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THE EFFECT OF ALTERED THYROID STATE ON ATRIAL INTRACELLULAR POTENTIALS

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SUMMARY

1. A group of rabbits was made hypothyroid by thyroidectomy, and another group was injected daily with L-thyroxine. After an appropriate interval respective alterations in thyroid state were confirmed by measurement of heart weight and of plasma iodine, and the animals' atria were isolated for recording.

2. Measurements were made of atrial contractions, conduction velocity, spontaneous heart rate and maximum driven frequency, and action potentials were recorded with intracellular micro-electrodes.

3. The resting potential and action potential heights were not affected by differences of thyroid state.

4. Atrial arrhythmias are common in hyperthyroidism, rare in myxoedema. The possibility that hypothyroidism might reduce the rate of rise of the action potential, as do anti-arrhythmic drugs, and hyperthyroidism increase it, was investigated. Although the rate of rise was slower in hypothyroid atria at some driving frequencies, this could not alone account for an anti-arrhythmic effect, because at frequencies near the spontaneous heart rate the rate of rise of the action potential was not reduced.

5. The duration of the repolarization phase of the action potential was greatly prolonged in atria from thyroidectomized rabbits, and was shortened in hyperthyroid atria. These changes could account for a reduced probability of arrhythmias in hypothyroidism, and the converse in hyperthyroidism.

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INTRODUCTION

Hyperthyroidism is a common cause of atrial fibrillation and other supraventricular arrhythmias, whereas in myxoedema irregularities of cardiac rhythm are rare (Goodall, 1920; Wilson, 1924; Scherf & Schott, 1953). Previous studies of the effect of anti-arrhythmic drugs upon intracellularly recorded atrial action potentials revealed that these agents had in common the property of reducing the rate of rise of the action potential, without altering the resting potential or significantly prolonging the duration of the action potential (Vaughan Williams, 1958*a*; Szekeres & Vaughan Williams, 1962). It seemed possible that hypothyroidism might be associated with effects similar to those produced by anti-arrhythmic drugs, and hyperthyroidism with changes in the opposite direction. Records of intracellular potentials were obtained from isolated spontaneously beating pacemaker tissue from the atria of hyper- and hypothyroid rabbits by Gargouïl, Lenfant & Tricoche (1966), but the heart rates were not the same in the different thyroid states, and measurements of the maximum rates of depolarization were not reported. Whether or not increased sympathetic activation plays a part in precipitating arrhythmias in hyperthyroidism has long been a topic of controversy (reviewed by Harrison, 1964). The observation that β-receptor blocking drugs are effective in reducing tachycardia in hyperthyroidism (Howitt & Rowlands, 1966; McDevitt, Shanks, Hadden, Montgomery & Weaver, 1968) provided an additional reason for investigating the direct effects of differences in thyroid state on cardiac intracellular potentials, since β-receptor blocking compounds also reduce the rate of rise of the atrial action potential (Vaughan Williams, 1964; Papp & Vaughan Williams, 1969*a*, *c*).

In the present experiments rabbits were made hypothyroid by surgical thyroidectomy, or hyperthyroid by daily injections of thyroxine. Thyroid state was estimated from growth rates, plasma-bound iodine, spontaneous heart rates, and heart weights expressed as percentages of body weight. Intracellular potentials were recorded from the isolated atria of these animals, and responses to various stimuli were measured.

METHODS

Thyroidectomy. Young rabbits (2–4 months old) of mixed breed and both sexes, weighing 800–1400 g, were used. One group was anaesthetized with 20 mg sodium pentobarbitone, and the method of G. W. Harris (personal demonstration) was used, involving tracheal intubation with a soft 6–8 French catheter, through which anaesthesia was maintained with ether. The technique was not aseptic, but penicillin was instilled into the wound, and 100,000 units injected intramuscularly. The thyroid gland, including the isthmus, was removed after ligation of the main thyroid artery on each side. In a second group ether anaesthesia only was employed, after injection

of atropine sulphate, 1 mg/kg, subcutaneously, without tracheal intubation, and strict asepsis was observed. The animals were used for experiment 4–6 weeks later.

Hyperthyroidism. Rabbits weighing 800–1400 g were given a large initial dose (100–200 μg) of thyroxine for 1 or 2 days, and repeated daily doses (20–200 μg) were subsequently administered in amounts sufficient to maintain the body weight within 10 % of the initial weight. They were used 1–3 weeks after the initiation of therapy.

Controls. These were kept on the same diet and under the same conditions as the others. Four were sham-operated; they were anaesthetized, the thyroid gland was dissected from its bed, and then replaced.

On the day of the experiment 1 mg heparin was injected into an ear vein, and the rabbit was stunned. A carotid artery was exposed and cut, and freely flowing blood was collected in iodine-free containers. The atria were dissected out, and set up for recording as previously described (Szekeres & Vaughan Williams, 1962). Measurements were made on the isolated atria at 32° C, of spontaneous heart rate, conduction velocity and maximum frequency at which a twice-threshold stimulus (1 msec, square wave) was followed. Electrical threshold and conduction velocity were measured at a frequency 10 % above the spontaneous frequency. Contractions were recorded with an RCA 5734 transducer. Intracellular potential records were obtained with 3 M-KCl-filled micropipettes from atria driven at a wide range of frequencies. The maximum rate of rise of the intracellular action potential was recorded with a differentiator (Papp & Vaughan Williams, 1969*b*). The nutrient solution contained (mM) NaCl, 125; KCl, 5·6; $CaCl_2$, 2·16; $NaHCO_3$, 25; glucose, 11, and was gassed with 95 % O_2, 5 % CO_2, with resultant pH 7·4.

Plasma-bound iodine was estimated by the method described by Riley & Gochman (1964) (determinations performed by Dr R. H. Wilkinson of the Radcliffe Infirmary, Oxford).

Statistics. Means have been given ± S.E. and significance calculated by Student's *t* test.

Drugs used: L-Thyroxine, sodium salt (B.D.H.), liothyronine, sodium salt (Glaxo), heparin, 166 i.u. mg^{-1} (Evans Medical).

RESULTS

Growth rates. The controls (mean initial weight 1003 g) gained weight at a slowly decreasing rate, averaging 35 g per day during the first 3 weeks, and 29 g during the second. The mean daily growth rate of the thyroidectomized animals (mean initial weight 1003 g) was 18·5 g in the first 3 weeks, and 16·3 g in the second. The rabbits injected with thyroid had a mean initial weight of 930 g, and the mean weight at the time of experiment was 1002 g.

The mean heart weight (wet) of twenty-three control rabbits, expressed as % body weight, was 0·2353 ± 0·0065 %. In the hypothyroid group (n = 10) it was 0·1843 ± 0·0044 %, which was highly significantly different (t = 5·2) from that of the controls. Conversely, the mean heart weight of the thyrotoxic animals (n = 10) was 0·335 ± 0·089 % body weight, the difference being again very highly significant (t = 8·8).

Plasma-bound iodine. The mean plasma-bound iodine in the blood of eleven control animals was 6·44 ± 0·431 μg/100 ml. (range 3·5–8·5). In the thyroidectomized group (n = 10) it was 3·321 ± 0·481 (range 1·0–5·5),

which differed significantly from the controls ($P < 0{\cdot}001$). In the hyperthyroid group ($n = 10$) the mean was $11{\cdot}141 \pm 1{\cdot}58$ μg/100 ml., which was again significantly different from the controls ($P < 0{\cdot}01$; range 7·4–20).

Heart rate. The spontaneous rates of the isolated atria beating in nutrient solution at 32 °C were measured at the beginning and end (figures given in brackets) of each experiment, and the means for the three groups were: hypothyroid, $100{\cdot}7 \pm 3{\cdot}47$ $(95{\cdot}3 \pm 4{\cdot}9)$ min^{-1}, euthyroid $128{\cdot}5 \pm 2{\cdot}3$ $(120{\cdot}0 \pm 3{\cdot}8)$ and hyperthyroid $201{\cdot}7 \pm 9{\cdot}9$ $(220 \pm 10{\cdot}0)$. The means of the groups were significantly different from each other ($P < 0{\cdot}001$), but the differences between the rates at the beginning and end of the experiments within each group were not statistically significant.

In the course of a previous study it was found that the spontaneous beating frequency of rabbit atria increased by 10–12 beats/°C (Vaughan Williams, 1958*b*), so that at 37 °C expected rates would have been 151–173 hypothyroid, 180–200 euthyroid, and 252–274 hyperthyroid. *In vivo* heart rates are hard to measure accurately by palpation, and if acute e.c.g. records are taken doubt arises how far the rates may have been increased by alarm caused by the presence of electrodes. The spontaneous heart rate of a thyroidectomized rabbit with chronically implanted e.c.g. electrodes was reported as being 130–150 beats/min under basal conditions by Guz, Kurland & Freedberg (1961). Previous e.c.g. records in adult rabbits indicated spontaneous rates in the range 200–230 beats/min. In the present series of experiments *in vivo* heart rates were counted by palpation in many of the animals just before they were killed, and the means were 172 (160–185) hypothyroid, 197 (185–225) euthyroid, and 278 (270–285) hyperthyroid.

In spite of these large differences in spontaneous heart rate, the maximum frequency at which the atria would follow a driving stimulus was within a few per cent in all three groups, $396 \pm 13{\cdot}7$ min^{-1} (controls), $382 \pm 22{\cdot}9$ (thyroidectomized), and $350 \pm 18{\cdot}7$ (hyperthyroid). The differences were not statistically significant.

Conduction velocity and electrical threshold. Conduction velocity was not significantly altered by the differences in thyroid state. In the controls it was $0{\cdot}484 \pm 0{\cdot}03$ m.sec^{-1}, in the thyroidectomized animals $0{\cdot}503 \pm 0{\cdot}01$ m.sec^{-1}, and in the hyperthyroid rabbits $0{\cdot}534 \pm 0{\cdot}022$ m.sec^{-1}. Measurements of electrical threshold (in volts) had no absolute meaning, but had relative significance since the electrodes and bath volume were similar in all experiments. The threshold in the hypothyroid atria ($2{\cdot}86 \pm 0{\cdot}38$ V) was the same as in the controls ($2{\cdot}93 \pm 0{\cdot}14$ V), but in the atria from the hyperthyroid animals the threshold was raised ($3{\cdot}53 \pm 0{\cdot}2$ V). Although this difference was significant ($0{\cdot}05 > P > 0{\cdot}02$), it was comparatively trivial, and could well have been merely a 'rate effect', as it

was not, of course, possible to measure electrical thresholds at low rates in hyperthyroid atria.

Intracellular records. Some parameters of intracellular action potentials are affected by driving frequency; for example, the duration of the action potential decreases as the heart rate increases, and the rate of rise is reduced at very high frequencies. It was, therefore, necessary to obtain records from the hypothyroid and control atria at heart rates at least as high as the spontaneous rate of hyperthyroid atria. In practice intracellular records were obtained from atria driven at various frequencies between

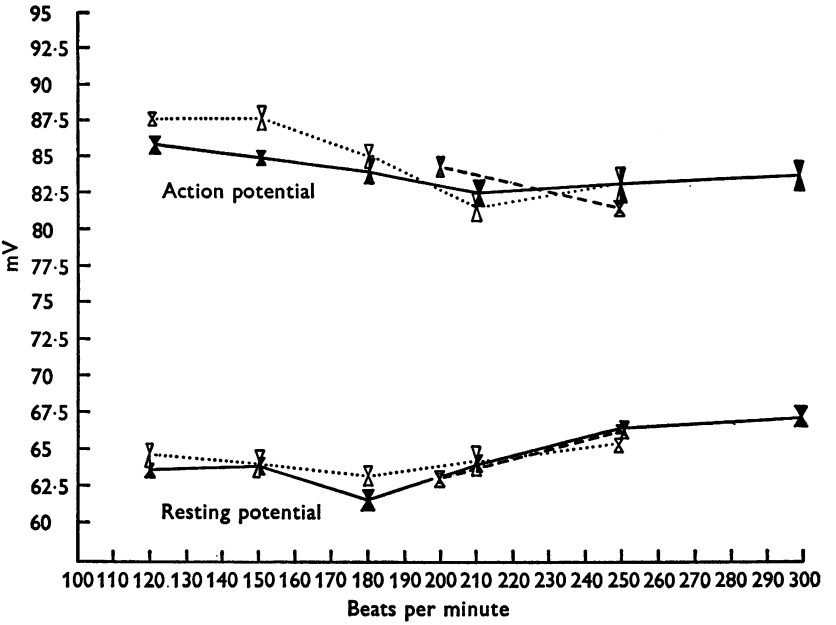

Fig. 1. Intracellularly recorded resting and action potentials. Ordinate: mV. Abscissa: driving frequency, beats/min. Controls: filled triangles and continuous line. Hypothyroid animals: open triangles and dotted line. Hyperthyroid animals: striped triangles and dashed line. The apices of the triangles meet at the mean, and the height of each triangle equals the S.E.

110 and 300 min^{-1}, but for simplicity of statistical analysis measurements taken at neighbouring frequencies have been pooled into groups separated by approximately 20 % intervals. Intracellular records were obtained from several different fibres in each experiment, and the results from all experiments have been pooled for presentation in Figs. 1 and 2. No point in either Figure is based on less than twenty-five individual measurements, and some represent the means of more than a hundred. The resting potential and height of the action potential were not affected by differences

of thyroid state (Fig. 1), and both were well maintained at the higher frequencies.

Measurements of the maximum rate of rise of the action potentials are presented in Table 1. In the control group the rate of rise was well maintained at frequencies up to 160 min^{-1}. Although at low frequencies the rate of rise in hypothyroid atria was significantly slower than that of the controls, the difference was small; and in the range 140–160 min^{-1} (which was close to the *in vivo* spontaneous heart rate in hypothyroid animals) the rate of rise was as fast as in the controls. Thus the low incidence of arrhythmias in hypothyroidism cannot fairly be attributed solely to a lower rate of rise, i.e. to a change in membrane activity similar to that induced by anti-arrhythmic drugs.

TABLE 1. Maximum rate of rise of action potential, V/sec

Driving frequency ... (beats/min)	110–130	140–160	180–220	250–300
Controls	90·48 ± 2·20	92·03 ± 1·91	78·22 ± 1·36	71·79 ± 1·95
Hyperthyroid	—	—	96·15** ± 4·09	70·61 ± 4·82
Hypothyroid	82·12* ± 1·69	95·33 ± 4·40	66·71** ± 2·75	58·04** ± 2·57

The figures marked with asterisks were significantly different from controls.
* = $0{\cdot}01 > P > 0{\cdot}001$. ** = $P < 0{\cdot}001$.

In marked contrast to the lack of effect on resting and action potentials, the duration of the repolarization phase was greatly altered by differences in thyroid state (Fig. 2). The action potential was very much longer in hypothyroid than in euthyroid atria at all driving frequencies, and was significantly shorter in the hyperthyroid atria. Indeed the time to 50 % repolarization in the hypothyroid hearts was only a few milliseconds shorter than the time required for 90 % repolarization in the hyperthyroid tissue.

Determination of the length of time an animal needs to be hyperthyroid or hypothyroid for changes in duration of the atrial action potential to occur will require a separate investigation. In one animal, 6 weeks after thyroidectomy, 500 μg liothyronine was injected subcutaneously, and recordings were made from the atria the following day, 16 hr after the injection. It was of interest that the duration of the action potential was 127·13 msec (time to 90 % repolarization, mean of sixteen fibres) at a driving frequency of 210/min. This was still very much longer than that of the controls, but already a few milliseconds less than the mean action potential duration in untreated hypothyroid animals.

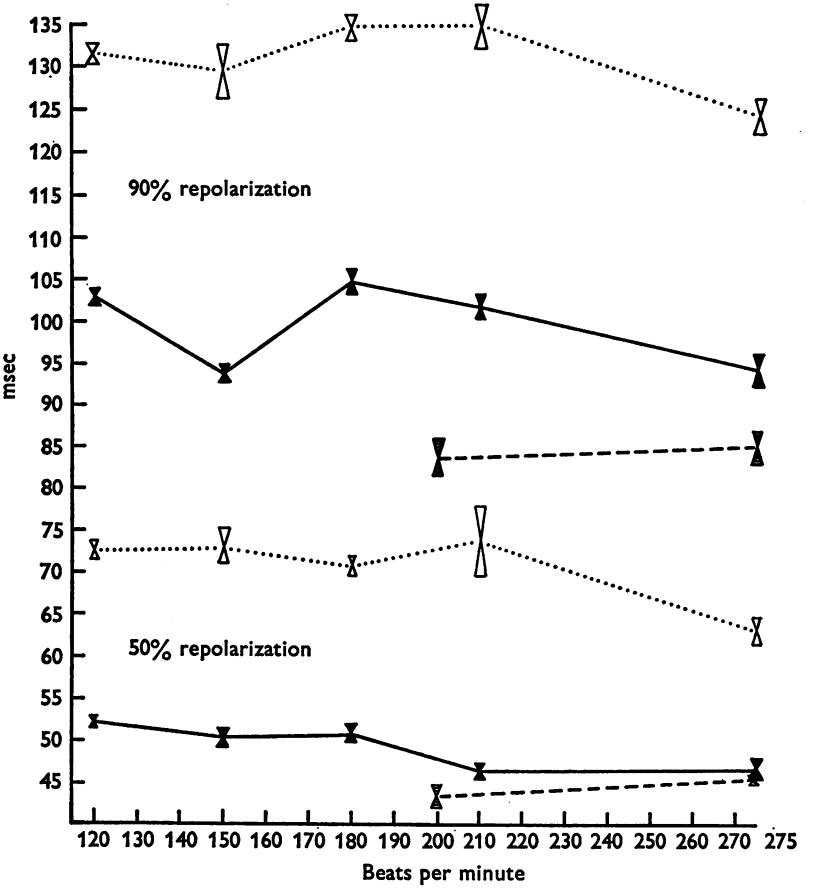

Fig. 2. Duration of action potential from peak to 90% repolarization (upper curves) and to 50% repolarization (lower curves). Ordinate: msec. Abscissa: driving frequency, beats/min. Symbols as for Fig. 1.

DISCUSSION

Sympathetic activation in thyrotoxicosis. Hyperthyroidism is a common cause of supraventricular arrhythmias, especially atrial fibrillation, whereas in myxoedema, apart from occasional ventricular extrasystoles associated with a very low sinus rate, irregularities of cardiac rhythm are rare (Goodall, 1920; Wilson, 1924; Wishart, 1929; Nahum & Hoff, 1935; Scherf & Schott, 1953). Arrhythmias in thyrotoxicosis may be associated with sympathetic activation, as are many other arrhythmias, even those induced by digitalis (Dohadwalla, Freedberg & Vaughan Williams, 1969). There have been many reports that injections of adrenaline and noradrenaline exert greater effects in hyperthyroid man and some animals than

in the euthyroid state (Schneckloth, Kurland & Freedberg, 1953 (in man); Brewster, Isaacs, Osgood & King (q.v. for further references), 1956 (in dogs); Wurtman, Kopin & Axelrod, 1963 (in rats)). It has also been suggested that increased background sympathetic activity may develop rather than sensitization of receptors, since heart rates in thyrotoxic patients and animals were immediately reduced by sympatholytic procedures (spinal anaesthesia (Brewster *et al.* 1956); reserpine (Thier, Gravenstein & Hoffman, 1962); guanethidine (Goldstein & Killip, 1965); propranolol (Howitt & Rowlands, 1966); oxprenolol (Turner & Hill, 1968; Darsinos, Katsilambros, Papamatheou & Papadoyanakis, 1968)).

In contrast, Benfey & Varma (1963), in cats, Hess & Shanfield (1965) in rats, and Margolius & Gaffney (1965) and Moran & van der Schoot (1967) in dogs, did not find that differences in thyroid state affected responsiveness to catecholamines. Increased sympathetic activity could not account for the whole of the cardiac effects of thyrotoxicosis in man, because propranolol (Howitt & Rowlands, 1966) did not reduce heart rates to normal values, and Wilson, Theilen & Fletcher (1964) observed that doses of pronethalol adequate to block isoprenaline-induced tachycardia did not alter heart rate, cardiac index or blood pressure in thyrotoxic patients. McDevitt *et al.* (1968), confirmed that DL-propranolol reduced heart rate in thyrotoxic patients, but found similar reductions by this drug in hypothyroidism. Since D-propranolol, which has one hundredth the β-receptor blocking activity of the L-isomer (Barrett & Cullum, 1968), also produced a small but significant fall in heart rate, at least part of the action of β-receptor blocking drugs may have been due to their non-specific action (Vaughan Williams, 1966).

Whatever the role of the sympathetic may be as an *additional* factor in thyrotoxicosis, it seems reasonable to conclude that differences in thyroid state directly influence the myocardium.

Conduction velocity and heart rate. Taylor, Covell & Ross (1969) suggested that the faster rate of development of tension found in hyperthyroid dogs might in part be attributed to 'an incrase in the rate of conduction of the depolarization wave, with greater synchronicity of conduction'. The present experiments indicated no significant difference in the conduction velocity of atria taken from hypothyroid (0·503 $m.sec^{-1}$), euthyroid (0·484 $m.sec^{-1}$) and hyperthyroid (0·534 $m.sec^{-1}$) rabbits.

The rate of decline of the spontaneous heart rate *in vitro* is relevant to the hypothesis that there may be increased sympathetic activity in hyperthyroidism which decays rapidly in isolated hearts (Brewster *et al.* 1956). Thier *et al.* (1962) found that the spontaneous rate of isolated rat atria declined quickly in Ringer lactate at 37 °C. In our experiments on rabbit atria at 32 °C, the spontaneous rate declined extremely slowly (not statisti-

cally significant after 3 hr), and was unaffected by differences of thyroid state.

Intracellular potentials. It was found that contracting atrial fibres, stimulated electrically over a wide range of frequencies, from hyperthyroid and hypothyroid rabbits had resting and action potentials similar to those of euthyroid atria. This observation contrasts with the findings of Lenfant, Gargouïl & Tricoche (1966) and of Gargouïl *et al.* (1966), that the maximum diastolic polarization in the sino-auricular nodes of isolated rabbit atria was increased in hypothyroid, decreased in hyperthyroid animals, but in their experiments the atria were beating spontaneously, and so the heart rates differed in accordance with thyroid state.

Many anti-arrhythmic drugs decrease the rate of rise of the action potential without affecting the resting potential (Vaughan Williams, 1958*a*; Szekeres & Vaughan Williams, 1962). It was of interest to discover, therefore, that at driving frequencies below 130 and above 180/min the rate of rise in atria taken from thyroidectomized rabbits was significantly reduced. The low incidence of arrhythmias in hypothyroidism cannot be attributed solely to a change of this kind, however, because at driving frequencies of 130–180/min, which covers the range of spontaneous heart rates of thyroidectomized rabbits *in vivo*, the rate of rise of the action potential was just as fast (95 $Vsec^{-1}$) in the hypothyroid atria, as in the hyperthyroid and euthyroid atria.

At driving frequencies up to 160/min in euthyroid atria the rate of rise of the action potential was over 90 $Vsec^{-1}$. Hyperthyroid atria could not be studied at such frequencies, but in the range 180–220/min the rate of rise was similar (96 $Vsec^{-1}$). At these higher frequencies the rate of rise in euthyroid atria had dropped to 78 $Vsec^{-1}$.

There were other thyroid-dependent changes in the intracellular atrial potentials which could account for the high incidence of arrhythmias in hyperthyroidism, and their rarity in myxoedema. Repolarization was greatly delayed in atria from thyroidectomized rabbits, and was significantly accelerated in the hyperthyroid animals, to the extent that, at the same driven frequency (200/min), the time for half-repolarization in the former (74 msec) was almost as long as the time for 90% repolarization in the latter (84 msec). Since these observations were made on isolated tissues, they cannot be attributed to differences of sympathetic tone, and, in any case, β-receptor blocking drugs do not prolong the duration of the action potential (Vaughan Williams, 1966).

Electrocardiogram. If the duration of the ventricular action potential also is prolonged in hypothyroidism, this should be apparent on the e.c.g., and it is of interest that Adams (1964) reported a large prolongation of the Q–T interval in a patient with myxoedema. Most authors have commented

on a change in shape of the *t*-wave (Wilson, 1924; Lerman, Clarke & Means, 1933; Aber & Thompson, 1963).

The most characteristic feature of the e.c.g. in myxoedema is a generalized low voltage (Zondek, 1918; McBrien & Hindle, 1963; Weir, Young & McGuinness, 1969). In our intracellular records there was no reduction in resting potential or total action potential height. The low voltage observed clinically may be due to extraneous factors such as interstitial fluid and pericardial effusion (Marks & Roof, 1953; Zondek, 1964).

Biophysical connotations. The large changes in duration of the atrial action potentials produced by differences of thyroid state require explanation in terms of the mechanisms controlling the flow of ions into and out of the fibres. Noble (1966) has discussed the evidence that Purkinje fibre action potentials can be reproduced by modified Hodgkin–Huxley equations, and more recently Noble & Tsien (1969) suggested that repolarization from the plateau involves the outward flow of ions through channels which open relatively slowly when the membrane potential becomes more positive than -50 mV. Thyroid hormone might therefore alter the characteristics of the membrane which determine the relation between membrane voltage and ion fluxes through this channel.

There are, however, important differences between conducting and contracting tissues. Purkinje fibres are 'nerve-like' in that they survive anoxia and cutting into small segments, and penetration is facilitated by their large size and absence of movement. Atrial fibres are small, succumb irreversibly to brief periods of anoxia, and their action potentials are of much shorter duration. Unlike nerve and skeletal muscle, in which bursts of activity alternate with long periods of rest, cardiac muscle cannot afford to go into 'ionic debt', and all the sodium which enters the fibres during systole must be pumped out long before the end of diastole. In other tissues there is evidence that the sodium pump is electrogenic (Thomas, 1969) and it is possible that active sodium extrusion could contribute part of the outward current required for repolarization. If so, thyroid hormone might be shortening the duration of the action potential by accelerating sodium extrusion as a secondary consequence of increased metabolic turnover. Conversely a reduced rate of extrusion in hypothyroidism would slow down repolarization, and it is pertinent that Dudel & Trautwein (1958) reported that digitalis, in the initial phase of a complex action, lengthened the duration of intracellularly recorded cardiac action potentials. Thyroid hormone induces changes in the metabolic activity of cardiac muscle (Hornbrook, Quinn, Siegel & Brody, 1965; Hess, 1967; Landsberg & Axelrod, 1968; McNeill & Brody, 1968), and its absence can relieve angina pectoris (Blumgart, Freedberg & Kurland, 1955).

Whatever the biophysical explanation may be for prolongation of the

action potential in hypothyroid atria and its shortening in hyperthyroid muscle, the practical consequences would be to decrease and increase respectively the probability of arrhythmia by concomitant effects on the absolute refractory period.

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