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EFFECTS OF TREATMENT WITH THYROXINE ON THE NORADRENALINE CONTENT OF THE RABBIT HEART

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It has been suggested that some of the signs of the hyperthyroid state can be attributed to increased activity of the sympathetic nervous system. This is supported by the observations that the symptoms of hyperthyroidism are alleviated by blockade of central sympathetic pathways by procaine injected into the subarachnoid space (Knight, 1945) or applied to the whole of the epidural area (Brewster, Isaacs, Osgood & King, 1956). The responses of the cardiovascular system to adrenaline and to noradrenaline are exaggerated in hyperthyroid animals, which suggests that the appearance of increased sympathetic activity may be due to an increased sensitivity to these catechol amines (Hoffmann, Hoffmann & Talesnik, 1947; Schneckloth, Kurland & Freedberg, 1953; Rosenblum, Hahn & Levine, 1933).

The increased heart rate produced by thyroid feeding or by thyroxine can be explained neither in terms solely of increased sympathetic activity since it is seen in the denervated or transplanted hearts of dogs (McIntyre, 1931; Priestley, Markowitz & Mann, 1931), nor by increased sensitivity to circulating catechol amines since isolated hearts removed from animals treated with thyroid hormone beat at a faster rate than those removed from normal animals (Lewis & McEachern, 1931; Hirvonen & Lybeck, 1956; Thier, Gravenstein & Hoffmann, 1962). This paper describes experiments on the responses of isolated atria to noradrenaline and on the relationship between the rate of beating of isolated atria and the noradrenaline content of the hearts from thyroxine-treated rabbits.

METHODS

Albino rabbits weighing 1.5 to 2.0 kg were used. Some were given thyroxine (2 mg/kg, intraperitoneally on each of three successive days and were used on the fourth day. Reserpine (2 mg/kg) was injected intravenously dissolved in a mixture of ethanol, propylene glycol and water (1 : 1 : 2); these rabbits were used 24 hr later. Some rabbits were given thyroxine for two days and both thyroxine and reserpine on the third day.

The heart was rapidly removed from the rabbit during ether anaesthesia. The atria were suspended in Tyrode solution in a 100-ml. organ-bath. The solution was bubbled with 95% oxygen and 5% carbon dioxide through a sintered glass disc and maintained at 38° C. The atrial beats were recorded on a smoked drum with an isotonic lever exerting a tension of 1.4 g and giving a fourfold magnification.

The stock solution of (−)-noradrenaline bitartrate was in 0.01 N-hydrochloric acid. Thyroxine (10^{-2} M) was dissolved in 0.05 N-sodium hydroxide solution. These solutions were diluted with Tyrode solution for addition of the drugs to the bath. The highest concentration of sodium hydroxide added to the bath

was 0.0005 N; this had no effect in control experiments. The disodium salt of edetic acid was dissolved in Tyrode solution.

The noradrenaline content of atria or ventricles was determined by the method of Shore & Olin (1958) after homogenization in two volumes of 0.01 N-hydrochloric acid.

RESULTS

Hearts from thyroxine-treated rabbits

Table 1 gives the mean rate of beating of atria and the mean catechol amine content of ventricles of hearts from normal and thyroxine-treated rabbits. Atria taken from thyroxine-treated rabbits beat consistently faster than those from normal rabbits and the difference between the mean rates of beating was statistically significant ($P<0.01$). The catechol amine content of the ventricles taken from thyroxine-treated rabbits was greater than that of ventricles from untreated rabbits, the difference in the mean contents being statistically significant ($P<0.01$).

TABLE 1

THE INITIAL RATE OF CONTRACTION OF THE ISOLATED ATRIA AND THE VENTRICULAR NORADRENALINE CONTENT OF HEARTS FROM RABBITS TREATED WITH THYROXINE, RESERPINE OR THYROXINE AND RESERPINE

The rabbits were assigned at random to the four groups. Thyroxine: this was injected intraperitoneally in a daily dose of 2 mg/kg for three successive days; the rabbits were used on the fourth day. Reserpine: this was injected intravenously in a dose of 2 mg/kg 24 hr previously. Thyroxine and reserpine: thyroxine was injected for three successive days with reserpine on the last day, in the above doses. Values are means and standard errors

Treatment	No. of rabbits	Initial rate of contraction (per min)	Catechol amine concentration (μg/g)
None	6	152·0±4·9	1·59±0·10
Thyroxine	6	203·0±4·6	3·43±0·14
Reserpine	6	117·0±2·2	0·21±0·02
Thyroxine and reserpine	6	124·0±2·0	0·37±0·06

The responses to noradrenaline of the atria isolated from normal and thyroxine-treated rabbits are given in Table 2. The effects of noradrenaline in increasing the rate of beating and the amplitude of the beat were greater in the atria from the thyroxine-treated rabbits. It was observed that the atria from the thyroxine-treated rabbits frequently beat arrhythmically after the addition of noradrenaline to the bath.

TABLE 2

THE EFFECTS OF NORADRENALINE ON RATE AND AMPLITUDE OF BEATING OF RABBIT ATRIA

The increases in the amplitude of contraction and in the rate of contraction after the addition of noradrenaline are expressed as percentages of the values before the addition of noradrenaline. Each figure is the mean of five or six experiments, followed by the standard error. * Significantly different ($P<0·01$) from the controls

	Increase in amplitude (%) with noradrenaline concentration (M)			Increase in rate (%) with noradrenaline concentration (M)		
Treatment	10^{-7}	5×10^{-7}	10^{-6}	10^{-7}	5×10^{-7}	10^{-6}
None	5·0±0·7	10·6±2·2	55·4±4·2	0	0	12·4±1·3
Thyroxine	22·0±2·8*	49·0±2·3*	78·7±3·2*	14·8±1·5	23·3±1·7	38·8±1·5*

Effects of thyroxine in vitro *on rabbit isolated atria*

Thyroxine added to the organ-bath in concentrations up to 10^{-4} M was without effect on the rate of beating of isolated atria. An increase in concentration of thyroxine resulted in a decrease both in the amplitude and in the rate of beating and sometimes caused arrhythmic beating. However, the responses to noradrenaline were augmented in the presence of thyroxine (5×10^{-5} M), as shown in Fig. 1.

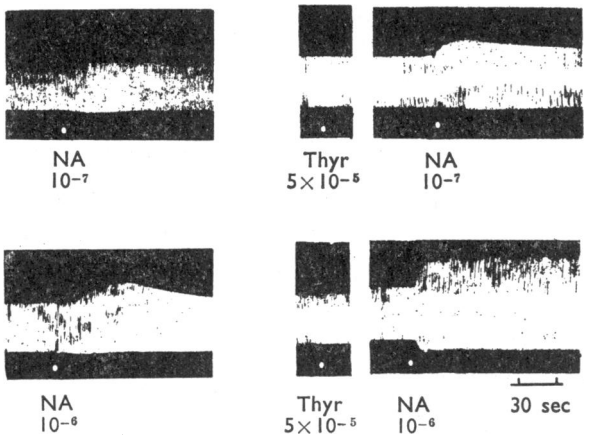

Fig. 1. The effects of thyroxine (Thyr) on the responses to noradrenaline (NA) in atria isolated from normal rabbits. Drugs were added at each white dot to provide the final concentration (M) indicated. The left-hand tracings illustrate the effect of noradrenaline before thyroxine. The preparations were repeatedly washed with Tyrode solution for more than 30 min between the left-hand and right-hand tracings. In the right-hand tracings, noradrenaline was added 10 min after the addition of thyroxine.

The potentiation of noradrenaline by thyroxine on other tissue has been shown to be due to chelation by thyroxine of copper, which is often present in small amounts in the water and reagents used for making Tyrode solution (Shemano & Fallon, 1960; Shida, Meyers & Barker, 1963). Therefore, the possibility was considered that the effect of thyroxine in enhancing the action of noradrenaline could be attributed to the capacity of thyroxine to chelate heavy metals. This was tested by comparing the effects of thyroxine and of edetic acid on the responses of the atria to noradrenaline. Edetic acid in a concentration of 10^{-5} M had no effect on the rate or amplitude of beating, but it markedly enhanced the action of noradrenaline. Another test was made by using carefully redistilled water and specially selected reagents for the preparation of the Tyrode solution, in order to keep the trace amounts of heavy metal contaminants as low as possible. When these precautions were taken the effects of thyroxine and of edetic acid in potentiating the responses of the atria to noradrenaline were largely reduced or disappeared. These results support strongly the possibility that the effect of thyroxine *in vitro* in enhancing the responses to noradrenaline is brought about indirectly by the removal by chelation of heavy metal contaminants in the bathing fluid rather than to a direct effect on the atria.

An attempt was made to examine the effect of thyroxine *in vitro* on the noradrenaline content of atria. Isolated atria were suspended in Tyrode solution containing 10^{-5} M

thyroxine for 6 hr, after which their noradrenaline content was determined. The content was decreased, but the decrease was no greater than occurred in atria suspended for 6 hr in Tyrode solution containing no thyroxine.

Relationship between the rate of beating and the noradrenaline content of atria

The rate of beating of atria isolated from thyroxine-treated rabbits gradually decreased as time elapsed, and after they had been in the bath for 6 hr their rate of beating was not significantly different from that of freshly isolated atria from untreated rabbits (Table 3). It was considered possible that this decline in rate may have been due to a gradual loss of the store of noradrenaline from the isolated atria. Consequently, when the atria from thyroxine-treated rabbits had been in the bath for 6 hr their noradrenaline content was determined. After 6 hr in the bath, atria from thyroxine-treated rabbits had a mean noradrenaline content which was not significantly different from that of freshly isolated atria from normal rabbits (Table 3).

TABLE 3

DECLINE IN RATE OF BEATING AND IN NORADRENALINE CONCENTRATION OF THE ISOLATED RABBIT ATRIA DURING A PERIOD OF 6 HR *IN VITRO*

The values are the means and standard errors from six or eight experiments. Initial values are from freshly isolated atria, and 6-hr values are from atria set up in the organ-bath and left for 6 hr. Thyroxine (2 mg/kg) was injected intraperitoneally for three successive days. Noradrenaline (2 mg/kg) was injected intramuscularly 4 hr before the experiment

	Rate of contraction			Catechol amine concentration		
Treatment	Initial (per min)	After 6 hr (per min)	Decrease (%)	Initial (μg/g)	After 6 hr (μg/g)	Decrease (%)
None	151·1±4·8	101·3±2·5	33·1	2·13±0·08	1·18±0·13	44·5
Thyroxine	210·7±5·5	143·0±6·4	32·2	3·94±0·11	2·14±0·15	38·8
Noradrenaline	197·5±6·9	130·3±1·8	34·3	3·87±0·14	2·15±0·23	45·0

The injection of noradrenaline into animals results in an increase in the noradrenaline content of the heart (Axelrod, Weil-Malherbe & Tomchick, 1959; Strömblad & Nickerson, 1961). Therefore, observations were made on the isolated atria from rabbits injected with noradrenaline in order to examine further the relationship between the rate of beating and the content. Rabbits were injected intramuscularly with noradrenaline (2 mg/kg) and they were killed 4 hr later. The hearts were removed immediately. The rate of beating of the isolated atria when they were first set up was significantly greater than that of atria from normal rabbits, and their catechol amine content was also significantly elevated. After these atria had been left in the organ-bath for 6 hr their rate of beating had decreased by 34.3% and their catechol amine content had decreased by 45%. The values they had reached in this time were not very different from those of freshly isolated atria from normal rabbits (Table 3).

Effects of reserpine

Reserpine exerts antithyroid activity (Moon & Turner, 1959; Mayer, Kelly & Morton, 1956; Felice, Smith & Dearborn, 1957) and depletes the stores of catechol amines in tissues, including the heart (Carlsson, Rosengren, Bertler & Nilsson, 1957; Paasonen & Krayer, 1958; Lee & Shideman, 1959). The effects of treatment with reserpine alone and of thyroxine and reserpine together on the rate of atrial beating and the noradrenaline content of the

ventricles are shown in Table 1. After reserpine the rate of beating of the atria and the noradrenaline content of the ventricles were significantly lower ($P<0.001$) than in untreated rabbits. Reserpine given to thyroxine-treated rabbits produced similar, but slightly smaller, effects.

DISCUSSION

There is a clear correlation between the rate of beating of rabbit isolated atria and the noradrenaline content of the ventricles from the same hearts. This has been observed after treatments with thyroxine and with reserpine, which produced opposite effects. There is also a correlation between the rate of beating of atria and the noradrenaline content of atria after injection of rabbits with thyroxine or noradrenaline, which both increased the rate of beating and the noradrenaline content, and after leaving the atria in the organ-bath for 6 hr, which decreased the rate of beating and the noradrenaline content.

These findings may explain the increased heart rate observed in hyperthyroid patients: in addition the hearts from thyroxine-treated rabbits exhibited an increased sensitivity to noradrenaline. In clinical practice, reserpine has been used to alleviate the symptoms of hyperthyroidism. This action of reserpine has been attributed to inhibition of thyrotrophin secretion (Moon & Turner, 1959), to inhibition of the binding of iodine (Mayer, Kelly & Morton, 1956), to the depression of oxygen consumption (Felice *et al.*, 1957) and to depression of food intake (Premachandra & Turner, 1960). The experiments reported here suggest that if some of the symptoms of hyperthyroidism are due to increases in the content of noradrenaline in tissues, then reserpine alleviated the symptoms by depleting the noradrenaline. Gaffney, Braunwald & Kahler (1961) observed that guanethidine decreased the heart rate of hyperthyroid patients, and this drug depletes noradrenaline from the heart (Cass, Kuntzman & Brodie, 1960). However, Thier *et al.* (1962) reported that reserpine had only a slight effect on the rate of beating of atria from hyperthyroid rats.

The increase in the noradrenaline content in the heart after treatment with thyroxine may result from the inhibition of monoamine oxidase by thyroxine (Burn, 1952; Spinks, 1952; Trendelenburg, 1953), since some drugs which inhibit monoamine oxidase cause an increase in the noradrenaline content of the rat heart (Crout, Creveling & Udenfriend, 1961).

There have been conflicting reports on the effect of thyroxine on isolated hearts. Harvey & MacRae (1931), using turtle heart, observed no immediate effect, and then a decrease in rate. Markowitz & Yater (1932) reported an increase in rate of beating of hearts from 2-day-old chick embryos. Cayaffa-Bonifaz & Vignolo-Puglia (1950) and Kleinfeld, Rosenthal & Stein (1958) observed a slight increase in rate of frog and toad hearts with low concentrations of thyroxine and a decrease with concentrations above 10^{-4} M. In our experiments, thyroxine up to 10^{-4} M had no effect on the rate of beating of rabbit atria; higher concentrations decreased the rate.

SUMMARY

1. Rabbits were injected with thyroxine (2 mg/kg, intraperitoneally) for three successive days. The atria isolated from these rabbits had a faster rate of beating than those from normal rabbits, and the catechol amine content of the heart was increased. The increase in the amplitude and rate of beating of these atria in response to noradrenaline was significantly greater than that of atria from normal rabbits.

2. Thyroxine added *in vitro* to isolated atria did not increase their rate of beating, but the response to noradrenaline was enhanced. Evidence was presented that this enhancement was the result of chelation by thyroxine of heavy metal contaminants in the bathing solution.

3. Isolated atria from rabbits which had been given an intraperitoneal injection of noradrenaline had an increased catechol amine content and they beat as fast as did atria from thyroxine-treated rabbits.

4. The ability of thyroxine to increase both the rate of atrial contraction and the catechol amine content of the heart were abolished after injection of reserpine.

5. The results indicate that the effects on the heart of treatment with thyroxine are largely dependent upon the capacity of thyroxine to increase the catechol amine content of the heart.

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