EFFECT OF ISOPROTERENOL ON PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN EUTHYROID AND HYPERTHYROID RAT HEARTS

by

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ABSTRACT.

The effect of triiodothyronine pretreatment on the physiological and biochemical properties of the rat heart was investigated in a number of cardiac preparations. Pretreatment with triiodothyronine for three days produced an increased chronotropic effect in the isolated right atrium, and a decreased inotropic effect in the isolated left atrium and Langendorff heart.

Following administration of isoproterenol there was a dose-dependent increase in rate of the right atrium. The absolute increase in rate was similar in euthyroid and hyperthyroid tissues; the hyperthyroid atria maintained a higher rate than the euthyroid atria, with no sign of supersensitivity.

In the electrically stimulated left atrium and right ventricle, isoproterenol produced a dose-dependent increase in tension. The absolute increase in tension was slightly greater in the euthyroid tissues than in the hyperthyroid tissues.

In all three preparations, the contractile changes produced by isoproterenol were accompanied by an increase in phosphorylase activity which was similar in euthyroid and hyperthyroid animals.

Five nanograms of isoproterenol produced a similar increase in tension in euthyroid and hyperthyroid Langendorff rat hearts. The increase in tension was accompanied by an increase in phosphorylase a activity. This effect of

isoproterenol on phosphorylase activation was potentiated in the hyperthyroid hearts. The possibility that the potentiation of phosphorylase activation in the perfused heart was a result of the increase in coronary blood flow, noted in hearts from hyperthyroid animals, was investigated. Coronary blood flow was reduced in a group of hearts from hyperthyroid animals to the same level as that of hearts from euthyroid animals, and the phosphorylase-activating effect of isoproterenol was again tested. Under these conditions the phosphorylase-activating effect of isoproterenol was still enhanced.

A pA_2 study carried out on the rat right atrium showed that beta-adrenoceptors in the euthyroid and hyperthyroid state were similar.

The data obtained in the present study suggest that the actions of thyroid hormones on the heart do not result in a supersensitivity to the chronotropic or inotropic effects of isoproterenol. In the Langendorff heart preparation a supersensitivity to the phosphorylase-activating effect of isoproterenol was detected, but this same potentiation could not be demonstrated in the right atrium or right ventricle. The reason for the absence of an isoproterenol-induced potentiation of phosphorylase activation in these two preparations, when it can be readily demonstrated in the isolated perfused heart, is not clearly understood but may be due to tissue damage incurred during dissection.

It is suggested that the greater resting rate of

hyperthyroid myocardial tissue might be due to a direct action of the thyroid hormones on calcium movements in the sino-atrial node.

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INTRODUCTION

Adrenal Medullary Involvement in Thyrotoxicosis.

The association of toxic goitre with rapid heart rate has been known for several centuries. In 1825, Caleb Parry, a practitioner in Bath, England, described eight cases which he saw between 1786 and 1814. Other early practitioners associating a rapid pulse with thyroid enlargement were Flajani (1802), Graves (1835), and von Basedow (1840). In 1865, it was suggested by Moore that the symptoms of thyrotoxicosis might be due to increased vasomotor or sympathetic actions. The introduction of the chemical synthesis of adrenaline in 1901 meant that the relationship between the thyroid gland and the adrenal medulla could be more closely investigated. Finally in 1908, Kraus and Friedenthal demonstrated that adrenaline could produce tachycardia and some of the eye signs of thyrotoxicosis.

In more recent studies there has been some support for this interaction between the two systems. Thier and co-workers (1962) showed an increase in heart rate over controls in hyperthyroid rat atria. This increase was significantly reduced when the animals were pretreated with reserpine. Cravey and Gravenstein (1965) also showed that atria from hyperthyroid rats beat faster than those from euthyroid rats. Following adrenalectomy, the hyperthyroid atria rates were significantly reduced, but returned to their

initial (nonadrenalectomized hyperthyroid) rates following corticosterone treatment. Lee, Lee and Yoo (1965) showed using rabbit atria that atria taken from thyroxine-treated animals beat at a significantly higher rate than those taken from euthyroid animals. Following administration of noradrenaline the increases in rate and amplitude were greatest in atria from the hyperthyroid animals.

However, the majority of evidence tends to suggest that this relationship is much less straightforward than it seems.

Initially Benfey and Varma(1963) showed that in spinal cats following treatment with triiodothyronine, intravenous adrenaline produced no greater effects on heart rate, blood pressure and contractile force than in control animals.

Margolius and Gaffney (1965) found that in the intact dog graded doses of noradrenaline produced no change in absolute or percentage increases in heart rate of euthyroid or hyperthyroid animals, although the initial heart rate was higher in the hyperthyroid dogs. Stimulation of the accelerator nerve produced similar frequency-dependent absolute increases in heart rate in euthyroid and hyperthyroid dogs. Van der Schoot and Moran (1965) working with open chest dogs found an increased initial heart rate in hyperthyroid animals as compared to controls. Adrenaline and noradrenaline changed the absolute and percentage increase in heart rate less in hyperthyroid than in euthyroid dogs. They also found less of an increase in contractile force in hyperthyroid than in euthyroid animals. In rat ventricle strips Van der Schoot

and Moran found that the initial contractile force of the strips from hyperthryoid rats was significantly lower than those from euthyroid rats. The positive inotropic effect of noradrenaline was similar in both groups. With rat atria, the initial contractile force was greater in euthyroid than in hyperthyroid animals. The positive inotropic effect of noradrenaline was less in the hyperthyroid than in the euthyroid animals if the response was expressed as grams tension. There was no significant difference between the two groups if the response was expressed as percent change. The initial rate of spontaneous contraction of atria from hyperthyroid rats was greater than that from euthyroid rats. In the presence of noradrenaline, the absolute increase in rate was the same in both groups; but if expressed as percent increase was less in hyperthyroid than in euthyroid rats.

Wilson and co-workers (1966) working with human volunteers, found that the major difference in the response of the euthyroid and hyperthyroid heart to isoproterenol was that the hyperthyroid heart had a higher initial rate; otherwise the increments in heart rate to graded doses of isoproterenol in the two states did not differ, and therefore the receptor sensitivity was unchanged. Following treatment with propranolol, isoproterenol did not produce a change in heart rate in either state.

Aoki and co-workers (1967), also working with human volunteers found that daily administration of 500 ug of triiodothyronine produced the typical symptoms of thyrotoxicosis,

including palpitation and pounding of the heart. Heart rate was significantly higher during the treatment regime than during the control periods. The response to noradrenaline infusion was similar during the two sessions.

Cairoli and Crout (1967) showed that hyperthyroid rats developed an increased metabolic rate and tachycardia. To test whether the tachycardia was due to increased adrenergic nerve activity or enhanced responsiveness of the pacemaker to noradrenaline, they studied the effect of propranolol on the resting heart rate of the unanesthetized rat. This produced less cardiac slowing in the hyperthyroid than in the euthyroid group, thus ruling out these two possibilities. Atropine was added to check whether cholinergic input was deficient (in which case there should have been little change); but there was a sharp increase in rate in both groups, indicating that vagal input to the sino-atrial node was unaffected by the hyperthyroid state. Treatment with reserpine reduced the hyperthyroid heart rate to that of the control animals. If atropine was added after reserpine pretreatment, the depression of heart rate was reversed. This suggested that reserpine could only "normalize" the hyperthyroid heart rate if vagal function was intact, and led to their conclusion that there was no evidence to suggest that the hyperthyroid state enhanced either adrenergic neural activity or the sensitivity of cardiac beta-receptors to noradrenaline, but rather that the tachycardia was due to a direct effect of the thyroid hormones on the pacemaker cell.

Thus, it can be clearly seen that treatment with thyroid hormones does raise resting heart rate above control values. Quite possibly if the data presented in the early studies were re-examined, taking into account the differing baselines, no catecholamine supersensitivity would be seen.

Cardiac Beta Receptor Involvement.

Clinically, beta-adrenoceptor blocking agents have been used to treat patients with thyrotoxicosis for many years. Employed in this manner, they control nervousness, palpitations, tachycardia, increased cardiac output, tremor and acute hyperthyroid crisis; but have no effect on the underlying hyperthyroid process itself (Levey, 1976; McDevitt, 1976; Brit. Med. J., 1977).

Kunos (1977) has suggested that since beta-adrenoceptor conversion seems to be promoted by an increase in metabolic activity (Buckley and Jordon, 1970; Kunos and Nickerson, 1976), changes in thyroid state could also alter adrenoceptor properties.

Williams and co-workers (1976) showed, using the beta-adrenoceptor blocking agent ^{3}H -dihydroalprenolol, that there was a 100 percent increase in the number of binding sites per milligram of protein in hyperthyroid myocardial membranes as compared to a similar preparation from euthyroid hearts. However the relative affinities of isoproterenol in the two groups were practically identical. In addition, Ciaraldi and Marinetti (1977) showed an increased number of beta-receptors and decreased number of alpha-receptors in heart ventricles from thyroxine-treated rats, without any change in the binding affinity of the beta-receptor.

Spaulding and Noth (1975) reported that in hyperthyroidism there is a decrease in the amount of noradrenaline released from sympathetic nerve endings. It has therefore been

suggested by Banerjee and Kung (1977) that this could support the hypothesis that the concentration of noradrenaline is important in regulation of beta-receptor density on mammalian myocardial membranes.

Another possible theory of the mechanism of action of beta-adrenoceptor blocking agents in hyperthyroidism was put forward by Melander and co-workers in 1975. They showed that in hyperthyroid mice, thyroid hormone secretion could be initiated by beta$_2$-, but not beta$_1$-adrenoceptor agonists. The induction of secretion could be abolished by pretreatment with L-propranolol. However, no clinical studies to date have demonstrated that beta-adrenoceptor blocking agents inhibit thyroid hormone secretion in hyperthyroid patients.

Most recently; Kempson, Marinetti and Shaw (1978) showed that triiodothyronine and thyroxine increase the number of beta-adrenoceptors in rat heart ventricle slices. These effects take place in two stages; the acute effect at 1.5 to two hours being a post-translational event; and the chronic effect at fifteen hours being a transcriptional or translational event. They suggest that there is a pool of catecholamine receptors located within the cytoplasm of the ventricular cells which may move from the pool into the plasma membrane following thyroid treatment.

Cyclic AMP and Phosphorylase Involvement.

Adenosine 3',5'-monophosphate (cyclic AMP), the enzyme which acts as a second messenger in many biological systems was first discovered by workers investigating the increased breakdown of glycogen to glucose in liver slices stimulated by adrenaline and glucagon.

Sutherland and Rall (1960) found that adrenaline acted on a membrane-bound enzyme, adenylate cyclase which was responsible for formation of cyclic AMP from ATP in the presence of Mg^{++}. Cyclic AMP in turn accelerated conversion of inactive phosphorylase b to active phosphorylase a, by the steps shown in figure 1. This then resulted in breakdown of glycogen to glucose. In heart muscle the same reaction occurs, but the finishing product is lactate. Inactivation of cyclic AMP was found to be brought about by a cyclic nucleotide phosphodiesterase, with subsequent formation of 5'-AMP (Sutherland and Rall, 1958).

Cyclic GMP (guanosine 3',5'-monophosphate) is formed in a similar manner from guanylate cyclase and GTP, and is also broken down by a phosphodiesterase.

Tissue levels of cyclic AMP depend on the balance between the activities of adenylate cyclase and phosphodiesterase. These activities may be stimulated or inhibited by a number of drugs. The methylxanthines caffeine and theophylline; papaverine, nitroglycerine, hydralazine, and some diuretics have been reported to be phosphodiesterase

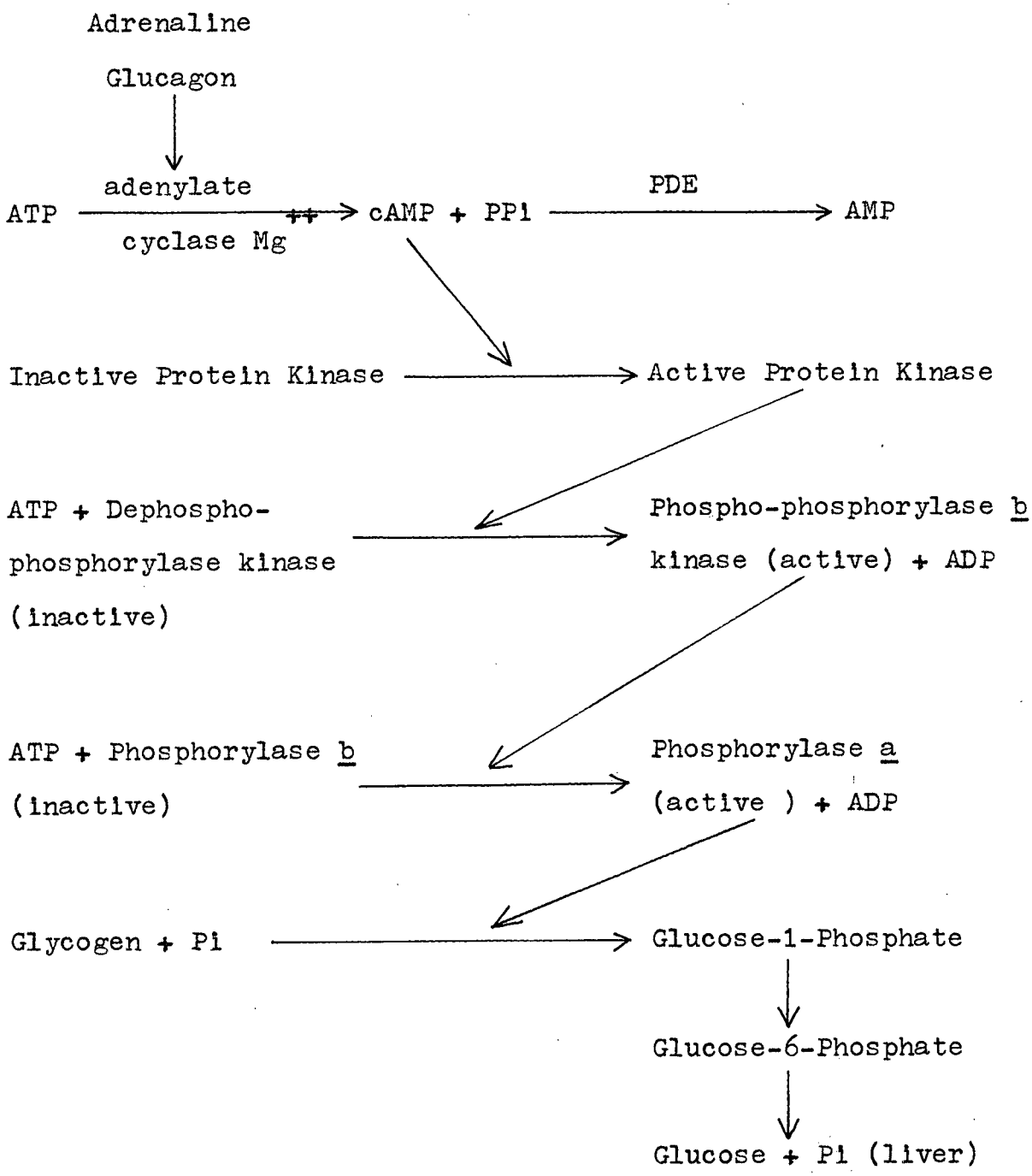


Figure 1. GLYCOGENOLYSIS

inhibitors (Øye, 1975). Their use results in increased cyclic AMP levels. The catecholamines; glucagon, ACTH, histamine, and prostaglandins are some substances which activate adenylate cyclase (Butcher, 1968) and increase cyclic AMP levels.

In the heart, cyclic AMP is thought to be the mediator of the inotropic response to catecholamines acting through the beta-adrenoceptor. It has been shown (Øye et al, 1964) that the inotropic response to adrenaline in perfused hearts was preceded by a rapid rise in cyclic AMP levels, and followed by a rise in phosphorylase a. Beta-adrenoceptor blocking agents have been shown to inhibit all the cardiac effects of the beta-agonists (Øye, 1975).

Other agents which cause an inotropic response of the heart, such as glucagon, histamine, dibutyryl cyclic AMP and some phosphodiesterase inhibitors have also been shown to increase cyclic AMP levels prior to the inotropic effect. Pretreatment with beta-adrenoceptor blocking agents has no effect on the response to these drugs(Butcher, 1968; Drummond and Hemmings, 1972; McNeill and Muschek, 1972; McNeill, Brenner and Muschek, 1974).

In heart muscle, the stimulation of glycogenolysis by these inotropic agents via cyclic AMP may provide some of the energy required to sustain the response, although it cannot be necessary for elicitation of the response since phosphorylase a levels only increase following the peak of the inotropic response (Williamson and Schaffer, 1976).

In initial studies, phosphorylase a, the enzyme which catalyzes the breakdown of glycogen was extensively studied.

Hornbrook and Brody (1963) showed that chronic thyroxine treatment (for fourteen to twenty days) produced an increase in anaesthetized rat cardiac phosphorylase a levels, and a decrease in cardiac glycogen levels when compared to control animals. In a subsequent paper (Hornbrook et al, 1965), they showed that infusion of noradrenaline potentiated the increase in % phosphorylase a seen in hyperthyroid animals. In the hyperthyroid rats, following reserpine administration, there was again potentiation of the noradrenaline-induced increase in cardiac phosphorylase a, compared to euthyroid rats pretreated with reserpine; although in the absence of noradrenaline the % phosphorylase a values in euthyroid and hyperthyroid rats pretreated with reserpine were essentially the same. This suggested to them that the catecholamine-induced responses seen in the hyperthyroid animals were due to some form of modulation of the "myocardial metabolic adrenergic receptor".

A series of studies by Hess and Shanfeld (1965), showed that the increase (from 30 to 50 percent) in cardiac phosphorylase a, brought about by chronic (more than three days) thyroxine pretreatment developed gradually over the time period studied, and that when treatment stopped, the phosphorylase a activity returned to control values in twenty days. Blood pressure and heart rate changes were shown to behave in a similar manner. In the presence of adrenaline,

% phosphorylase a increased in both euthyroid and hyperthyroid rat hearts, but the absolute increase (allowing for the higher basal activity in the hyperthyroid animals) was similar. They therefore concluded that thyroxine did not potentiate the effects of adrenaline on cardiac phosphorylase a.

These results differ from those of Hornbrook and co-workers (1965), who did show noradrenaline potentiation. Firstly, the % phosphorylase a values of Hess and Shanfeld in the absence of adrenaline (30 percent for euthyroid and 50 percent for hyperthyroid) are very high compared to Hornbrook's values (14.2 and 32.8 percent respectively). Secondly, the doses of noradrenaline used by Hornbrook were not able to produce a significant increase in % phosphorylase a levels in the euthyroid animals over euthyroid controls; although in the hyperthyroid animals, the same doses did produce a significant increase in phosphorylase a.

To test the effect of "antiadrenergic" drugs on thyroid hormone pretreatment, Hess and Shanfeld studied the response of % phosphorylase a to acetylcholine, reserpine and pronethalol. They found that in euthyroid rats acetylcholine had little effect on phosphorylase activity, but in hyperthyroid animals it produced a significant decrease in % phosphorylase a compared to values in the presence of thyroxine alone; the levels being reduced to euthyroid control values. Pretreatment for five days with reserpine reduced the usual increase in phosphorylase activity seen in hyperthyroid hearts when compared to euthyroid controls. In euthyroid rats, reserpine produced no change in

cardiac phosphorylase a activity. Pronethalol, a beta-adrenoceptor blocking agent produced no change in the phosphorylase activity of euthyroid rat hearts one minute after administration; although it produced a significant decrease in heart rate. In hyperthyroid rats, pronethalol produced a significant decrease in cardiac phosphorylase a activity, as well as a decreased heart rate. These results seemed to indicate some relationship between the catecholamines and thyroid hormones, which at this time was postulated to be due to increased adenylate cyclase activity.

McNeill and Brody (1968) used cardiac phosphorylase a as a marker to test whether thyroid hormones enhanced catecholamine actions by blocking uptake into the neuron. This was tested using cocaine, since if hyperthyroidism produced blockade of catecholamine uptake, subsequent administration of cocaine would have no further effect. Initially, the hyperthyroid animals receiving cocaine had significantly higher % phosphorylase a values than those receiving triiodothyronine alone. Following infusion of noradrenaline, the hyperthyroid animals pretreated with cocaine again had significantly higher % phosphorylase a values than those receiving either triiodothyronine or cocaine alone.

Tyramine is a sympathomimetic amine which utilizes the catecholamine uptake system to enter the sympathetic neuron; therefore its actions would be affected if triiodothyronine

were to have an effect on catecholamine uptake. However, triiodothyronine pretreatment enhanced the effect of tyramine on % phosphorylase a compared to controls, although in a later study (Young and McNeill, 1974), it was shown that in the guinea pig Langendorff heart, triiodothyronine did not enhance the phosphorylase activating, nor cyclic AMP increasing effect of tyramine. It is therefore unlikely that pretreatment with thyroid hormones decreases catecholamine uptake.

In a follow-up study, McNeill, Muschek and Brody (1969) showed that while pretreatment of rats with triiodothyronine significantly increased cardiac phosphorylase a over control values, both in the absence and presence of adrenaline; the thyroid hormone treatment had little effect on adenylate cyclase activity in the same hearts. Cyclic AMP levels in euthyroid and hyperthyroid hearts were also essentially similar. It was therefore apparent that the greater increase in % phosphorylase a seen in the hyperthyroid animals was not due to any changes in adenylate cyclase activity. This was further supported by Sobel, Dempsey and Cooper (1969) who showed that adenylate cyclase activity was similar in euthyroid and hyperthyroid rat hearts.

However Levey and Epstein (1968) working with cat ventricle homogenates found that administration of triiodothyronine and thyroxine in vitro to the incubation mixture would increase adenylate cyclase activity as measured by cyclic AMP production, and that this could be achieved

without any inhibition of phosphodiesterase. A later study (Levey and Epstein, 1969) showed that in the presence of propranolol, triiodothyronine and thyroxine still increased adenylate cyclase activity, although the same concentration of propranolol abolished the stimulatory effect of noradrenaline on adenylate cyclase. They also showed that the combination of maximal stimulatory doses of thyroxine and noradrenaline produced an additive effect on cyclic AMP production. This evidence would seem to point towards the existence of two separate adenylate cyclase systems; one responsive to thyroid hormones, and the other to noradrenaline with the ability to be blocked by beta-adrenoceptor antagonists. McNeill, LaRochelle and Muschek (1971) were, however, unable to confirm these findings using adenylate cyclase from rat heart.

Levey also carried out some studies (Levey, Skelton and Epstein, 1969) with euthyroid and hyperthyroid cats. They showed that when left ventricle muscle was incubated with noradrenaline, the effect on adenylate cyclase was similar in the two groups, although the control values of adenylate cyclase were significantly lower in hyperthyroid than in euthyroid animals. This is similar to McNeill's findings in the anaesthetized rat (McNeill et al, 1969), where adrenaline increased adenylate cyclase activity in a similar manner in euthyroid and hyperthyroid animals. The only difference being that although hyperthyroid control adenylate cyclase activity was less than euthyroid control adenylate cyclase

activity, it was not significantly so. This is because the euthyroid activity in the Levey et al experiment was much higher than in the study of McNeill et al; the hyperthyroid values being essentially the same.

In the study carried out by McNeill, LaRochelle and Muschek (1971) it was also shown that following pretreatment with triiodothyronine and theophylline, or triiodothyronine and tripelennamine; addition of noradrenaline resulted in an additive effect on % phosphorylase a, and that a combination of the three treatments resulted in a further enhancement. This indicated that the treatments enhanced the responses of phosphorylase by different mechanisms.

In 1974, Young and McNeill showed in the rat Langendorff heart that although control values were similar, the phosphorylase-activating effect of noradrenaline was enhanced by triiodothyronine pretreatment, but that cyclic AMP levels at these doses were similar in both euthyroid and hyperthyroid animals.

In order to determine more accurately the point in the phosphorylase activation cycle (figure 1) where the thyroid hormones act, Fraser, Hess and Shanfeld (1969) investigated phosphorylase b kinase, the enzyme responsible for the conversion of phosphorylase b to phosphorylase a. They found that although thyroxine pretreatment resulted in an increase in phosphorylase a and phosphorylase b kinase at pH 8.2 in anaesthetized rat hearts; there was no overall activation of the enzyme, since the activity at this pH is due to both active and inactive forms. The ratio of activity

at pH 6.8 (active only) to pH 8.2 (active plus inactive) which was used as an index of activation, was in fact lower in hyperthyroid than in euthyroid hearts. No change in cyclic AMP levels was detected. The effect of thyroxine pretreatment differed from that of adrenaline administration, since following injection of 2 ug per kg i.v. of adrenaline, there was an increase in cyclic AMP, phosphorylase a and phosphorylase b kinase activity. This therefore suggests that the mechanism for thyroid hormone-induced elevation of cardiac phosphorylase a differs from that of adrenaline. It was postulated that since both electrical stimulation and perfusion with a high Ca^{++} medium can also elevate phosphorylase a without a concommitant increase in cyclic AMP or activation of phosphorylase b kinase, some mechanism requiring calcium might be involved.

Although most investigations were restricted to interactions of thyroid hormones with catecholamines, McNeill and Schulze (1972) carried out a study on the interaction of histamine with triiodothyronine. They showed that histamine could increase cardiac phosphorylase a, and that this effect was potentiated by prior treatment with triiodothyronine. The activating action of histamine was thought to be brought about by stimulation of adenylate cyclase at a different site than that for the catecholamines.

A new hypothesis was put forward by Hornbrook and Cabral (1972) who, noted again that pretreatment of rats with triiodothyronine increased cardiac phosphorylase a compared

to euthyroid rats, and that administration of noradrenaline produced a greater activation of phosphorylase *a* in hyperthyroid than in euthyroid rats although the contractile response was similar. They therefore proposed that the interaction of thyroid hormones and catecholamines occurred at a site other than the adrenoceptor. This would probably be somewhere in the biochemical chain between adenylate cyclase and phosphorylase (figure 1). They suggested that creatine phosphate (creatine-P) might be involved, since its levels were found to be lower in hyperthyroid than in euthyroid hearts. They also demonstrated a negative correlation between phosphorylase and creatine-P levels following administration of noradrenaline.

McNeill (1977a,b) carried out a series of experiments to determine whether pretreatment with thyroid hormones sensitized a step in the activation of phosphorylase by cyclic AMP. Langendorff rat hearts were injected with dibutyryl cyclic AMP and assayed for phosphorylase *a* at various times following its administration. Phosphorylase *a* levels increased steadily following injection of dibutyryl cyclic AMP, reaching a maximum at eight minutes (McNeill, 1977a). At all times studied, activation was greatest in hearts from triiodothyronine-pretreated rats. When the Langendorff hearts were frozen five minutes after injection, dibutyryl cyclic AMP was shown to produce a dose-dependent increase in phosphorylase *a*. Again, activation was greatest in hearts from triiodothyronine-pretreated animals (McNeill,

1977a,b). To test the hypothesis that dibutyryl cyclic AMP produced a greater response in hyperthyroid animals because more drug could enter the heart cells, tritiated dibutyryl cyclic AMP was injected and the amount of activity retained in the heart af five minutes was measured. Hyperthyroid hearts retained significantly less activity than euthyroid hearts, thus discounting this theory (McNeill, 1977b). It was suggested that activation of protein kinase might be the key step in the supersensitivity of phosphorylase a in the hyperthyroid heart.

The next component of the phosphorylase activation cycle to be investigated was accordingly cyclic AMP-dependent protein kinase, the enzyme responsible for conversion of inactive phosphorylase b kinase to its active form. Gibson, Tichonicky and Kruh (1975) found that following triiodothyronine pretreatment, cytosol protein kinases from rat heart were not significantly different from those obtained from euthyroid animals, nor was cyclic AMP stimulation of these protein kinases affected by triiodothyronine pretreatment. However when non-histone proteins were extracted from cardiac nuclei of animals pretreated with triiodothyronine, there was a rapid increase in protein kinase activity. Following administration of the hormone there was an increase in activity commencing at two hours, reaching a maximum after three days treatment, and declining to control levels after one week of treatemnt. This increase in protein kinase activity was thought to represent an early step in the development of

cardiac hypertrophy, through a regulatory action on the genes responsible for RNA production.

Katz and co-workers (1977) working with Langendorff hearts from rats pretreated with triiodothyronine for three days (at a three-fold higher dose than that used by Gibson and co-workers), found no difference in protein kinase activity from the 12,000x g supernatant fraction, when compared to euthyroid controls. In the presence of noradrenaline there was a significant increase in protein kinase activity in both groups of animals; the degree of activation being significantly less in hearts from hyperthyroid animals than in those from the controls. It therefore seems certain that the increase in phosphorylase a activity seen in hyperthyroid animals cannot be explained on the basis of a change in the supernatant fraction protein kinase activity.

The supernatant fraction of Katz and co-workers may be similar to the cytosol fraction of Gibson and co-workers, since the two studies agree that these fractions showed no difference in protein kinase activation between the two groups. Although an increase in nuclear protein kinase activity was seen by Gibson's group, this had returned to control values by one week of treatment with triiodothyronine. Since Katz used a three-fold greater dose of triiodothyronine; by three days, nuclear protein kinase levels might well have been low if they had been studied. However, it is very difficult to compare the two studies critically, due to the differences of dosage, and the different protein kinase

extraction methods used.

Friesen, Allen and Valadares (1967) showed that calcium was capable of activating cardiac phosphorylase a, and it has since been shown (Hartley and McNeill, 1976) that this activation can occur without any changes in cyclic AMP levels. In addition, the elevation in % phosphorylase a following calcium administration was shown to be greater in hearts from hyperthyroid than euthyroid rats, up to ten seconds after the peak in contractile force. By thirty seconds after the peak force change, % phosphorylase a levels from hyperthyroid hearts were significantly lower than those from euthyroid hearts. Propranolol had no effect on the calcium-induced increase in phosphorylase a in either group, indicating that the beta-adrenoceptor was not involved.

A study was also carried out by Aronson (1976); the results of which closely agree with Hartley and McNeill showing that although calcium did increase cardiac phosphorylase a, there was little difference between euthyroid and hyperthyroid animals.

Nemecek and Hess (1974) carried out a study to determine the effect of altered sympathetic activity on the metabolic actions of the thyroid hormones. Groups of animals used were those treated with nerve growth factor (NGF), to increase sympathetic innervation; nerve growth factor antiserum (AS), to produce a permanent immunological sympathectomy; and 6-hydroxydopamine (6-OHDA), to destroy adrenergic nerve terminals reversibly or irreversibly. It was shown that the immunosympathectomized and NGF-treated mice still responded to triiodothyronine

pretreatment with tachycardia, increased oxygen utilization and increased phosphorylase a activity; but the AS-treated mice did not develop cardiac hypertrophy. Increasing the density of sympathetic innervation with NGF resulted in potentiation of the triiodothyronine-induced increases in heart rate. A drug, P-286, which blocks the release of catecholamines from AS-resistant chromaffin tissues such as the adrenal medulla, was used to eliminate responses due to circulating catecholamines. P-286 did not prevent either the increase in heart rate or % phosphorylase a seen following triiodothyronine pretreatment. Neonatal pretreatment with AS combined with P-286 administration resulted in significant bradycardia, but no change in % phosphorylase a compared to euthyroid controls. Surgical demedullation and/or treatment with 6-OHDA prevented the thyroxine-induced increase in phosphorylase a, and decreased cardiac noradrenaline levels without affecting the tachycardia and increased oxygen consumption and heart weight.

It therefore seems that the thyroid hormone-induced increases in cardiac phosphorylase a are dependent on the presence of catecholamines; while the calorigenic and chronotropic effects are independent of the sympathetic nervous system.

One interesting point to come out of the work on phosphorylase a is that if the control % phosphorylase a is measured in an anaesthetized animal (Frazer et al, 1969; Hess and Shanfeld, 1965; Hornbrook and Brody, 1963;

Hornbrook et al, 1965; McNeill and Brody, 1968; McNeill et al, 1969; McNeill et al, 1971; and Nemecek and Hess, 1974), the resting levels in the hyperthyroid animals are significantly greater than in the euthyroid animals. However, if control % phosphorylase a is measured in the Langendorff heart preparation (Aronson, 1976; Hartley and McNeill, 1976; Hornbrook and Cabral, 1972; McNeill, 1977a; McNeill and Schulze, 1972; and Young and McNeill, 1974), resting levels in the hyperthyroid animals are not significantly different from the euthyroid animal, indicating that phosphorylase a in the anaesthetized heart is probably being activated by endogenous catecholamines. After administration of exogenous catecholamines to both groups, there is a significantly greater increase in % phosphorylase a in the hyperthyroid heart.

Hess and Shanfeld (1965) showed that pretreatment with reserpine reduced the increased % phosphorylase a seen in anaesthetized hyperthyroid rats compared to control euthyroid levels. Also Nemecek and Hess (1974) showed that adrenal demedullation and chemical sympathectomy of thyroid hormone-pretreated animals, followed by anaesthetization and measurement of cardiac phosphorylase a levels did not produce the increase which they had previously seen in thyroid hormone-pretreated animals compared to euthyroid controls. This evidence would support the notion that circulating catecholamines increase cardiac phosphorylase a levels in the anaesthetized, thyroid hormone-pretreated animal.

Involvement of the Parasympathetic Nervous System.

Differing responses between euthyroid and hyperthyroid heart tissues are also noticable when agents which affect the parasympathetic nervous system are studied.

Cairoli and Crout (1967), found that in thyroxine-treated rats, stimulation of the vagus at varying frequencies produced a decrease in heart rate which was less than that observed in euthyroid controls. A larger study by Frazer and Hess (1969) also showed that the bradycardia produced by vagal stimulation in hyperthyroid rats was less than in euthyroid controls. They also noted that the more thyrotoxic the rat became, the less the heart rate decreased in response to vagal stimulation. The bradycardia seen in the hyperthyroid hearts was not accompanied by any change in phosphorylase a levels. Myocardial acetylcholinesterase activity was measured to see if there was increased enzyme activity in the hyperthyroid animals resulting in a rapid breakdown of acetylcholine and a smaller response, but the levels were similar in euthyroid and hyperthyroid animals.

To determine whether thyroxine pretreatment produced a decreased sensitivity of the cardiac muscarinic receptors, acetylcholine was injected in vivo and caused an equal depression of cardiac phosphorylase a, heart rate and blood pressure in both groups of animals. A follow-up study (Hess and Bilder, 1972) showed that choline acetyltransferase (choline acetylase- the enzyme which catalyses the acetylation of choline)

activity was similar in both euthyroid and hyperthyroid rat hearts; thus ruling out any decrease in synthesis of acetylcholine in the hyperthyroid animals which might have accounted for the reduced bradycardia and phosphorylase activation seen in the hyperthyroid animals following vagal stimulation. They also showed that although free plasma calcium was lower in hyperthyroid than in euthyroid rats, this was not responsible for the decreased response to vagal stimulation, since lowering the free plasma calcium in normal rats by infusion with EDTA did not change the response normally seen in the euthyroid animals.

Finally, choline infusion in the hyperthyroid rats during stimulation of the vagus resulted in greater cardiac slowing than in euthyroid controls. Since the resting heart rate in both groups was unchanged by the choline infusion, the response of the hyperthyroid hearts to vagal stimulation is unlikely to be due to a muscarinic action of choline. From these findings it was postulated that thyroxine treatment might lower free extracellular choline, and/or inhibit uptake of choline into parasympathetic neurons.

Direct Action of Thyroid Hormones.

An easy answer to the problem of why thyrotoxic hearts have different contractile characteristics than their euthyroid counterparts would be that the thyroid hormones themselves have a direct action on the heart. Although it has been demonstrated that single cardiac cells or heart fragments will respond to thyroid hormones *in vitro*; few reports show a response by intact hearts or heart portions. The reason for this may be because the tachycardia and other manifestations of hyperthyroidism shown following *in vivo* administration of thyroid hormones do not become apparent until several hours or days later (Brewster *et al*, 1956; Hess and Shanfeld, 1965; Hirvonen and Lang, 1962; Markowitz and Yater, 1932; Priestley, Markowitz and Mann, 1931; Skelton, Karch and Wildenthal, 1973; van der Schoot and Moran, 1965; Wildenthal, 1971, 1972; and Yater, 1931).

Markowitz and Yater (1932) demonstrated that explanted heart fragments from two day chick embryos which were devoid of functioning nerve tissue responded to 3×10^{-5} M thyroxine with an increased number of pulsations, although adrenaline and acetylcholine had no effect. This response could also be demonstrated in fragments from chick embryos up to nine days old, which had developed functioning nerve elements and were capable of responding to adrenaline and acetylcholine. More recently, Wollenberger (1964a,b; 1975) has shown that a twenty-four hour culture of chick embryo ventricle cells responds to 6.6×10^{-7} M triiodothyronine within ten minutes,

reaching a maximal increase of twenty beats per minute and remaining at that rate until washed. This action could be blocked by concentrations of veratramine and pronethalol which did not affect the rate of beat of the cultured heart cells in triiodothyronine-free medium (Wollenberger, 1964a,b), but this may be due to an unspecific antagonistic effect.

Wildenthal (1972) working with cultured foetal mouse whole hearts could find no significant difference in rate between controls and hearts exposed to 5×10^{-7} M and 5×10^{-6} M triiodothyronine for $2\frac{1}{2}$ to 3 hours or two days. However, if the drug was added to the culture medium for four to ten days (Wildenthal, 1971), a significant increase in rate over controls was observed.

In hearts exposed to 5×10^{-6} M triiodothyronine for two days, 1×10^{-8} M and 1×10^{-7} M noradrenaline produced an increase in rate which was significantly greater than in the group exposed to 5×10^{-7} M triiodothyronine or controls. At 1×10^{-6} M noradrenaline, all groups showed a maximal increase in rate with no difference between them.

Early workers showed that thyroxine had no effect on the frog Straub heart rate (Kalnins, 1928), or on the anaesthetized dog heart rate (Coelno and Rocheta, 1929).

Later work in the isolated frog heart by Kleinfeld, Rosenthal and Stein (1958) showed that 0.2 mg of thyroxine or triiodothyronine produced a slight increase in heart rate; triiodothyronine being more potent than thyroxine. Higher doses produced a decrease in heart rate. The methods do not describe

how long the hearts were in contact with the hormones, but it is unlikely that it was greater than a few minutes. Harvey and MacRae (1931) showed that administration of 1×10^{-5} M thyroxine to turtle hearts had no effect on heart rate for up to several days of treatment. Rabbit Langendorff hearts perfused with 5×10^{-7} M and 5×10^{-6} M triiodothyronine or thyroxine were also shown to be unaffected (Lewis and McEachern, 1931). More recently, Lee, Lee and Yoo (1965) found that doses of thyroxine up to 1×10^{-4} M had no effect on the rate of spontaneously beating rabbit atria. Baird and Spilker (1970) reported that 1.4×10^{-5} M to 8.3×10^{-5} M triiodothyronine produced dose-related increases in the tension developed by isometrically contracting guinea pig left atria. At the peak, the tension increase averaged only 12 percent, but was statistically different from the tension increase at lower doses. Thyroxine (1×10^{-5} M to 6×10^{-5} M) had no effect on inotropic activity. Conversely, Skelton, Karch and Wildenthal (1973) found that neither 1×10^{-6} M to 1×10^{-4} M thyroxine nor triiodothyronine had any effect on the tension developed by isometrically contracting cat papillary muscle, or left atria; or guinea pig left atria during periods of observation of up to eight hours. Experimental conditions of the study were almost identical to those of Baird and Spilker, but the results do not agree. No explanation was given for this difference.

Therefore, although thyroid hormones have been shown to affect heart rates of cultured isolated cardiac cells fairly quickly; in isolated mammalian hearts this cannot easily be

demonstrated. However, cultured foetal hearts exposed to thyroid hormones for periods of time longer than four days do demonstrate an increase in heart rate over controls, similar to *in vivo* induction of tachycardia by thyroid hormones. This would tend to indicate that there may be membrane or ultrastructural damage in the preparation of the cell cultures, resulting in an immediate tachycardia when the thyroid hormones are added to the medium, and therefore that these preparations do not represent a truly physiological situation.

Muscle Mechanics.

As well as affecting rate, tension development and enzyme content, thyrotoxicosis has been shown to affect the intrinsic contractile properties of the heart, and produce changes in the contractile protein content.

In 1965, Inchiosa and Freedberg described experiments which demonstrated that pretreatment of rabbits with triiodothyronine or thyroxine produced a 35 percent hypertrophy of the ventricles of the heart accompanied by a 49 percent increase in the contractile protein actomyosin.

Later work (Yazaki and Raben, 1975), demonstrated that thyroxine treatment increased cardiac myosin ATPase activity (Ca^{++}-activated) over a period of two weeks. The cardiac myosin formed had altered enzymatic properties when compared to myosin extracted from normal rabbits, suggesting a structural difference. This difference was specific for the heart, since skeletal myosin was identical in the two groups. The synthesis of this new myosin might therefore mediate the thyroid hormone-induced changes in contractility. However, in the rat the properties were different. Cardiac myosin from thyroidectomized rats showed a pattern of activity similar to the myosin from euthyroid rabbits. With euthyroid and thyroxine-treated rats, cardiac myosin activity was similar to that from thyroxine-treated rabbits. Rat cardiac myosin therefore seems to be more sensitive to thyroid hormone than rabbit cardiac myosin; since the structural

changes, which in the rabbit required thyroxine treatment to occur, were observed in the rat even in the euthyroid state. Since analysis of the amino acid content and molecular weight of cardiac myosin demonstrate no significant changes between the hypothyroid and hyperthyroid state, it is not yet possible to determine where the structural change occurs and what form it takes, although it is unlikely to be a major difference.

The contractile properties of the myocardium have been more extensively studied than the mechanisms behind them. In 1967, Buccino and co-workers found that isolated papillary muscles from anaesthetized hyperthyroid cats demonstrated an augmented velocity of shortening and rate of tension development, and decreased duration of active state compared to euthyroid muscles. Isometric tension was slightly higher in the muscles from hyperthyroid animals and lower in the muscles from euthyroid animals. This was supported by Levey, Skelton and Epstein (1969), and Skelton, Su and Pool (1976). No significant difference was found between papillary muscle levels of creatine-phosphate and ATP levels in anaesthetized euthyroid and hyperthyroid cats. This differs from the results of Hornbrook and Cabral (1972), who found that in both anaesthetized and perfused rat heart ventricles, creatine-phosphate levels were significantly lower in the hyperthyroid animal. ATP levels were similar in euthyroid and hyperthyroid perfused hearts, and lower in hyperthyroid non-perfused hearts.

Later work by Buccino and co-workers (1968) showed that pretreatment with reserpine did not significantly alter the increased velocity of shortening in either euthyroid or hyperthyroid cat papillary muscles. That is, the velocity of shortening was still significantly increased in the hyperthyroid animals compared to euthyroid controls, and does not seem to depend upon endogenous catecholamine stores.

A series of studies by Strauer and Scherpe were carried out to measure various indices of contractility. They demonstrated an increase of both the maximum rate of left ventricular pressure developemnt (dp/dt_{max}) and pressure fall (dp/dt_{min}) in anaesthetized hyperthyroid cats compared to euthyroid controls. The time interval from the beginning of ventricular contraction to peak dp/dt_{max} was found to be shortened in hyperthyroidism. These changes were considered by them to be evidence for a direct increase of contractility in hyperthyroidism (Strauer and Scherpe, 1975a). In cat papillary muscles, the isotonic contraction velocity (dl/dt_{max}) was significantly higher in the hyperthyroid state, as was the maximal isometric tension rise velocity (dT/dt_{max}), (Strauer and Scherpe, 1975b,d). Treatment with propranolol <u>in vitro</u> reduced the velocity values of isotonic (dl/dt_{max}) and isometric (dT/dt_{max}) contraction in both euthyroid and hyperthyroid animals in a dose-dependent manner. Hyperthyroid animals were much more responsive to the negative inotropic effect of propranolol, the fall in dl/dt_{max} being much greater in hyperthyroid than in euthyroid tissues. Heart rate <u>in vivo</u> fell in a dose-dependent

manner following administration of propranolol to both groups, but in hyperthyroid animals the fall in heart rate was much smaller than the fall in maximal pressure rise velocity of the left ventricle (dp/dt_{max}). The greater fall of dp/dt_{max} in the hyperthyroid heart could explain the increased responsiveness of the hyperthyroid papillary muscles. This decrease of contractility seen after use of propranolol, accompanied by a decrease in myocardial oxygen consumption (Strauer and Scherpe, 1975d), makes the use of propranolol in the clinical situation quite appropriate (Strauer and Scherpe, 1975c).

Therefore, myocardial tissues from hyperthyroid animals demonstrate an enhanced rate of tension development and a decreased time to peak tension. Whole hearts demonstrate an increased rate of left ventricular pressure development and pressure fall, resulting in an overall increase in contractility of both the contraction and relaxation phase.

Involvement of the Peripheral Blood Vessels.

Much of the early work on the effect of thyroid hormone pretreatment on peripheral blood flow is not quantitative, but nevertheless as early as 1931, it was noted that in four out of five rabbit Langendorff hearts, there was an increased coronary blood flow following perfusion with 3.2×10^{-6} M thyroxine. A decreased flow was seen in one heart. "These changes were not striking," (Lewis and McEachern, 1931). In 1933, Herrick and co-workers carried out experiments which demonstrated "a tremendous increase" in blood flow through the unanaesthetized dog femoral artery following ingestion of dessicated thyroid gland. From his data, a t-test performed shows the increase in blood flow following thyroid gland ingestion to be significantly greater than that in control animals at $P < 0.05$. The same group of workers (Essex et al, 1936), found that intravenous injection of thyroxine (1mg/kg) into unanaesthetized dogs resulted in increased coronary blood flow forty-eight hours later, but no significant change at twenty-four hours. Wurtman and co-workers, using ^{42}K as a marker, determined that cardiac blood flow was increased in hyperthyroid unanaesthetized rats. This increase in blood flow through the heart paralleled the increase in heart weight, so flow per unit mass remained unchanged (Wurtman et al, 1963). Administration of adrenaline increased the percent of cardiac output delivered to the heart in a dose dependent manner in both euthyroid and hyperthyroid animals. Hyperthyroid hearts

exhibited a steeper dose-response curve to adrenaline than the euthyroid hearts. This was probably due to increased delivery of catecholamines to the heart (Wurtman et al, 1964).

Although thyroid hormone treatment did not significantly alter hind limb blood flow of cats and dogs; following adrenaline and noradrenaline infusion hyperthyroid animals demonstrated a less marked reduction of blood flow than did euthyroid animals (Zsoter, Tom and Chappel, 1964). In contrast, the vasoconstriction produced by angiotensin and vasopressin was not significantly affected, suggesting an enhanced sensitivity of beta-adrenoceptors.

In summary, experimental hyperthyroidism produces tachycardia, accompanied by an increased rate of tension development, decreased time to peak tension and increased velocity of shortening. Tension developed is reduced compared to euthyroid controls.

Absolute increases in rate in response to catecholamines are similar in euthyroid and hyperthyroid hearts, but there is an augmented activation of phosphorylase a in the hyperthyroid animals. No difference in activity of cyclic AMP, adenylate cyclase, protein kinase or phosphorylase b kinase has been detected between euthyroid and hyperthyroid animals; although in one study, creatine-P, a source of high energy phosphate, has been shown to be lower in hyperthyroid than in euthyroid hearts.

Propranolol and other beta-adrenoceptor blocking agents

produce either no difference, or less slowing in the hyperthyroid heart when compared to the euthyroid heart.

Reserpine pretreatment may reduce heart rate in hyperthyroid animals to that of the euthyroid controls, but does not affect the noradrenaline-induced potentiation of phosphorylase a.

Vagal stimulation of hyperthyroid hearts results in less bradycardia than is seen in euthyroid hearts at the same frequency. This bradycardia was not associated with changes in myocardial acetylcholinesterase or choline acetyltransferase levels.

The cardiac manifestations of hyperthyroidism are unlikely to be due to an immediate direct action of thyroid hormones since few studies have demonstrated this in the intact heart.

Finally in hyperthyroidism, peripheral blood flow has been shown to be increased, including flow through the coronary blood vessels.

Purpose of the Investigation.

This study set out to investigate contractile and enzymatic changes in the atria and right ventricle of the hyperthyroid rat heart. Previously the majority of work had been carried out on the Langendorff or intact heart, with few instances of studies where the separate portions were used (Cravey and Gravenstein, 1965; Their, Gravenstein and Hoffman, 1962; van der Schoot and Moran, 1965). We felt that it would be interesting to follow the isoproterenol-induced increase in phosphorylase a in both euthyroid and hyperthyroid myocardial tissues and determine whether any differences could be seen.

We also wished to determine the effect of using rate-controlled tissues, such as the left atrium and right ventricle, where factors such as changes in rate and coronary blood flow could be controlled.

Following studies in the right and left atria and right ventricle, where contractility changes occurred following isoproterenol administration that were significantly different between the two groups, but were not accompanied by significant differences in phosphorylase a activity, we decided to work with the Langendorff heart preparation.

It has been shown in the Langendorff heart preparation (Hornbrook and Cabral, 1972; McNeill and Schulze, 1972; and Young and McNeill, 1974) and anaesthetized rat heart (Hess and Shanfeld, 1965; Hornbrook et al, 1965; McNeill and Brody, 1968; McNeill, LaRochelle and Muschek, 1971; and McNeill, Muschek and Brody, 1969), that catecholamine

administration results in an increase in myocardial phosphorylase a, which is significantly higher in the hyperthyroid animals. It was therefore surprising that no differences in phosphorylase a activity could be detected between the two groups in our study of atria and ventricles.

To test whether the higher level of phosphorylase a found in the hyperthyroid whole heart was due to an increased coronary blood flow, resulting in increased levels of catecholamine being made available for beta-adrenoceptor binding, a study was undertaken in which the perfusion pressure was decreased so coronary blood flow in the hyperthyroid hearts was now the same as that of the euthyroid hearts. The response of phosphorylase a to isoproterenol was then determined, to see if phosphorylase a activation was now similar to that of the euthyroid hearts.

Finally, a study was carried out to determine whether the receptors through which the inotropic effect of isoproterenol was mediated in the right atrium, were the same in the euthyroid and hyperthyroid state. This study took the form of a pA_2 test as described by Schild (1957), and utilized propranolol as the antagonist.

MATERIALS AND METHODS.

Methods.

Male Wistar rats weighing 250-350g were used throughout the study and received food (Purina Lab Chow) and water ad libitum.

Half of the animals were made hyperthyroid by subcutaneous injection of 3,3',5'-triiodo-L-thyronine (500 mg per kg) in alkaline saline, administered daily for three days. This treatment has previously been shown to make rats hyperthyroid (McNeill et al, 1969). All studies carried out involved both euthyroid and hyperthyroid rats.

The animals were pretreated with heparin sodium (8 mg per kg s.c.), ten minutes prior to sacrifice. They were stunned by a blow to the head, and the hearts were rapidly removed.

Preparation of Atria.

Atria were dissected free by the method of Levy (1971), and suspended by the method of Katzung (1968) in organ baths containing Chenoweth-Koelle solution at 37°, (Chenoweth and Koelle, 1946). The solution was aerated with 95% oxygen, 5% carbon dioxide. Contractile force and rate were measured by means of a Palmer clip placed on the apex of the atrium and connected to a Grass force displacement transducer, and recorded on a Grass model 79D polygraph.

Diastolic tension was adjusted to 1 g. The atria were allowed to equilibrate for 30 minutes before the drug was added.

Right atria were allowed to contract spontaneously. Left atria were driven at 1Hz with 5 millisecond square wave pulses, using one to four volts, by a Grass model S6 stimulator. Dose-response curves were measured using the cumulative method described by van Rossum and van der Brink (1963), and van Rossum (1963). Rates were measured 75-90 seconds after drug administration for right atria. For left atria, the maximun tension attained was recorded.

Preparation of Ventricles.

Rat right ventricles were dissected free from the left ventricles and suspended in the organ baths of Chenoweth-Koelle solution at 37^{0}, aerated with 95% oxygen, 5% carbon dioxide from an electrode designed in our laboratory, (Figure 2). Diastolic tension was adjusted to 1g. The ventricles were driven at 3Hz with 5 millisecond square wave pulses using one to six volts, by a Grass model S6 stimulator. Dose-response curves were obtained as described above, and maximun tension attained was recorded.

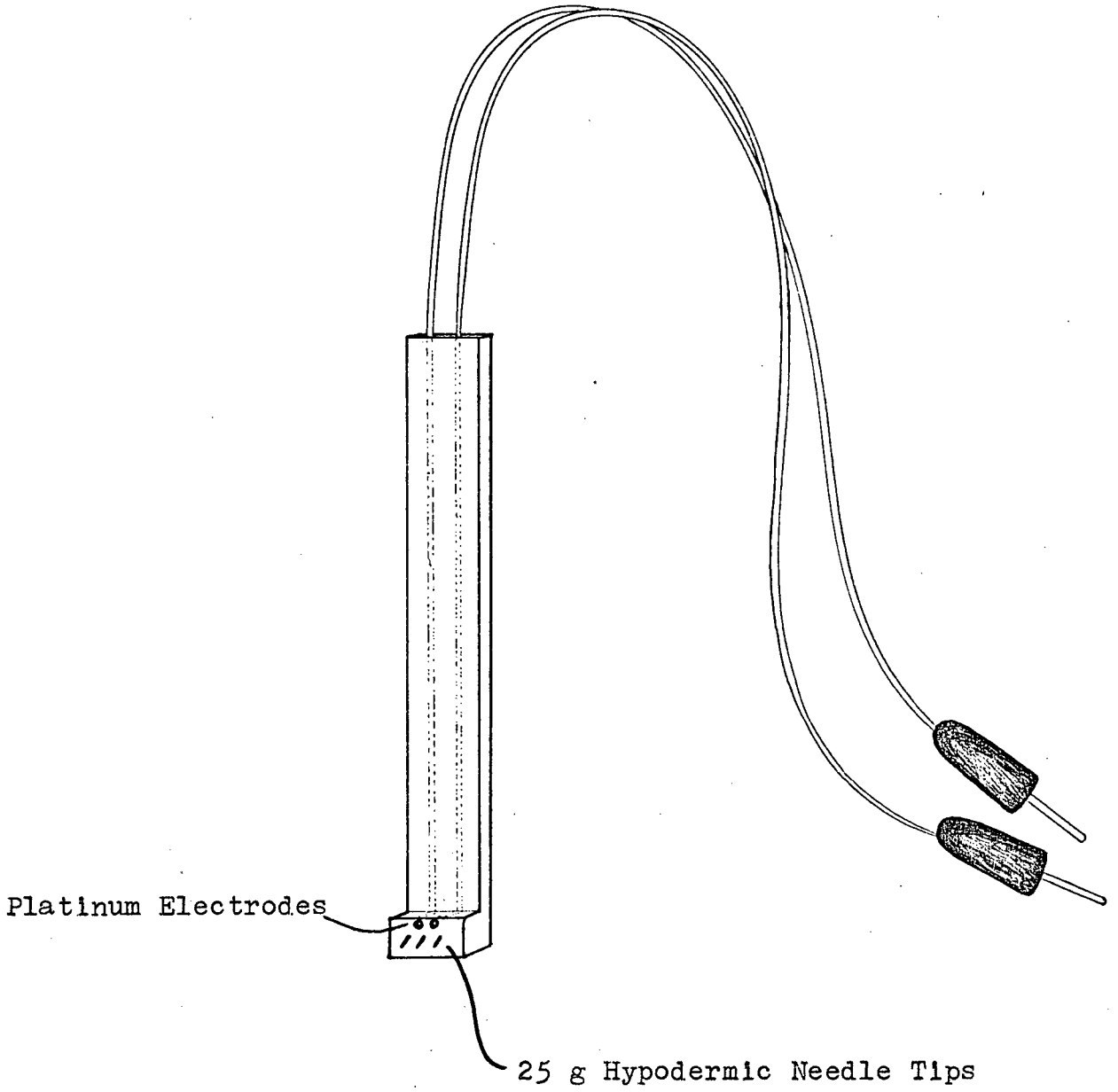
Preparation of Langendorff Hearts.

Whole hearts were set up by the method of Langendorff (1895), using a constant pressure apparatus similar to that described by Chenoweth and Koelle (1946), at a reservoir height of 40cm of perfusate, Chenoweth-Koelle solution at a reservoir temperature of 37.5^{0} and aerated with 95% oxygen, 5% carbon dioxide was used as perfusate. Diastolic tension was

Figure 2.

Electrode used for Ventricle Stimulation.

(Actual Size).



adjusted to 1g. Contractile force and rate were measured by means of a Palmer clip placed on the apex of the heart and connected to a Grass force displacement transducer, and recorded on a Grass model 79 polygraph. Hearts were allowed to equilibrate until rate and contractile force were constant before drugs were added.

For all studies, dose-response curves to isoproterenol were carried out initially; and a sub-maximal dose was chosen for a time-response study.

To determine the biochemical responses; at various times following administration of the chosen sub-maximal dose, the atria and Langendorff hearts were frozen by clamping them with tongs previously chilled in a mixture of 2-methyl butane and dry ice (Wollenburger et al, 1960). The ventricles were rapidly removed from the bath and dropped into the 2-methyl butane mixture. All samples were stored at -80^{0} until assayed for phosphorylase.

Measurement of pA_2

A pA_2 study was carried out using right atria prepared as described above, using the method of Schild (1957). The agonist used was isoproterenol, and the antagonist propranolol. Doses of antagonist used were 2×10^{-7} M, 5×10^{-7} M, and 1×10^{-6} M. ED_{50} values were calculated for each tissue. From the pooled ED_{50} values at each dose of antagonist, the pA_2 values for euthyroid and hyperthyroid rat right atria were calculated.

Measurement of Coronary Blood Flow.

Coronary blood flow determinations were carried out using the Langendorff heart technique described above. Care was taken to ensure that there were no leaks in the system, and the volume of perfusate leaving the heart per unit time was measured as described by Lewis and McEachern (1931). This volume was taken as coronary blood flow. By raising or lowering the height of the reservoir the volume of perfusate leaving the heart could be changed.

Phosphorylase Assay.

The phosphorylase assay used is a modification of the method of Cori and Cori (1940). Liberation of inorganic phosphate during the synthesis of glycogen from glucose-1-phosphate is used as an indicator of phosphorylase activity.

The method followed is that of Diamond and Brody (1965). Heart samples weighing 70-100 mg were homogenised using a Polytron (Brinkmann Instruments, Rexdale, Ont.) in 200 volumes of a solution containing 0.05M Tris buffer (pH 6.8), 0.001M EDTA, 0.01M NaF, and 0.3% serum albumin. All procedures were carried out at 0-4^{0}. After centrifugation of the homogenate at 10,000g for 10 minutes, 0.2 ml aliquots of the supernatant were incubated at 37.5^{0}. in test tubes containing 0.05M Tris buffer (pH 6.8), 0.4% glycogen, 0.01M glucose-1-phosphate, 0.001M EDTA, 0.01M NaF, and 0.3% serum albumin in a final volume of 1.0 ml. Duplicate samples of

the supernatant solutions were incubated in the same reaction mixture containing, in addition, AMP in a final concentration of 0.001M. The reaction was terminated by the addition of 2.0 ml of 10% tri-chloroacetic acid. The samples were then centrifuged at 2,500g for 10 minutes, and the supernatants assayed for inorganic phosphate by the method of Fiske and SubbaRow (1925).

The rate of liberation of inorganic phosphate was linear over the time studied in all tissues, and was proportional to enzyme concentration. Incubation times chosen were 16 minutes for the right ventricle and whole heart, and 30 minutes for the right and left atria.

When the glycogen primer was omitted from the reaction mixture, the liberation of inorganic phosphate was negligible.

The amount of inorganic phosphate liberated in the absence of AMP represented phosphorylase a, and the amount liberated in the presence of AMP represented total phosphorylase. Results are given as % phosphorylase a, which is;

$$\frac{\text{activity in the absence of AMP}}{\text{activity in the presence of AMP}} \times 100$$

Statistical Methods.

Statistical analysis was done by the Student's t-test for unpaired data. A probability of $p < 0.05$ was taken as the criterion for significance.

Materials.

Drugs used were DL-Isoproterenol, DL-Propranolol HCl, and 3,3',5'-triiodo-L-thyronine, (all from Sigma Chemical Corporation).

RESULTS.

Effect of Three Day Pretreatment with 3,3',5-Triiodo-L-Thyronine on Rat Body Weight.

Control and test rats were weighed before pretreatment with drug or vehicle for three days, and again on day four, prior to sacrifice (figure 3).

Control rats gained weight in a linear manner, from 275g to 295g over the four day period. The weights on days three and four were significantly different from that on day one. Conversely the test rats did not show any significant increase in weight, with a mean weight of 283g on both day one and day four. On day four, the control and hyperthyroid animal weights differed significantly from each other.

Effect of Isoproterenol on Rate of Right Atria from Euthyroid and Hyperthyroid Rats.

Isoproterenol produced dose-related increases in rate in both euthyroid and hyperthyroid rat hearts (figure 4).

In the absence of drug, the two groups had significantly different initial heart rates. The hyperthyroid atria had significantly higher rates than the euthyroid atria throughout the extent of the isoproterenol dose-response curve.

Despite the differing initial heart rates, the absolute increase in rate following isoproterenol administration was similar in both groups.

Figure 3.

Effect of Three Day Pretreatment with 3,3',5'-Triiodo-L-Thyronine on Rat Body Weight.

Rats were weighed daily before pretreatment with drug or vehicle for three days, and again on day four, prior to sacrifice.

Each point represents the mean weight (g) ± S.E.M. of 38-42 observations.

a Significantly greater than day one value at $P < 0.05$.

b Euthyroid significantly greater than hyperthyroid at $P < 0.05$.

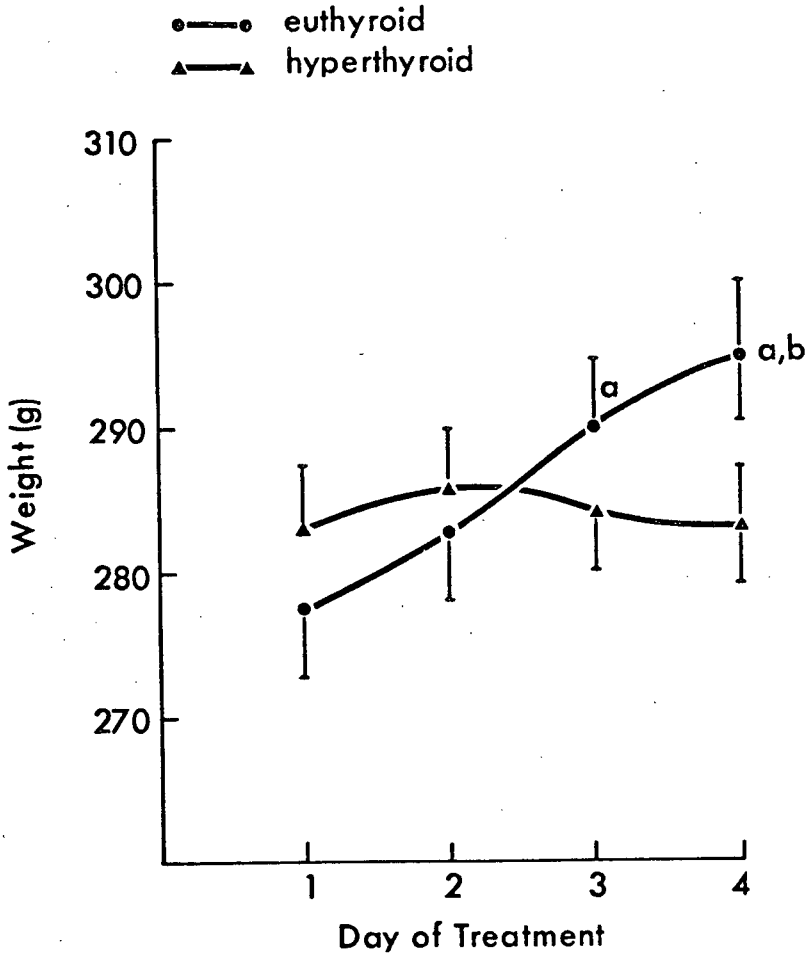

euthyroid
hyperthyroid
310
300
290
280
270
Weight (g)
a
a,b
1
2
3
4
Day of Treatment

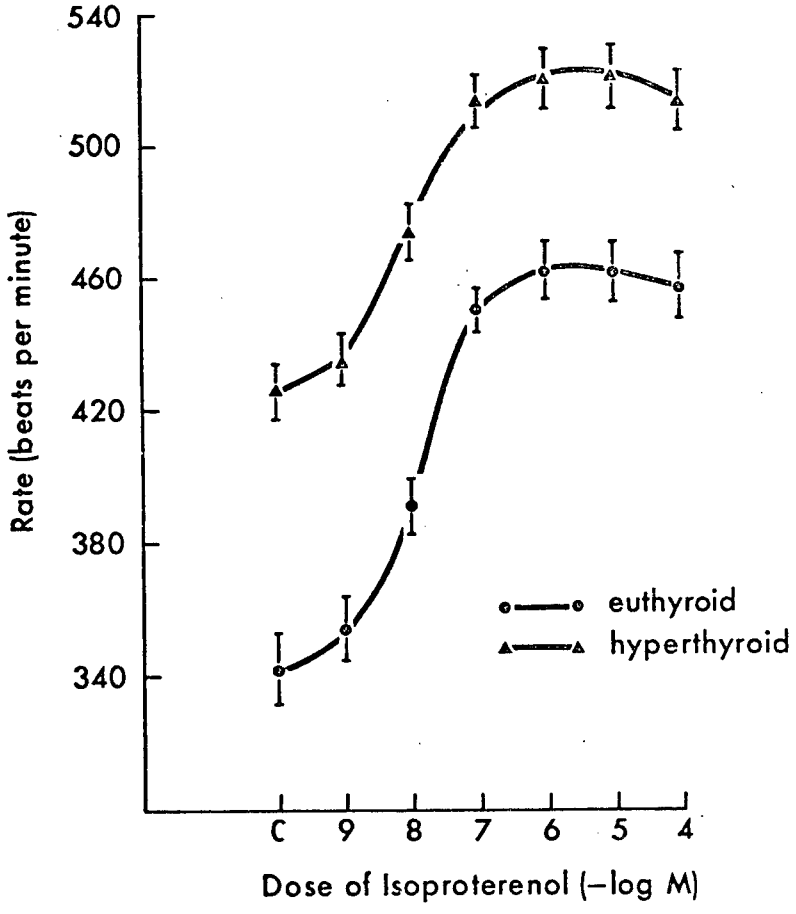
Figure 4.

Effect of Isoproterenol on Rate of Right Atria from Euthyroid and Hyperthyroid Rats.

The plot depicts the effect of various doses of isoproterenol on rate in euthyroid and hyperthyroid rat right atria. Rates were measured 75-90 seconds after drug administration.

Each point represents the mean rate (beats per minute) $\pm$ S.E.M. of 17-19 observations.

All points were significantly different from the corresponding control value at $P < 0.05$.


540
500
460
420
380
340
Rate (beats per minute)
euthyroid
hyperthyroid
C
9
8
7
6
5
4
Dose of Isoproterenol (–log M)

Effect of Isoproterenol on % Increase in Rate of Right Atria from Euthyroid and Hyperthyroid Rats.

When the data was expressed as absolute rate, the increase in rate in response to isoproterenol was the same in the two groups. However, plotting the same data in terms of % increase in rate put a different aspect on the results. In this form, the percent increase was significantly less in the hyperthyroid than in the euthyroid tissues (figure 5).

Effect of Isoproterenol on Tension of Left Atria from Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

Isoproterenol produced a dose-related increase in tension in the left atria from both euthyroid and hyperthyroid rats, as shown in figure 6. The tension development was greater in euthyroid than in hyperthyroid atria, both in the absence and presence of isoproterenol. This difference was significantly greater at doses of isoproterenol over 1×10^{-7} M.

Changes in Rate Following Administration of 5×10^{-8} M Isoproterenol to Euthyroid and Hyperthyroid Rat Right Atria.

From the dose-response curve in figure 4, 5×10^{-8} M isoproterenol was chosen as a sub-maximal dose for a time-response study. Rates were studied in euthyroid and hyperthyroid rat right atria at various times following administration of the drug.

The difference in rate between euthyroid and hyperthyroid

Figure 5.

Effect of Isoproterenol on % Increase in Rate of Right Atria from Euthyroid and Hyperthyroid Rats.

The plot depicts the effect of various doses of isoproterenol on the % increase in rate of right atria from euthyroid and hyperthyroid rats.

Each point represents the mean % increase in rate ± S.E.M. of 17-19 observations.

a Euthyroid significantly greater than hyperthyroid at $P < 0.05$.

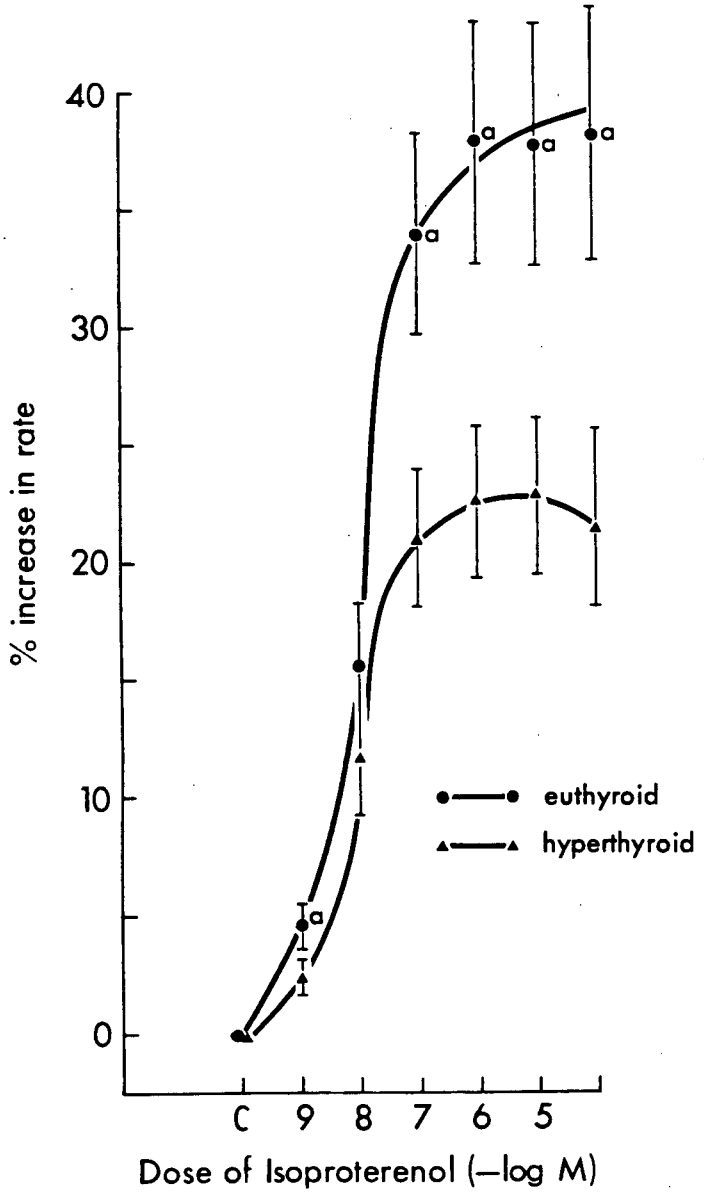

40
30
20
10
0
% increase in rate
a
a
a
a
a
euthyroid
hyperthyroid
C 9 8 7 6 5
Dose of Isoproterenol (—log M)

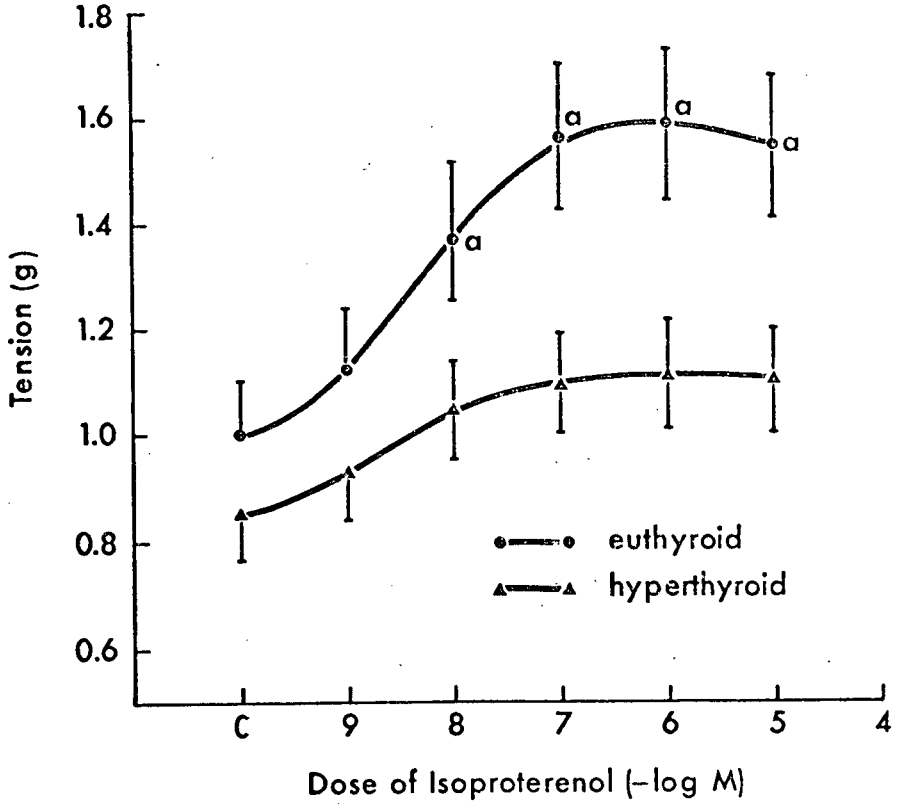
Figure 6.

Effect of Isoproterenol on Tension of Left Atria from Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

The plot depicts the effect of various doses of isoproterenol on the absolute tension of left atria from euthyroid and hyperthyroid rats. Maximal tension attained was recorded.

Each point represents the mean tension (g) $\pm$ S.E.M. of 17-20 observations.

a Euthyroid significantly greater than hyperthyroid at $P < 0.05$.


1.8
1.6
1.4
1.2
1.0
0.8
0.6
Tension (g)
a
a
a
euthyroid
hyperthyroid
C
9
8
7
6
5
4
Dose of Isoproterenol (–log M)

atria was maintained throughout the time-response curve (figure 7). Rates of the hyperthyroid atria were significantly higher than those of the euthyroid atria at all times studied.

Changes in % Phosphorylase a Following Administration of $5x10^{-8}$ M Isoproterenol to Euthyroid and Hyperthyroid Rat Right Atria.

The increase in % phosphorylase a following administration of $5x10^{-8}$ M isoproterenol in rat right atria is shown in figure 8.

In the absence of drug, % phosphorylase a levels were significantly greater in the hyperthyroid atria, but this was not maintained when isoproterenol was added to the bath. At all other times studied, no significant difference was found between % phosphorylase a levels in euthyroid or hyperthyroid rat right atria. However, in both groups, % phosphorylase a levels increased significantly following administration of the drug, compared to the appropriate control; reaching maximal values at eighty to one hundred seconds.

Figure 7.

Changes in Rate Following Administration of 5×10^{-8} M Isoproterenol to Euthyroid and Hyperthyroid Rat Right Atria.

The plot depicts the change in rate of euthyroid and hyperthyroid rat right atria, at various times following administration of a sub-maximal dose of isoproterenol.

Each point represents the mean rate (beats per minute) $\pm$ S.E.M. of 5-88 observations.

All points were significantly different from the corresponding control value at $P < 0.05$.

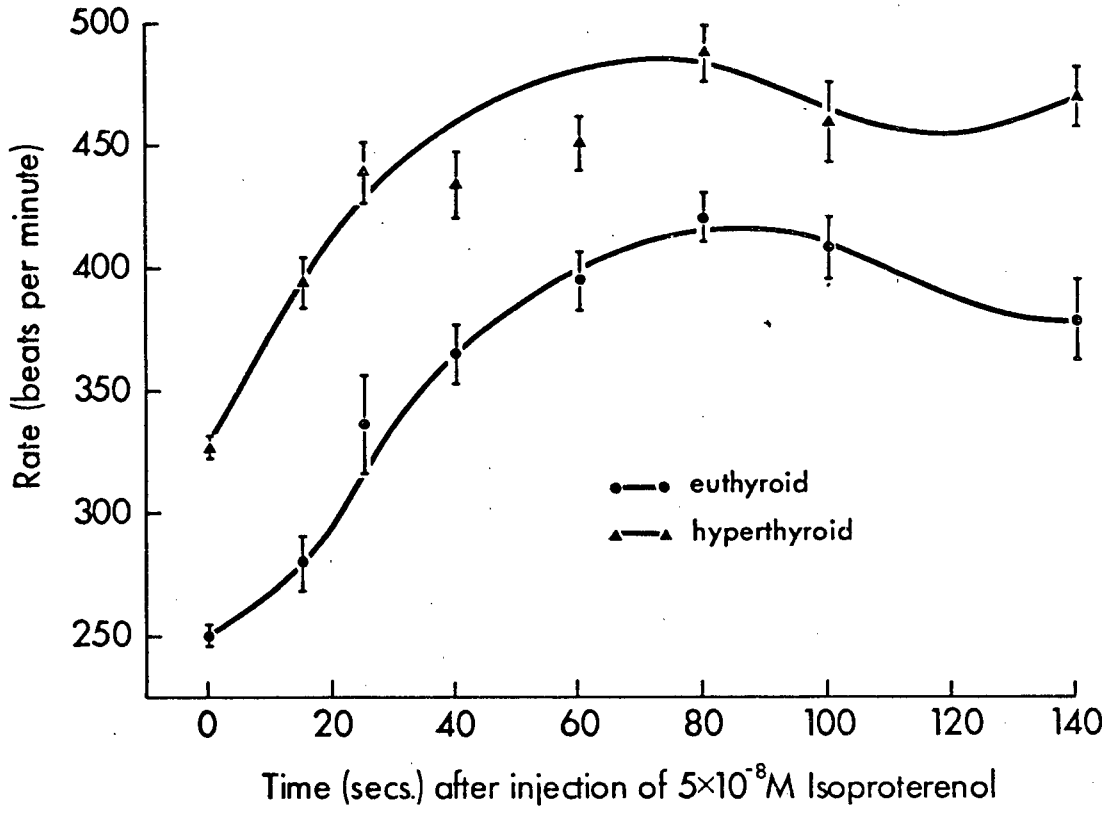

500
450
400
350
300
250
Rate (beats per minute)
0
20
40
60
80
100
120
140
euthyroid
hyperthyroid
Time (secs.) after injection of 5×10⁻⁸M Isoproterenol

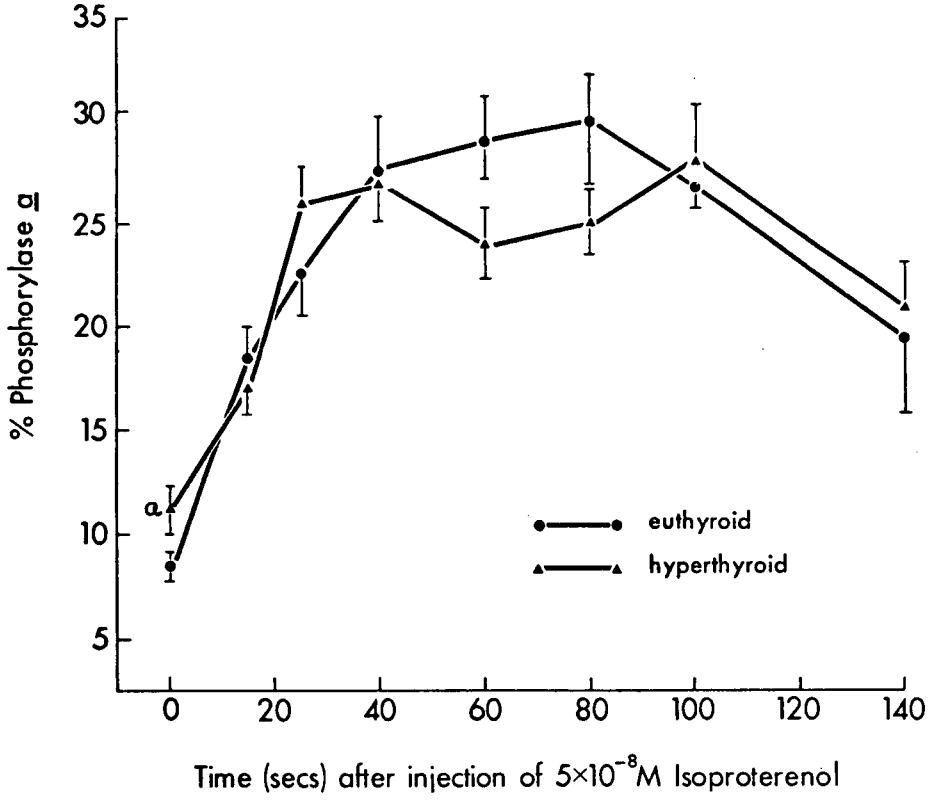
Figure 8.

Changes in % Phosphorylase a Following Administration of 5×10^{-8} M Isoproterenol to Euthyroid and Hyperthyroid Rat Right Atria.

The plot depicts the changes in % phosphorylase a content of euthyroid and hyperthyroid rat right atria, frozen at various times following administration of a sub-maximal dose of isoproterenol.

Each point represents the mean % phosphorylase a ± S.E.M. of 8-18 observations.

a Hyperthyroid significantly greater than euthyroid at $P < 0.05$.


% Phosphorylase a
35
30
25
20
15
10
5
a
0
20
40
60
80
100
120
140
euthyroid
hyperthyroid
Time (secs) after injection of 5×10⁻⁸M Isoproterenol

A Comparison of pA_2 Values for Propranolol in Euthyroid and Hyperthyroid Rat Right Atria.

The Schild plot used to determine the pA_2 values for propranolol in euthyroid and hyperthyroid rat right atria is shown in figure 9. There was no significant difference between the two values; euthyroid atria having a pA_2 of 7.0996, and hyperthyroid atria a pA_2 of 7.1180. This indicates that the receptors involved in the inotropic response of the rat right atrium to isoproterenol are the same in euthyroid and hyperthyroid animals.

Effect of Isoproterenol on Changes in Tension of the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

Isoproterenol produced a dose-related increase in tension in the right ventricles from both euthyroid and hyperthyroid rats as shown in figure 10. The tension developemnt was significantly greater in euthyroid than in hyperthyroid ventricles at doses of $1x10^{-6}$ M and $1x10^{-5}$ M isoproterenol. In the absence of drug, the tension developed by the two groups of ventricles was indistinguishable.

Figure 9.

A Comparison of pA_2 Values for Propranolol in Euthyroid and Hyperthyroid Rat Right Atria.

The plot depicts the log dose of antagonist versus log (DR-1) for euthyroid and hyperthyroid rat right atria.

Each point represents the log (DR-1) calculated from the mean ED_{50} of 7-19 observations.

The lines were not significantly different from each other.

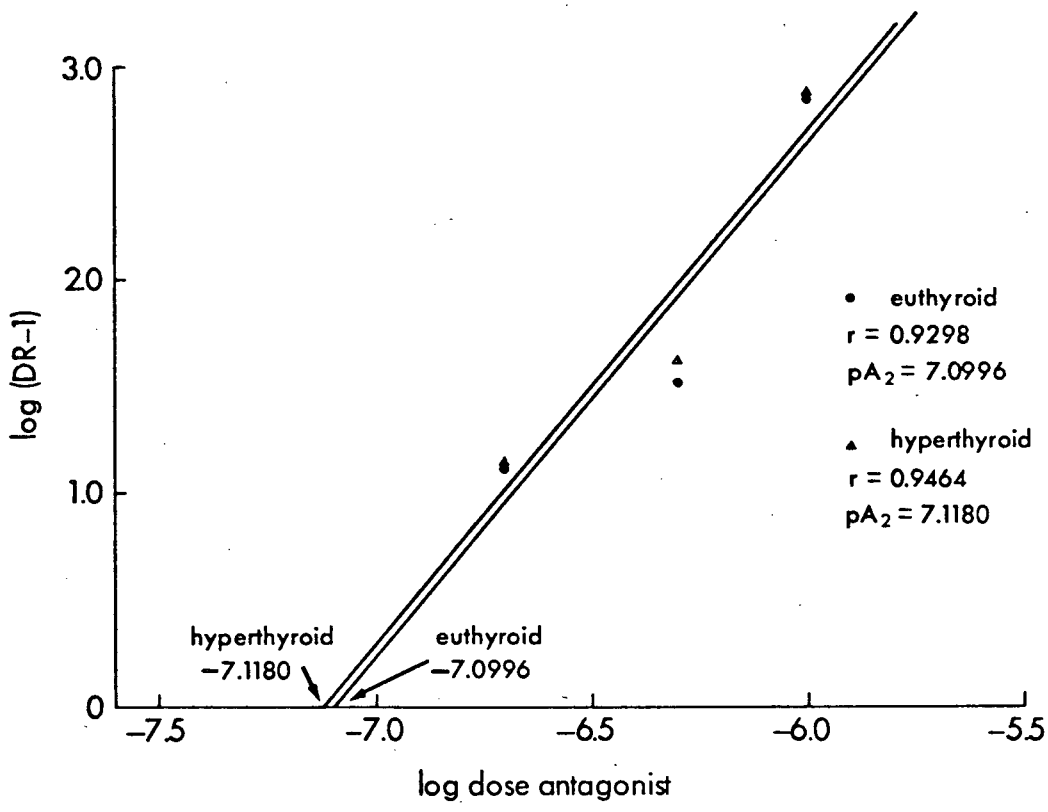

3.0
2.0
1.0
0
log (DR-1)
-7.5
-7.0
-6.5
-6.0
-5.5
log dose antagonist
euthyroid
r = 0.9298
pA2 = 7.0996
hyperthyroid
r = 0.9464
pA2 = 7.1180
hyperthyroid
-7.1180
euthyroid
-7.0996

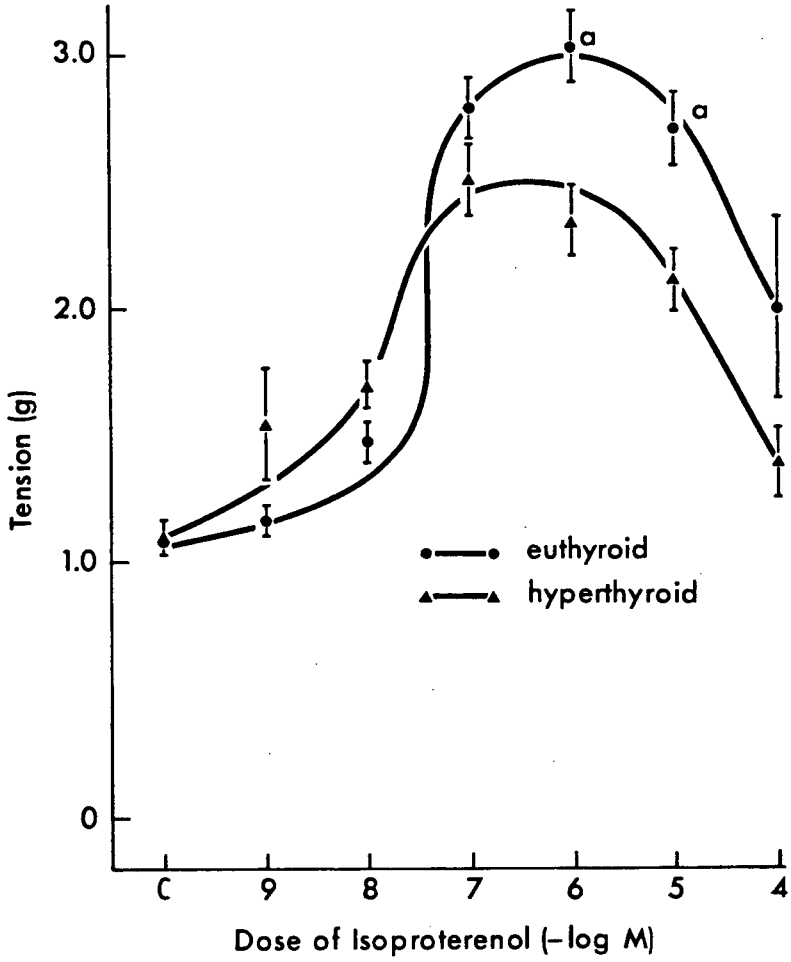
Figure 10.

Effect of Isoproterenol on Changes in Tension of the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

The plot depicts the effect of various doses of isoproterenol on absolute tension of euthyroid and hyperthyroid rat right ventricles, following stimulation at 3Hz. Maximal tension attained was recorded.

Each point represents the mean tension (g) $\pm$ S.E.M. of 5-44 observations.

a Euthyroid significantly greater than hyperthyroid at $P < 0.05$.


3.0
2.0
1.0
0
Tension (g)
a
a
euthyroid
hyperthyroid
C
9
8
7
6
5
4
Dose of Isoproterenol (-log M)

Changes in Tension Following Administration of 5×10^{-8} M Isoproterenol to the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

From the dose-response curve in figure 10, 5×10^{-8} M isoproterenol was chosen as a sub-maximal dose for a time-response study. Tension was studied in euthyroid and hyperthyroid rat right ventricles at various times following administration of the drug.

In the absence of drug, the tension developed by the two groups was indistinguishable; but by fifteen seconds after administration of the drug the euthyroid ventricles had a significantly higher developed tension than the hyperthyroid ventricles (Figure 11). This significant difference was maintained until forty seconds, although the euthyroid ventricles had consistently higher developed tensions at all times studied.

Changes in % Phosphorylase a Following Administration of 5×10^{-8} M Isoproterenol to the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

The response of % phosphorylase *a* activity to administration of 5×10^{-8} M isoproterenol in rat right ventricles is shown in figure 12.

In the absence of drug there was no significant difference between % phosphorylase *a* content in euthyroid or hyperthyroid rat right ventricles. Following administration of 5×10^{-8} M

Figure 11.

Changes in Tension Following Administration of 5×10^{-8} M Isoproterenol to the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

The plot depicts the change in absolute tension of euthyroid and hyperthyroid rat right ventricles, at various times following administration of a sub-maximal dose of isoproterenol.

Each point represents the mean tension (g) ± S.E.M. of 7-55 observations.

a Euthyroid significantly greater than hyperthyroid at $P < 0.05$.

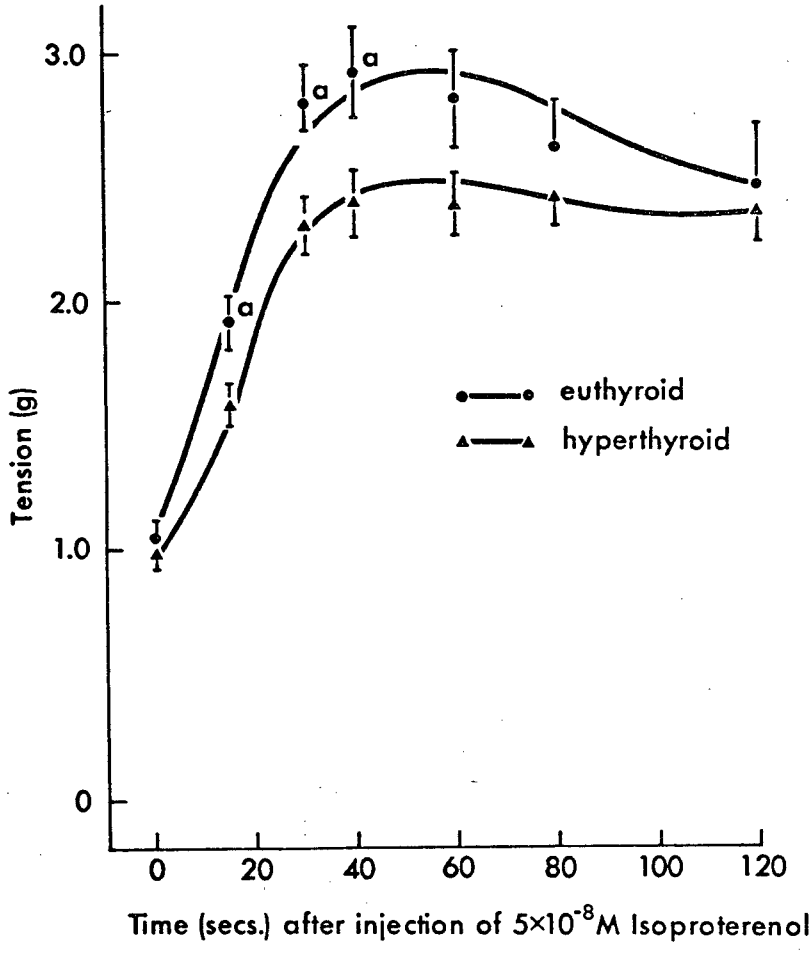

3.0
2.0
1.0
0
Tension (g)
a
a
a
euthyroid
hyperthyroid
0
20
40
60
80
100
120
Time (secs.) after injection of 5×10⁻⁸M Isoproterenol

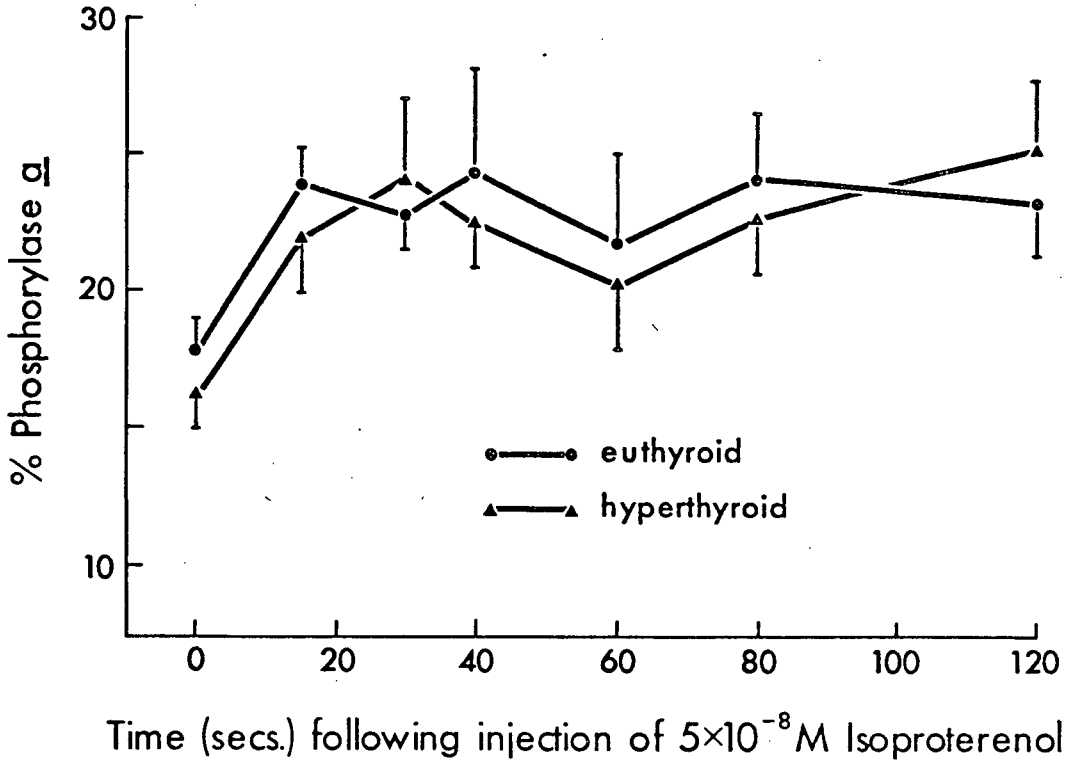
Figure 12.

Changes in % Phosphorylase a Following Administration of 5×10^{-8} M Isoproterenol to the Right Ventricle of Euthyroid and Hyperthyroid Rats while stimulating at 3Hz.

The plot depicts the change in % phosphorylase a content of euthyroid and hyperthyroid rat right ventricles, frozen at various times following administration of a sub-maximal dose of isoproterenol.

Each point represents the mean % phosphorylase a ± S.E.M. of 6-13 observations.

There was no significant difference between the two groups at any time studied.


30
20
10
% Phosphorylase a
0
20
40
60
80
100
120
euthyroid
hyperthyroid
Time (secs.) following injection of 5×10⁻⁸M Isoproterenol

isoproterenol there was a small time-related increase in % phosphorylase a, but at no time studied was there a significant difference between levels in euthyroid or hyperthyroid tissues. Only in euthyroid ventricles were the levels of % phosphorylase a following drug administration significantly greater than in the absence of drug.

Differences Between Euthyroid and Hyperthyroid Rat Langendorff Hearts.

Using the constant pressure Langendorff heart perfusion apparatus, it was found that there was a significantly higher coronary blood flow in the hyperthyroid hearts than in the euthyroid hearts (figure 13A). Euthyroid hearts having a coronary blood flow of 3.72 ± 0.177 ml per minute, and hyperthyroid hearts having a flow of 5.08 ± 0.134 ml per minute.

Associated with this, the hyperthyroid hearts also demonstrated a significantly higher wet weight (euthyroid: 1.00 ± 0.034 g; hyperthyroid: 1.25 ± 0.056 g) (figure 13B), and significantly higher resting heart rates (euthyroid: 197.54 ± 4.826; hyperthyroid: 280.75 ± 7.019) (figure 13C), than the euthyroid hearts.

Conversely, euthyroid hearts demonstrated a significantly greater developed tension than did the hyperthyroid hearts, with a value of 5.02 ± 0.210 g against the hyperthyroid value of 4.18 ± 0.116 g, (figure 13D).

Figure 13.

Differences Between Euthyroid and Hyperthyroid Rat Langendorff Hearts.

A: Differences in coronary blood flow (ml per minute).

B: Differences in wet heart weight (g).

C: Differences in resting rate.

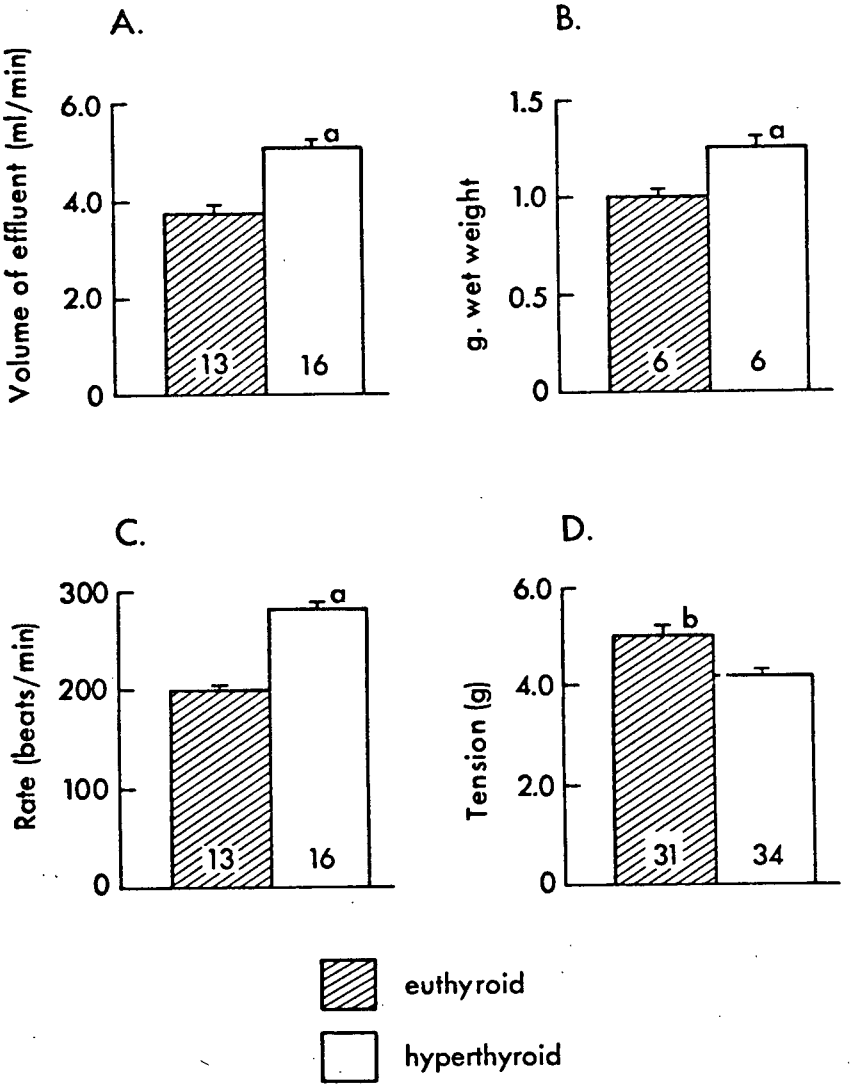
D: Differences in basal tension development.

The bars represent mean value ± S.E.M.

The number within the bar represents the n values.

a Hyperthyroid significantly greater than euthyroid at $P < 0.05$.

b Euthyroid significantly greater than hyperthyroid at $P < 0.05$.


A.
Volume of effluent (ml/min)
6.0
4.0
2.0
0
13
16
a
B.
g. wet weight
1.5
1.0
0.5
0
6
6
a
C.
Rate (beats/min)
300
200
100
0
13
16
a
D.
Tension (g)
6.0
4.0
2.0
0
31
34
b
euthyroid
hyperthyroid

Effect of Changes in Coronary Blood Flow on % Phosphorylase a Activation.

No significant difference in % phosphorylase a levels was found in Langendorff hearts from euthyroid and hyperthyroid rats perfused at the regular reservoir height of forty cm, and hyperthyroid hearts perfused at a reduced reservoir height so that the coronary blood flow was reduced to that of the euthyroid hearts ± two S.E.M. (figure 14). In these same hearts basal tension was greater in the euthyroid hearts than in the hyperthyroid hearts regardless of reservoir height (euthyroid: 5.05 ± 0.436 g, n=8; hyperthyroid at regular reservoir height: 4.68 ± 0.280 g, n=11; hyperthyroid at reduced reservoir height: 4.21 ± 0.229 g, n=13).

Following the bolus injection of five ng isoproterenol, there was a significant increase in tension and % phosphorylase a in all treatment groups compared to the appropriate control in the absence of drug. The absolute increase in tension was similar in all groups; the hearts from euthyroid animals maintaining a greater tension development than those from hyperthyroid animals (euthyroid: 7.38 ± 0.758 g, n=4; hyperthyroid at regular reservoir height: 6.72 ± 0.505 g, n=6; hyperthyroid at reduced reservoir height: 5.50 ± 0.257 g, n=7). The increase in % phosphorylase a in both hyperthyroid groups was significantly greater than the increase in hearts from euthyroid animals following administration of the drug, but not significantly different from each other.

Figure 14.

Effect of Changes in Coronary Blood Flow on % Phosphorylase a Activation.

The plot depicts the % phosphorylase a activity in euthyroid and hyperthyroid rat hearts, perfused at the regular reservoir height, and in hyperthyroid rat hearts perfused at a reduced reservoir height; thirty seconds after injection of 5 ng of isoproterenol or vehicle.

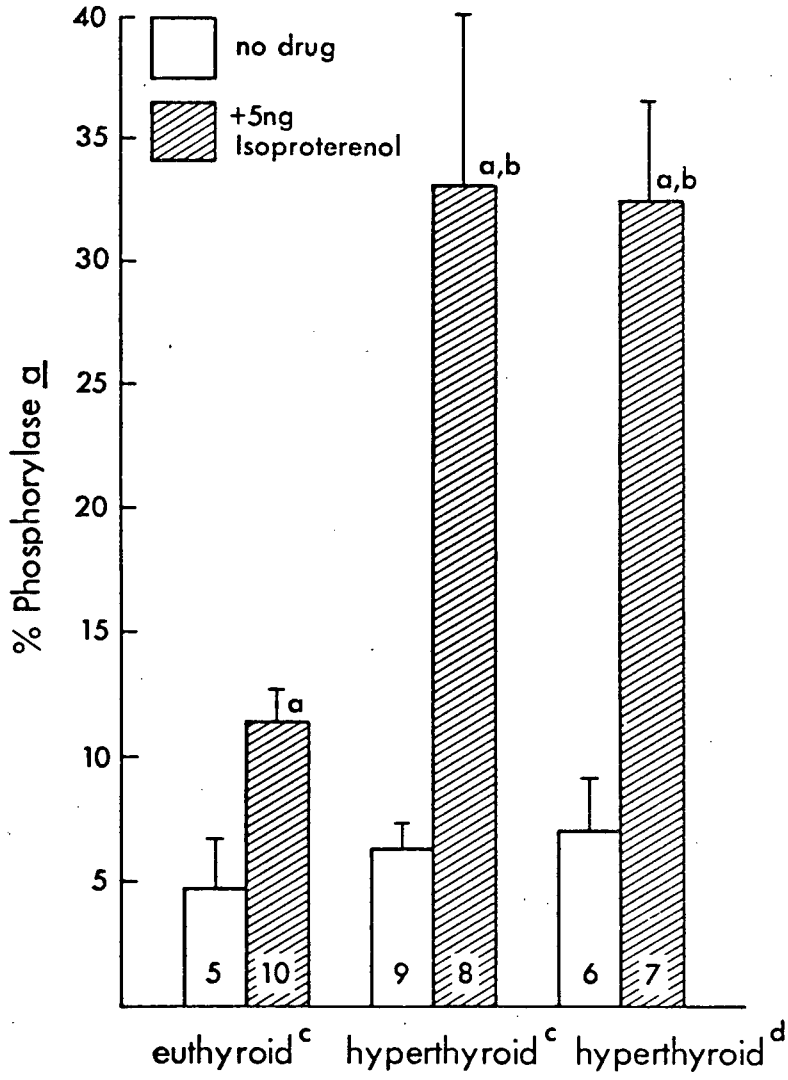
The bars represent mean % phosphorylase a ± S.E.M. The number within the bar represents the n value.

a Significantly greater than control value at $P < 0.05$.

b Hyperthyroid + drug significantly greater than euthyroid + drug at $P < 0.05$.

c Euthyroid and hyperthyroid hearts perfused at regular reservoir height (40cm).

d Hyperthyroid hearts perfused at reduced reservoir height.


40
35
30
25
20
15
10
5
% Phosphorylase a
no drug
+5ng
Isoproterenol
a,b
a,b
a
5
10
9
8
6
7
euthyroid c
hyperthyroid c
hyperthyroid d

DISCUSSION.

To date, then, our results have shown that cardiac tissues from hyperthyroid rats differ from those of euthyroid rats in that they exhibit an increased chronotropic and decreased inotropic response in their basal states. In response to isoproterenol administration, in the right atrium there is a dose-related increase in rate which is significantly greater in the hyperthyroid animal; in the left atrium and right ventricle there is a dose-related increase in contractile force which is significantly greater in the euthyroid animal. No supersensitivity to these effects was noted in the hyperthyroid tissues. The responses to isoproterenol, while accompanied by an increase in phosphorylase a activity, demonstrate no differences in enzyme activity between the two groups. In the Langendorff heart however; isoproterenol besides producing an inotropic response, increased phosphorylase a to a significantly greater extent in the hyperthyroid animals. The greater resting rate and lower basal tension of the hyperthyroid Langendorff hearts is accompanied by a significant increase in coronary blood flow as compared to euthyroid hearts. Reduction of the blood flow in the hyperthyroid hearts to the same level as in the euthyroid hearts had no significant effect on the phosphorylase activating effect of isoproterenol.

The pA_2 study which was carried out showed that any difference in the responses of euthyroid and hyperthyroid hearts to catecholamines (whether exogenous or endogenous) was not due

to any changes in receptor similarity, since the values obtained were practically identical in the two groups.

It is well documented that treatment of animals with thyroid hormones produces a reduction in whole body weight and increase in heart weight, resulting in an increased heart weight to body weight ratio when compared to euthyroid controls (Aronson, 1976; Cairoli and Crout, 1967; Frazer, Hess and Shanfeld, 1969; Gibson, Tichonicky and Kruh, 1975; Hornbrook and Cabral, 1972; Hornbrook et al, 1965; Inchiosa and Freedberg, 1965; Levey, Skelton and Epstein, 1969; Margolius and Gaffney, 1965; McNeill, Muschek and Brody, 1969; Nemecek and Hess, 1974; Skelton, Su and Pool, 1976; van der Schoot and Moran, 1965; and Yazaki and Raben, 1975). This was also found in the present study. Hyperthyroid rats did not gain any overall weight during the four day treatment period in contrast to control rats which gained an average of twenty grams (figure 3). Wet heart weight was significantly greater in the hyperthyroid than in the euthyroid rats, by a factor of 25 percent (figure 13B). Since the heart weight and body weight studies were not carried out on the same animals, heart weight to body weight ratios were not determined, but would almost certainly have been increased in the hyperthyroid animals.

The myocardial hypertrophy of hyperthyroidism has been shown to be accompanied by an enlargement of the myocardial cells; and an increase in the number, size and complexity of the mitochondria (DeGroot, 1972). Triiodothyronine has been

shown to augment the synthesis of both nuclear and cytosol liver proteins within five hours of administration to thyroidectomized rats (Bernal, Coleoni and DeGroot, 1978), and to cause mitochondrial swelling, probably due to increased permeability of cations (Marzoev and Vladimirov, 1978). These findings could all account for the cardiac hypertrophy found following thyroid hormone treatment,

Detection of loss of body weight or cardiac hypertrophy is an easy index of thyrotoxicosis to measure, Other indices which are often used include increases in oxygen consumption (Cairoli and Crout, 1967; Frazer, Hess and Shanfeld, 1969; Hornbrook and Cabral, 1972; Nemecek and Hess, 1974; and van der Schoot and Moran, 1965), or increases in protein-bound and total iodine (Buccino et al, 1967; Levey, Skelton and Epstein, 1969; Margolius and Gaffney, 1965; and Melander et al, 1975). A significant difference in body weight was found in our experiment after only four days, and was used as the index of thyroid hormone activity.

Right atria from hyperthyroid rats beat consistently faster than those from euthyroid rats (figure 4). The mean rate of atria from hyperthyroid rats was 25 percent higher than for those from euthyroid animals. Following administration of isoproterenol there was an increase in rate in both groups, the hyperthyroid atria maintaining consistently higher rates than the euthyroid atria. This is also well supported in the literature (Cravey and Gravenstein, 1965; Lee, Lee and Yoo, 1965; Thier, Gravenstein and Hoffman, 1962;

and van der Schoot and Moran, 1965). Although these investigators used adrenaline and noradrenaline, the results were similar; the initial heart rate was greater in atria from hyperthyroid rats than in those from euthyroid rats, and following catecholamine administration there was a dose-dependent increase in rate in both groups. Atria from hyperthyroid rats maintained consistently higher rates than those from the euthyroid group.

When the data were expressed as percent of maximal increase, we found that the results were similar to those of van der Schoot and Moran (1965), who showed that although the noradrenaline-induced increase in rate was similar in euthyroid and hyperthyroid rat atria (allowing for the different basal rates), when the data were expressed as percent of maximal increase, the response in the hyperthyroid atria was less than in the euthyroid atria (figure 5).

It therefore seems that, similar to the suggestion of Wilson et al, (1966); the chief difference between the euthyroid and hyperthyroid animals is the starting level of the heart rate. The increase in intrinsic heart rate may be due to a direct action of the thyroid hormones on the pacemaker cells as was suggested by Cairoli and Crout (1967).

Dratman (1974) has suggested that the mechanism of action of thyroxine may be similar to that of tyrosine, (which thyroxine is an amino acid analogue of). She postulated that thyroxine forms false neurohumours in adrenergic nerves, to be released instead of catecholamines (or with catecholamines) when the nerve is stimulated. It has been

shown that in hyperthyroidism there are decreased tissue and blood levels of adrenergic neurotransmitters (Dratman, 1974), although the body as a whole behaves as if there was increased sympathetic stimulation. Dratman suggests that this is due to iodothyronine-derived neurohumours of high biological activity which are released following nerve stimulation, and produce various manifestations of thyrotoxicosis. There is no direct evidence to support this hypothesis however.

In our experiment with right atria there was definitely no supersensitivity development to the chronotropic effect of isoproterenol in the hyperthyroid animal, since the increase in rate of the euthyroid atria was slightly more than that of the hyperthyroid atria (figure 4).

In the rat left atrium, right ventricle and Langendorff heart, basal tension was greater in euthyroid tissues than in hyperthyroid controls. In the left atrium, the euthyroid tissues had a mean basal tension which was 23 percent greater than that of the hyperthyroid tissues (figure 6). Following administration of isoproterenol, there was a dose-related increase in tension in both groups; but the tension development in the euthyroid tissues became significantly greater than that in the hyperthyroid tissues.

In the rat right ventricle there was no difference between basal tension in euthyroid and hyperthyroid tissues. Following administration of isoproterenol, there was a dose-dependent increase in contractile force, euthyroid tissues having a

significantly greater tension development than hyperthyroid tissues (figure 10). These results would therefore rule out the possibility of thyroid hormone-induced supersensitivity to the inotropic effects of isoproterenol in the rat left atrium and right ventricle.

The mean basal tension of euthyroid rat Langendorff hearts was significantly greater than that of hyperthyroid hearts (figure 13D). Dose-response curves to isoproterenol were not performed, but doubtless the data would have been similar to that of the left atrium and right ventricle since previous studies with other adrenergic amines have shown that no enhancement of the inotropic response occurs in this preparation. Young and McNeill(1974) showed that noradrenaline produced a dose-dependent increase in tension and phosphorylase a activity in both euthyroid and hyperthyroid rat Langendorff hearts. They found no significant difference in force or cyclic AMP activation between the two groups, but phosphorylase a activation was potentiated in the hyperthyroid hearts.

Van der Schoot and Moran (1965) suggested that the reduced inotropic force found in hyperthyroid cardiac tissues might be due to hypoxia of the myocardium, as a result of the increased oxygen comsumption induced in hyperthyroid animals. Dobson et al (1974) carried out a study using isolated perfused hearts, in situ hearts, right ventricle strips and papillary muscles from guinea pigs. On the basis of comparison of several metabolic and physiologic parameters measured in these tissues, they concluded that the right ventricle was prone to protein loss due to damage during the dissection period, and to tissue hypoxia;

rendering it unsuitable for studies of the mechanisms of myocardial contractility. These factors would probably be accentuated to an even greater extent in a hypermetabolic state such as hyperthyroidism.

In both the spontaneously contracting rat right atrium (figures 7 and 8), and the electrically driven rat right ventricle (figure 10 and 11); a sub-maximal dose of isoproterenol (in both cases, 5×10^{-8} M) produced a change in contractile force which was significantly different between euthyroid and hyperthyroid tissues, without producing a significant difference in phosphorylase a activity. However, in the rat Langendorff heart, 5 ng of isoproterenol produced a significantly greater increase in phosphorylase a in hyperthyroid than in euthyroid hearts. That this difference in phosphorylase a activity was not a result of the increased coronary blood flow detected in the hyperthyroid animals was demonstrated by the fact that reducing the coronary blood flow of the hyperthyroid hearts to that of the euthyroid hearts had no effect on the isoproterenol-induced phosphorylase activation (figure 14).

In our study, coronary blood flow was measured by the oldest and easiest method available; collection and measurement of the effluent from the coronary blood vessels, following retrograde perfusion through the aorta. Other more sophisticated methods are available and are discussed in a book on coronary vasodilators by R. Charlier, (1961); as are other variations of the Langendorff method. In our

case the method used was the most practical, since it was relatively easy to perform, and we did not require long-term measurements to be made. Other methods available include involvement with cinematography (Stehle, 1932), elaborate cannulations (Lu and Melville, 1950), radioactive methods using ^{42}K or ^{3}H-labelled drugs (Wurtman, Kopin and Axelrod, 1963; Wurtman et al, 1964), or the thermostromuhr method of Rein (Charlier, 1961; Essex, Baldes and Mann, 1936), in which a small cuff containing a diathermy unit and two thermocouples is fitted around a coronary artery. Part of the heat supplied by the unit is removed by the blood stream, providing a difference in temperature between the two thermocouples which is a function of the blood flow and can be recorded with a galvanometer. The advantage of this method is that it can be used to record coronary blood flow continuously, and can be used in the normal unanaesthetized animal. Its disadvantages are that it requires surgical installation, a recovery period, and may be dislodged; generally with fatal results.

It is possible that the apparent potentiation of phosphorylase activation seen in the Langendorff heart could be due to a number of factors. Firstly, the physical process of dissection carried out to obtain the separate portions of the heart could damage cells, although it would appear likely that only a minority of cells would be affected, and that this is therefore unlikely to be a major cause of the discrepancy. Secondly, in the isolated organ bath,

penetration of the tissues by drug is unlikely to be as complete as when the drug is introduced into the coronary circulation, as is the case in the Langendorff heart. However, we demonstrated in the Langendorff heart that even when coronary blood flow was equal in the euthyroid and hyperthyroid hearts there was still potentiation of the phosphorylase-activating effect of isoproterenol.

It has recently been shown (Herd, 1978), that thyroxine directly affects the kinetics of calcium transfer across mitochondrial membranes. This change may be due to alteration of the number of ions transported per transfer cycle, altered mobility of the calcium in the membranes, and/or altered rate of release of the transport calcium. This action of thyroxine on calcium may also alter calcium-dependent metabolic processes such as ADP translocation, which is stiumlated in the presence of thyroid hormone. Friesen, Allen and Valadares (1967), and Hartley and McNeill (1976) showed that calcium can activate phosphorylase directly in the perfused heart; although the sensitivity of phosphorylase a to calcium is not changed by thyroid hormone treatment.

pA_2 studies (Schild, 1957) are widely used to quantify the affinity of antagonists for a receptor site, and also to characterize receptor types in vitro. The pA_2 is defined as the negative logarithm of the molar dose of antagonist which reduces the effect of a double dose of agonist to that of a single dose. In the present study we wished to determine if the beta-adrenoceptors of the spontaneous rat right

atrium were the same in their affinities for beta-agonists and antagonists whether the heart was obtained from a euthyroid or hyperthyroid animal. If pA_2 values are the same, then it is usually considered that the receptors are the same. From the data we present here (figure 9), it can be seen that the pA_2 values for the receptor interaction were almost identical, regardless of the thyroid state. This would tend to indicate that the difference in intrinsic rate and other cardiac manifestations of thyrotoxicosis are not due to any changes in receptor sensitivity, although it is possible that changes in beta-receptor number do take place (Williams et al, 1977).

In summary, we have confirmed that catecholamine-induced phosphorylase activation is potentiated in the isolated perfused hyperthyroid rat heart. However, in isolated portions of the heart no potentiation of phosphorylase activation in hyperthyroid animals could be found. We have also determined that the effect is not due to the increased coronary blood flow found in the hyperthyroid hearts.

A similar situation has been shown to occur with reserpine supersensitivity. McNeill and Schulze (1972) showed in the guinea pig Langendorff heart that pretreatment with reserpine for forty-eight hours, resulted in the development of supersensitivity to both the phosphorylase-activating and the inotropic effects of noradrenaline and histamine. However, in the guinea pig right atrium (Westfall and Fleming, 1968), and the rat ventricle strip (McNeill, unpublished data) supersensitivity to

catecholamines after reserpine pretreatment cannot be readily demonstrated. Westfall and Fleming suggested that the ability to demonstrate reserpine-induced supersensitivity in myocardial tissues is inversely related to the extent of mechanical manipulation of the muscle. This anomaly seems to be analogous to the situation found in the heart following pretreatment with thyroid hormones; which could also be due to damage caused during dissection of the various heart portions.

Future studies could include investigation of phosphorylase phosphatase, the enzyme which converts phosphorylase a back into the inactive phosphorylase b; to see if the potentiation of phosphorylase activity seen in hyperthyroid hearts is due to alterations in the activity of this enzyme. The method devised by Rall and Sutherland (1962) for estimation of phosphorylase phosphatase is quite similar to that which we use for phosphorylase determinations (Diamond and Brody, 1965), and could therefore be carried out.

It would also be of interest to investigate electrophysiological changes in the sino-atrial node of the right atrium of euthyroid and hyperthyroid animals, and to determine the effect of calcium on the resting membrane potential and action potential in cardiac tissues from the two groups to determine whether the thyroid hormones alter the permeability of nodal tissue to calcium. Previous studies (Hartley and McNeill, 1976) have indicated that a change of calcium permeability in the sino-atrial node is a definite possibility.

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