length of 150 ms (horizontal line below record). Under the condition of $[Ca^{2+}]_o = 9.6$ mM, the hyperthyroid preparation developed several triggered action potentials followed by a small delayed afterdepolarization, while the euthyroid preparation developed only a small delayed afterdepolarization. $[Ca^{2+}]_o$ = external Ca²⁺ concentration.

Table 3 Effect of hyperthyroidism on the incidence of triggered activity in papillary muscle cells

[Ca ²⁺] _o	Train pulses*	No. developing triggered activity**		
		Euthyroid	Hyperthyroid	
2.4	10	0	1	NS
	20	0	1	
	40	0	1	
4.8	10	0	1	<i>P</i> < 0.05
	20	0	4	
	40	4	9	
9.6	10	0	1	<i>P</i> < 0.01
	20	0	6	
	40	3	10	

* Number of pulses per train at a cycle length of 150 ms.

** Each number represents the fraction of ten euthyroid and ten hyperthyroid preparations that developed triggered activity under conditions of altered Ca²⁺ concentration.

[Ca²⁺]_o: external Ca²⁺ concentration (mM); NS: not significant at *P* = 0.05. Statistical significance in the incidence of triggered activity was determined between euthyroid and hyperthyroid groups by the Chi-squared test.

figure, following 40 preceding driven action potentials at a cycle length of 150 ms, triggered responses were evoked and followed by a delayed after-depolarization in the hyperthyroid preparation exposed to the solution containing 9.6 mM Ca²⁺, whereas the euthyroid preparation failed to develop triggered activity under the same experimental conditions. Table 3 summarizes the frequency of occurrence of triggered activity when the muscles were exposed to the three solutions in order of increasing concentration of Ca²⁺ (2.4, 4.8 and 9.6 mM). The data show that the hyperthyroid myocardium developed triggered activity more frequently than the euthyroid myocardium under conditions of increased external Ca²⁺ concentration. In the preliminary experiments, triggered activity did not develop in hyperthyroid muscles at cycle lengths of stimulation longer than 300 ms even with the highest concentration (9.6 mM) of external Ca²⁺. Triggered

activity in the muscles from euthyroid rats was also examined during incubation with 1×10^{-6} M L-thyroxine at a calcium concentration of 9.6 mM. These muscles did not show enhanced triggered activity (data not shown).

Reperfusion arrhythmias

During the occlusion period, both euthyroid and hyperthyroid groups exhibited general phenomena associated with ischaemia, i.e., LVP was lessened rapidly just after coronary occlusion with an increase in the height of the QRS wave followed by elevation of the ST-T segment. Under our experimental conditions, no severe arrhythmias apart from a few ventricular premature contractions (VPCs) were observed during the 10 min period of coronary ligation. Comparison of arrhythmias elicited during occlusion and reperfusion periods between euthyroid

Table 4 Effect of hyperthyroidism on arrhythmias during occlusion and reperfusion

	Occlusion	VPCs**	VT (s)*	Reperfusion	VT + VF (s)*
	VPCs*			VF (s)*	
Euthyroid (<i>n</i> = 14)	14.8 ± 3.9	16.8 ± 4.5	5.5 ± 0.9	8.5 ± 1.5	14.0 ± 2.1
Hyperthyroid (<i>n</i> = 14)	9.8 ± 5.1 NS	40.7 ± 8.8 <i>P</i> < 0.01	4.0 ± 0.8 NS	111.0 ± 38.1 <i>P</i> < 0.001	115.1 ± 38.6 <i>P</i> < 0.001

* Number of VPCs during 10 min occlusion. ** Number of VPCs during first 10 min after reperfusion. # Duration of arrhythmias during first 10 min after reperfusion.

Results are expressed as mean ± s.e.mean.

VPCs: ventricular premature contractions; VT: ventricular tachycardia; VF: ventricular fibrillation; NS: not significant at *P* = 0.05.

Statistical significance was determined by the Mann-Whitney U-test.

Figure 2 Effect of drugs on enhanced reperfusion arrhythmias in hyperthyroid hearts. The severity of arrhythmias was estimated as the total duration of ventricular tachycardia (VT) and fibrillation (VF). Each column represents the mean, and the vertical bars represent s.e.mean. Con = control (no treatment); Prop = propranolol (3×10^{-7} M); Nado = nadolol (3×10^{-7} M); Praz = prazosin (3×10^{-7} M); Verap = verapamil (3×10^{-8} M); Lign = lignocaine (1×10^{-5} M). Asterisks indicate data significantly reduced from the hyperthyroid control value, * $P < 0.001$ (Mann-Whitney U-test).

and hyperthyroid preparations are represented in Table 4. Ventricular tachycardia (VT) and/or ventricular fibrillation (VF) were induced within 20 s after reperfusion in both of the groups.

Effect of drugs on enhanced reperfusion arrhythmias in hyperthyroid hearts

The effects of drugs on the prolonged duration of arrhythmias (total duration of VT + VF) developed in hyperthyroid preparations are shown in Figure 2. Propranolol (3×10^{-7} M) reduced the duration of arrhythmias significantly, but nadolol (3×10^{-7} M), another β -adrenoceptor blocker which has no membrane stabilizing activity, failed to shorten the duration of arrhythmias. Prazosin (3×10^{-7} M), an α_1 -selective adrenoceptor blocker, did not have any anti-arrhythmic effect. Both verapamil (3×10^{-8} M) and lignocaine (1×10^{-5} M) showed significant reduction of reperfusion arrhythmias in hyperthyroid preparations.

Effect of heart rate on reperfusion arrhythmias

Of the drugs tested, lignocaine decreased heart rate significantly from 358 ± 26 beats min^{-1} (before drug treatment) to 290 ± 13 beats min^{-1} (15 min after drug treatment) ($P < 0.05$, $n = 7$), and verapamil

reduced heart rate from 368 ± 8 beats min^{-1} to 333 ± 12 beats min^{-1} ($P < 0.05$, $n = 8$) and LVP from 111.6 ± 5.8 mmHg to 83.1 ± 5.5 mmHg ($P < 0.01$, $n = 8$). The other drugs did not show any statistically significant effect either on heart rate or on LVP at least at the concentration tested. To evaluate the effect of increased heart rate on the severity of reperfusion arrhythmias, euthyroid heart preparations were driven by electrical pacing at the fixed rate of 375 beats min^{-1} which was nearly equal to the heart rate observed in hyperthyroid preparations. The electrically paced group exhibited significant ($P < 0.01$) prolongation of the duration of VT + VF (34.8 ± 6.1 s, $n = 8$) as compared with the non-paced euthyroid control (14.0 ± 2.1 s, $n = 14$), but it was still significantly ($P < 0.01$) milder than that observed in the hyperthyroid control group (115.1 ± 38.6 s, $n = 14$).

Discussion

Comparison of electrophysiological characteristics of papillary muscle cells revealed that APD and ERP were significantly prolonged in the hyperthyroid preparations compared with the euthyroid ones, whereas there were no significant differences in the other parameters measured. Such prolonged APD was also observed in the myocardium from streptozotocin-induced diabetic rats (Nordin *et al.*, 1985) and in hypertrophied myocardium from renal hypertensive rats (Gulch *et al.*, 1979). As prolongation of APD and ERP is one of the explanations of the effect of antiarrhythmic drugs such as quinidine and amiodarone, our results obtained from the experiments with reperfused hearts seem paradoxical. This suggests that other factors may be involved in the increased tendency to develop arrhythmias in hyperthyroid preparations.

Delayed afterdepolarization and triggered activity occur under conditions assumed to increase the concentration of intracellular Ca^{2+} , such as the presence of cardiac glycosides or high Ca^{2+} in the extracellular solution (Hashimoto & Moe, 1973; Ferrier & Moe, 1973; Allen *et al.*, 1984). In our study of triggered activity, the results suggest that the myocardium from hyperthyroid rats is much more prone to develop triggered activity than that from euthyroid ones under our conditions believed to cause intracellular Ca^{2+} overload. The enhanced triggered activity is not caused by thyroid hormone itself but by thyroid hormone-induced hypertrophy or by some modification of myocardial function, because the papillary muscles from the euthyroid rat did not show enhanced triggered activity during the incubation with thyroxine.

The present study with rat isolated perfused hearts demonstrates that hyperthyroidism enhances the severity of reperfusion arrhythmias expressed as the total duration of VT and VF. The enhanced arrhythmias observed in hyperthyroid preparations were significantly reduced by propranolol (3×10^{-7} M). In contrast, nadolol failed to reduce the enhanced reperfusion arrhythmias in hyperthyroid preparations at a concentration of 3×10^{-7} M which was the same concentration as propranolol and was sufficient to block β -adrenoceptors (Vukovich *et al.*, 1979). This discrepancy agrees with results which showed that atenolol (Kane *et al.*, 1984; Rochette *et al.*, 1984) and (-)-propranolol (Lamontagne *et al.*, 1986) were ineffective on reperfusion arrhythmias, although propranolol and acebutolol (Rochette *et al.*, 1984) were effective. As nadolol is a β -adrenoceptor blocker that lacks membrane stabilizing activity (MSA) (Lee *et al.*, 1975), our results may support the explanation that MSA of some β -blocking agents is of importance in reducing reperfusion arrhythmias (Rochette *et al.*, 1984), and that β -adrenoceptors are probably not involved in the genesis of arrhythmias of the reperfused ischaemic myocardium, although the responses mediated by β -adrenoceptors are enhanced by hyperthyroidism (Kunos, 1977).

α_1 -Adrenoceptor blockade with prazosin failed to reduce the prolonged duration of reperfusion arrhythmias in hyperthyroid preparations. Inhibitory effects of prazosin on reperfusion arrhythmias were reported in dogs (Lamontagne *et al.*, 1986) and in cats (Sheridan *et al.*, 1980) *in vivo*. However, Bralet *et al.* (1985) showed that prazosin did not prevent reperfusion arrhythmias after a 10 min period of coronary ligation in rat hearts on a Langendorff perfusion system and explained that this contradiction might be derived from the difference in the duration of the occlusion period during which the increase of the number of α_1 -adrenoceptors took place. In the experiments in dogs and cats, the occlusion period was 20 and 30 min respectively, while in the experiments in rats *in vitro*, the period was only 10 min which was equal to our experimental protocol. It has also been reported that the increase in the number of α_1 -adrenoceptors in ischaemic myocardium began to occur after 15 min of ischaemia without an alteration of affinity (Corr *et al.*, 1981). Thus, our result could be explained in a similar manner to the result of Bralet *et al.*, but because responses mediated by α -adrenoceptor are attenuated and those mediated by β -adrenoceptor are potentiated in hyperthyroid animals (Kunos, 1977; Hashimoto & Nakashima, 1978), there is the possibility that attenuation of responses mediated by α -adrenoceptors may conceal the effect of prazosin on α_1 -adrenoceptors. Although the detailed reason why prazosin is ineffective in our

model is not clear, it seems apparent that α - and β -adrenoceptors may not participate in the enhancement of reperfusion arrhythmias in hyperthyroid preparations under our experimental conditions.

Lignocaine caused a marked reduction of the prolonged duration of reperfusion arrhythmias in hyperthyroid preparations. Similar results have been reported in experiments in normal rats; drugs that can block the fast inward sodium current in cardiac tissue (Class I of the Vaughan Williams classification), such as Org 6001, bepridil (Kane *et al.*, 1984), lignocaine, quinidine and procainamide (Lubbe *et al.*, 1978; Bergey *et al.*, 1982), reduced the severity of reperfusion arrhythmias.

On the other hand, the effect of the calcium entry blocker verapamil in hyperthyroid rats produced a conflicting result compared with the results obtained from the euthyroid rat by other authors. Verapamil reduced the enhanced reperfusion arrhythmias in our model, whereas calcium entry blockers such as verapamil, nifedipine and prenylamine failed to show antiarrhythmic effects on the reperfusion arrhythmias in the euthyroid rat (Kane *et al.*, 1984). Nevertheless, it is generally accepted that calcium antagonists inhibit triggered activity (El-Sherif *et al.*, 1983), which may be involved in the antiarrhythmic effect of verapamil observed in the present study. Considering the enhanced triggered activity and the antiarrhythmic effect of the calcium antagonist in the hearts of hyperthyroid rats, electrophysiological alteration, especially an increased propensity to develop triggered activity, may be involved in the enhanced reperfusion arrhythmias in our hyperthyroid model.

On the other hand, ventricular arrhythmias which developed during the 10 min period of coronary ligation were not enhanced by hyperthyroidism. As the dominant mechanism of genesis, at least of the first phase of early arrhythmias in ischaemic hearts is that of re-entry (Russell, 1982), hyperthyroidism may not affect re-entrant activation within the myocardium during the ischaemic period under our experimental conditions.

As hyperthyroid hearts have faster heart rates than euthyroid hearts, and lignocaine and verapamil significantly reduced this, we investigated whether increased heart rate was responsible for the enhancement of reperfusion arrhythmias in electrically paced euthyroid hearts. Euthyroid hearts which were paced at a rate approximately equal to that of hyperthyroid hearts showed enhanced reperfusion arrhythmias compared with non-paced euthyroid controls, but these were still less severe than those observed in hyperthyroid hearts. Moreover, propranolol shortened the prolonged duration of arrhythmias without a significant fall in heart rate in the hyperthyroid hearts under our experimental conditions. It has also

been reported that atenolol, which had a marked negative chronotropic effect, failed to reduce reperfusion arrhythmias in normal rats *in vivo* (Kane *et al.*, 1984). Thus, increased heart rate may be a part of the cause, but other factors may be more important in the enhancement of reperfusion arrhythmias in hyperthyroid hearts.

In summary, the severity of reperfusion arrhythmias in perfused rat hearts was significantly enhanced in hyperthyroid preparations compared with that observed in euthyroid ones. Neither α - nor β -adrenoceptors were involved in the genesis of enhanced reperfusion arrhythmias, and the increased

heart rate could only be a part of the cause. As cardiac hypertrophy occurs in our hyperthyroid model, it is uncertain whether the enhanced reperfusion arrhythmias are the result of hyperthyroidism itself and/or cardiac hypertrophy. Although the precise mechanism of enhancement of reperfusion arrhythmias in hyperthyroid hearts is not apparent, increased susceptibility to development of triggered activity, which was observed in our electrophysiological study under conditions assumed to increase intracellular Ca^{2+} , may be at least one possible explanation for the cause of enhanced reperfusion arrhythmias in the hyperthyroid preparations.

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(Received December 6, 1988

Revised February 7, 1989

Accepted March 9, 1989)