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Additional evidence against universal modulation of β-adrenoceptor responses by excessive thyroxine

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1 The effect of prolonged, excessive thyroxine on β-adrenoceptor-mediated relaxation of guinea-pig trachea was studied.

2 Thyroxine did not significantly affect the potency of the β_1-adrenoceptor agonist, noradrenaline, or the β_2-adrenoceptor agonist, terbutaline.

3 Thyroxine did not significantly affect the apparent K_B values of the selective β_1-adrenoceptor antagonist, practolol, or the selective β_2-adrenoceptor antagonist, butoxamine.

4 Thyroxine did not significantly affect the maximum response to noradrenaline. The maximum response to terbutaline in tissues from the thyroxine-treated animals was only slightly lower than the maximum response in tissues from paired control animals.

5 These results suggest that excessive thyroxine does not significantly affect the β-adrenoceptor-mediated relaxation of guinea-pig trachea and that the reported modulation of β-adrenoceptor-mediated responses in other tissues is specific for the given tissue rather than common to all β-adrenoceptor systems.

Introduction

Several laboratories have reported that thyroid hormone increases the sensitivity of cardiac tissue to β-adrenoceptor agonists. This is accompanied by an increase in the number of β-adrenoceptor binding sites with no change in affinity (Wildnethal, 1974; Tsai & Chen, 1978; Tse, Wrenn & Kuo, 1980). In fat cells and adipose tissue, thyroid hormone appears to increase β-adrenoceptor-induced cyclic adenosine 3′,5′-monophosphate (cyclic AMP) accumulation (Malbon, Moreno, Cabelli & Fain, 1978; Fain, 1981). Its major effect on cyclic AMP in adipose tissue appears to be mediated through regulation of the ability of β-adrenoceptor agents to activate adenylate cyclase rather than through an effect on binding characteristics. On the other hand, lymphocyte β-adrenoceptor responses are not affected by thyroid hormone (Williams, Guthrow & Lefkowitz, 1979; Smith, Silas & Yates, 1981).

One can conclude from the studies cited above that the ability of thyroid hormone to modulate β-adrenoceptor systems varies with the type of tissue or cell. An open question remains as to whether this variability is due to the tissue *per se* or to the β-adrenoceptor subtype invloved. The β-adrenoceptors of the heart and adipose tissue, tissues whose β-adrenoceptor responsiveness is altered by thyroid hormones, are generally agreed to be of the β_1-subtype while those of lymphocytes appear to be β_2 (Williams, Snyderman & Lefkowitz, 1976; Gilman, Goodman & Gilman, 1980; Smith, Silas & Yates, 1981).

The purpose of the present study was to examine the effect of prolonged, excessive thyroid hormone on a tissue that possesses both β_1- and β_2-adrenoceptors. Guinea-pig trachea was chosen since Furchgott (1976) has reported that it possesses both β-adrenoceptor subtypes. The experiments were designed to show any independent modification of β_1- or β_2-adrenergically mediated relaxation of the trachea by thyroxine.

Treatment of guinea-pigs

Male, albino guinea-pigs (Hartley, 300–600 g) were used in these experiments. The animals were paired by weight. An experimental animal was chosen randomly from each pair and a Model 2002 ALZET mini-osmotic pump (Alza Corp.) was implanted subcutaneously. The pump was loaded with a solution of levothyroxine in 50% dimethylsulphoxide to provide a constant infusion of 0.4–0.6 μg/g daily for 13 days. Preliminary experiments showed a 0.4 μg/g daily in-

fusion to be sufficient to raise the resting metabolic rate by 100% by the 4th day after implantation. This was accompanied by a significant weight loss as compared to sham-operated controls, and consequently, effectiveness of treatment was determined by difference in weight change relative to the paired control animal.

Preparation of tracheae

Tracheae were removed from the guinea-pigs, cleaned, halved and mounted on clips in tissue baths bubbled with 5% CO_2 in O_2 and maintained at 37° and pH 7.4. The modified Krebs solution contained the following (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, CaCl 2.5, $NaHCO_3$ 25, glucose 11, disodium edetate 0.02, as well as phentolamine (0.003), cocaine (0.01) and metanephrine (0.03) to block α-adrenoceptors, neuronal uptake and extraneuronal uptake, respectively.

Experimental procedure

Pharmacological isolation was used to allow independent examination of β_1- and β_2-adrenoceptor-mediated relaxation (Taylor, 1982). Basically, this entails obtaining the concentration-response relationship for an agonist selective for one of the β-adrenoceptor subtypes in the presence of an effective concentration of an antagonist selective for the complementary β-adrenoceptor subtype. Noradrenaline was the selective β_1-agonist and terbutaline was the selective β_2-agonist used. The β_2-adrenoceptors were blocked with butoxamine (4×10^{-6} M; approximate pA_7 vs. terbutaline) while the β_1-adrenoceptors were blocked with practolol (5×10^{-6} M; approximate pA_{12} vs. noradrenaline).

After the tissues had equilibrated for 2 h at 5–6 g tension, cumulative concentration-response curves (CCRCs) were obtained by first contracting the tracheae with carbachol (2.5×10^{-7} M) and then adding the β–adrenoceptor agonist to the baths in a cumulative fashion. Change in isometric tension was measured with a Narco Model F-60 Myograph and recorded on a Narco Physiograph. Response was calculated as the relaxation (i.e. the decrease in g tension from the carbachol baseline) caused by each concentration of agonist expressed as a percentage of the relaxation caused by a maximally effective concentration of aminophylline given at the end of each run.

In addition, the effect of thyroxine on the affinities of the β_1- and β_2-adrenoceptors for the appropriate selective antagonist (practolol and butoxamine, respectively) was examined indirectly by measuring the apparent dissociation constant, K_B (Furchgott, 1976). The ED_{50} of the second of two initial CCRCs was taken as the control for determining the concentration-ratio caused by the antagonist. After the trachea had equilibrated in the presence of the antagonist for 1 h, a third CCRC was obtained, and from it the ED_{50} in the presence of the antagonist was determined. Analysis of variance of data from preliminary studies following this same experimental protocol, but without addition of the antagonist, indicated that any change in sensitivity to the agonist with time and exposure was negligible.

The effect of thyroxine on the maximum responses produced by the two agonists as an indirect indicator of changes in functioning receptor number was also examined. Since the presence of spare receptors would obscure such a comparison, the maxima were obtained against a physiologically antagonistic concentration of carbachol (2.5×10^{-5} M) in an attempt to require activation of all functional receptors present for maximum relaxation (Buckner & Saini, 1975).

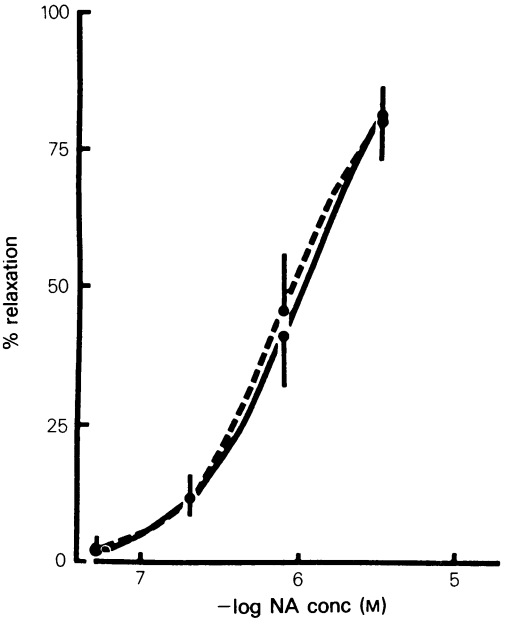

Figure 1 Cumulative concentration-response curves for noradrenaline (NA)-induced relaxation of trachea taken from thyroxine-treated and paired control guinea-pigs. Isolated tracheal preparations were contracted with carbachol; when the response had stabilized, noradrenaline was added in a cumulative manner. Relaxation is expressed as a percentage of the response to a maximally effective concentration of aminophylline. Butoxamine 4×10^{-6} M was present to block β_2-adrenoceptors. Dashed line: thyroxine-treated group; solid line: paired control group. Each point represents the mean of 7 tissues; the vertical lines indicate s.e. mean.

Statistical methods

Differences between the individual pD_2, K_B and maxima values of the paired animals were tested by analysis of variance (Sokal & Rohlf, 1969).

Drug solutions

All agonist and antagonist solutions were made fresh from the crystalline powder on the day of the experiment in Krebs solution and were kept on ice during the experiment.

The sources of drugs used were: (−)-noradrenaline HCl ((−)-arterenol; Sigma), (±)-practolol (gift of ICI), (±)-butoxamine HCl (gift of Burroughs Wellcome), (±)-terbutaline sulphate (Brethine; gift of Geigy Pharmaceuticals), phentolamine HCl (Regitine, gift of Ciba Pharmaceutical), cocaine HCl, (±)-metanephrine HCl (Sigma), disodium edetate (dihydrate; Sigma), carbamylcholine chloride (carbachol; Sigma), aminophylline ($(theophylline)_2$-ethylenediamine; Sigma). Glucose, NaCl, KCl, $MgSO_4$, KH_2PO_4 and $NaHCO_3$ were obtained from Mallinckrodt.

Results

Thyroxine had no effect on the CCRC of either noradrenaline or terbutaline (Figures 1 and 2, respectively). Analysis of variance of the pD_2 values obtained from the individual CCRCs for each of the paired animals also showed that thyroxine had no significant effect on the potency of either of the two agonists. The means and standard errors calculated from these values are shown in Table 1.

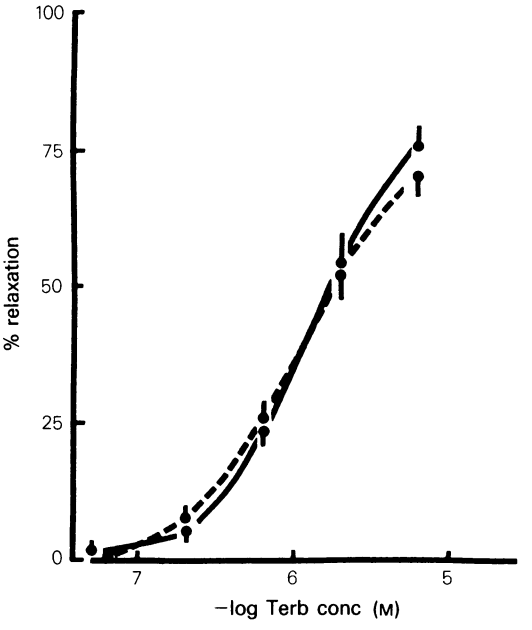

Figure 2 Cumulative concentration-response curves for terbutaline-induced relaxation of trachea taken from thyroxine-treated and paired control guinea-pigs. Conditions were similar to those of Figure 1 except that 5×10^{-6} M practolol was present to block β_1-adrenoceptors. Dashed line: thyroxine-treated group; solid line: paired control group. Each point represents the mean of 8 tissues, the vertical lines indicate s.e. mean.

Similarly, Table 1 shows that thyroxine had no effect on the apparent dissociation constants for butoxamine vs. terbutaline or practolol vs. norad-

Table 1 Lack of effect of thyroxine on parameters of β-adrenoceptor-mediated relaxation of guinea-pig trachea

	Noradrenaline[1]		*Terbutaline*[2]	
	Control	*T_4-treated*	*Control*	*T_4-treated*
pD_2[3]	6.12 ± 0.13 (8)	6.09 ± 0.12 (8)	5.77 ± 0.08 (8)	5.72 ± 0.09 (9)
K_B[4] Practolol	6.66 ± 0.62 (8)	7.33 ± 0.95 (8)	----------	----------
K_B[4] Butoxamine	----------	----------	9.59 ± 1.23 (8)	8.21 ± 0.89 (8)
Maximum[5]	48.3 ± 4.2 (7)	49.3 ± 2.2 (7)	19.9 ± 2.2 (8)	15.3 ± 1.9 (8)

Each value is the mean ± s.e. calculated from individual cumulative concentration-response curves. The number in parentheses is the number of animals.

[1] From noradrenaline CCRC in the presence of 4×10^{-6} M butoxamine.
[2] From terbutaline CCRC in the presence of 5×10^{-6} M practolol.
[3] Negative log of the molar concentration which produced half-maximal response.
[4] Apparent dissociation constant ($\times 10^7$ M) calculated from the equation: K_B = antagonist concentration/(dose ratio − 1), where the dose ratio is the concentration of agonist required to produce the same level of effect in the presence and absence of the given concentration of antagonist (5×10^{-6} M practolol and 4×10^{-6} M butoxamine).
[5] Estimated ceiling effect of the CCRC for respective agonist vs. 2.5×10^{-5} M carbachol.

renaline. Analysis of variance confirmed that the individual K_B values obtained in the paired tissues were not significantly different.

Finally, analysis of variance of the maxima of the individual paired CCRCs vs. a physiologically antagonistic concentration of carbachol showed no evidence for a significant difference between the maxima of tissues from paired animals, either to noradrenaline or terbutaline. However, the terbutaline CCRC constructed from the mean responses of the tissues from the control animals appears to approach its ceiling level at 6.2×10^{-5} M while the CCRC for the tissues from the thyroxine-treated animals reaches its maximum at 2.0×10^{-5} M (Figure 3). An analysis of variance of responses obtained at 6.2×10^{-5}, 2.0×10^{-4} and 3.4×10^{-4} M terbutaline in the tissues from the control animals led to acceptance of the null hypothesis that these data belonged to the same population. Similar results were obtained for the responses at 2.0×10^{-5}, 6.2×10^{-5} and 2.0×10^{-4} M terbutaline in the tissues from the thyroxine-treated animals. The responses to the above three doses of terbutaline obtained in the tissues from the control animals were thus pooled as were the respective responses obtained in the tissues from the thyroxine-treated animals. Analysis of variance of these pooled data showed that the ceiling level of the CCRC from the thyroxine-treated group was significantly different ($P < 0.05$) from that of the CCRC from the control group (Table 2).

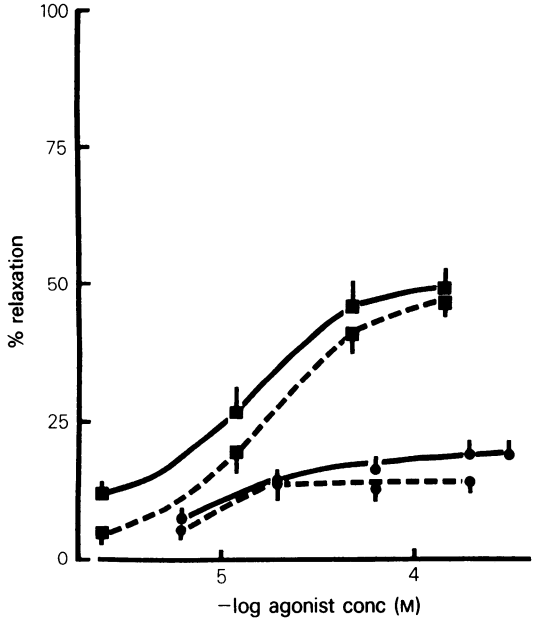

Figure 3 Cumulative concentration-response curves for noradrenaline- and terbutaline-induced relaxation of trachea taken from thyroxine-treated and paired control guinea-pigs under conditions of physiologic antagonism. Conditions were similar to Figures 1 and 2, except that tone was induced with a 100× greater concentration of carbachol. (■-----■), thyroxine-treated group, noradrenaline agonist ($n = 7$); (■———■), paired control group, noradrenaline agonist ($n = 7$); (●-----●), thyroxine-treated group, terbutaline agonist ($n = 8$); (●———●), paired control group, terbutaline agonist ($n = 8$).

Table 2 Analysis of variance and means ± s.e. of pooled responses to maximally effective concentrations of terbutaline

Source	*d.f.*	*Sum of squares*	*Mean square*	*F*
Among*	1	212.08	212.08	4.98[1]
Within	42	1790.38	42.63	
Total	43	2002.46		

*Mean ± s.e. (n)

Controls[2]	T_4-treated[3]
17.9 ± 1.2 (20)	13.5 ± 1.5 (24)

[1] $P < 0.05$.
[2] Calculated from responses to 6.2×10^{-5}, 2.0×10^{-4}, and 3.4×10^{-4} M terbutaline in tissues from paired control animals.
[3] Calculated from responses to 2.0×10^{-5}, 6.2 ± 10^{-5}, and 2.0×10^{-4} M terbutaline in tissues from thyroxine-treated animals.

Discussion

Previous studies have shown that excessive thyroid hormone increases the inotropic sensitivity of various *in vitro* heart preparations to β-adrenoceptor agents (Wildnethal, 1974; Hashimoto & Nakashima, 1978; Guarnieri, Filburn, Beard & Lakatta, 1980). Binding studies with broken cell preparations have shown an increased number of β-adrenoceptor binding sites (Ciaraldi & Marinetti, 1977; Tse *et al.* 1980). In adipose tissue and isolated fat cells results vary with respect to the effect of excessive thyroid hormone on adrenoceptor mediated lipolysis. Some studies have shown an increase in lipolysis while others have reported no effect (Debons & Schwartz, 1961; Goswami & Rosenberg, 1978; Malbon *et al.*, 1978). Differences in types of preparations may account for some of the discrepancies. There also appear to be differences in the effects of thyroid hormone on cyclic AMP accumulation between species; thyroid hormone increases β-adrenoceptor-mediated cyclic AMP accumulation in adipose tissue and adipocytes from rats, but decreases it in adipocytes from hamster (Fain, 1981). β-Adrenoceptor responses of human lymphocytes are unaffected by thyroid hormone (Williams *et al.*, 1979; Smith *et al.*, 1981).

The lack of significant effect of chronic exposure to

high doses of thyroxine on the concentration-response relationships of both noradrenaline and terbutaline indicates that thyroxine does not modify either β_1- or β_2-adrenoceptor-mediated relaxation of guinea-pig trachea. In light of the numerous reports of the potentiation of β_1-adrenoceptor-mediated responses in heart and adipose tissue caused by thyroid hormone, the lack of effect of thyroxine on β_1-adrenoceptor-mediated relaxation of guinea-pig trachea was somewhat unexpected. The apparent lack of effect of thyroxine on β_2-adrenoceptor-mediated relaxation agrees with the similar lack of effect on β_2-adrenoceptor-mediated cyclic AMP accumulation in human lymphocytes.

While potency is only indirectly related to the affinity of an agonist for its receptor, the lack of significant effect of thyroxine on the pD_2 values for noradrenaline and terbutaline implies that thyroxine did not change the affinities of the above selective agonists for their respective receptors. Neither did thyroxine significantly affect the affinities of the selective antagonists, practolol and butoxamine, for their respective receptor subtypes as measured by the apparent K_B values.

The lack of significant effect of thyroxine on the ceiling level of the noradrenaline CCRC under conditions of physiological antagonism suggests that it did not change the number of functional β_1-adrenoceptors. The ceiling level of the terbutaline CCRC from the trachea of thyroxine-treated guinea-pigs was slightly lower than that of the CCRC from the trachea of the control animals. This difference was statistically significant if the responses to all doses after the curves had plateaued were pooled, suggesting that thyroxine may have decreased the number of functional β_2-adrenoceptors or decreased the response to receptor activation. However, the magnitude of this difference was small and, although the maxima of the terbutaline CCRCs under conditions of minimal carbachol tone were not determined, it is doubtful that such a change is of any practical significance.

The above finding raises the obvious point that failure to show a statistically significant difference and the resulting acceptance of the null hypothesis that the data are samples drawn from a common population does not prove nonexistence of an effect. Even though of low probability, the possibility remains that the null hypothesis is false but that the test is not sensitive enough to determine this.

One measure of the sensitivity of the tests used in the present study is the 'least significant difference' for *a priori* planned comparisons which can be calculated for each parameter from the standard errors and numbers of animals given in Table 1 (Sokal & Rohlf, 1979). Given the variability of the data presented, a difference between the mean pD_2 values of the treated and control groups of at least 0.38 for noradrenaline and 0.25 for terbutaline would have been required to reject the null hypothesis ($P < 0.05$). The data presented in several of the studies cited above which showed significant effects of excessive thyroxine on various β-adrenoceptor-mediated responses reported potency differences between treated and control groups greater than either of the above values. For example, the reported magnitudes of the thyroxine-induced increase in sensitivity to the inotropic and chronotropic effects of isoprenaline in rat isolated atria and to its inotropic effects in rabbit isolated papillary muscle as measured by the difference in pD_2 values calculated from the data presented were 0.86, 0.90 and 0.43, respectively (Hashimoto & Nakashima, 1978; Wildenthal, 1974). Adrenaline-induced accumulation of cyclic AMP by isolated fat cells and stimulation by isoprenaline of adenylate cyclase in rat heart homogenates were significantly increased by thyroxine (Malbon *et al.*, 1978; Tse *et al.*, 1980). The magnitudes of the pD_2 differences were 1.5 and 0.57, respectively.

Similarly, the least significant differences required between the mean ceiling responses of the tracheae from hyperthyroid relative to control guinea-pigs in the present study are 10 and 6 percentage units for noradrenaline and terbutaline, respectively. Tse and coworkers measured isoprenaline-induced isotonic contractile force development expressed as a percentage of initial force in rat isolated ventricular strips from hyperthyroid and control animals (Tse *et al.*, 1980). They found that the mean maximal force development of strips from hyperthyroid animals was 20 percentage units less than that of control animals. Guanieri and coworkers reported similar changes in perfused interventricular septa from hyperthyroid rats with respect to isoprenaline-induced mean maximal force development (71 percentage units less than the control group), rate of force development (92 percentage units less than the control group), and shortening of duration of contraction (20 percentage units less than the control group) (Guarnieri *et al.*, 1980).

Few studies have reported a significant change in binding characteristics of β-adrenoceptors caused by thyroxine, and those studies that have shown significant changes have involved binding of radiolabelled ligands in broken cell preparations. The author is unaware of any studies of the effects of excessive thyroxine on the binding characteristics of β-adrenoceptors using intact preparations as in the present study. However, it is generally accepted that a difference of one order of magnitude between apparent K_B values, as measured by the method used in the present study, is required to be meaningful (Furchgott, 1970). The least significant differences between the mean apparent dissociation constants of

the treated and control groups for practolol vs. noradrenaline and butoxamine vs. terbutaline are 2 and 3, respectively.

In conclusion, this study has shown no evidence of independent modulation of β_1- or β_2-adrenoceptor-mediated activity by thyroxine in a tissue possessing both β-adrenoceptor subtypes. From the above discussion, it can be reasonably concluded that the results of this investigation suggest that the interaction of thyroxine with β-adrenoceptor systems in other tissues is a target specific phenomenon and not a general effect on the β_1- or β_2-adrenoceptor systems.

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