Quantitative changes in β-adrenergic responses of isolated atria from hyper- and hypothyroid rats[1]

H. P. Rub, H. Thommen and H. Porzig

Pharmakologisches Institut der Universität, Friedbühlstrasse 49, CH-3010 Bern (Switzerland), 22 May 1980

Summary. Concentration-response curves for the chronotropic and inotropic effects of isoprenaline, in the absence and presence of propranolol, were obtained on heart atria isolated from normo- or dysthyroid rats. Hyperthyroidism increased the chronotropic potency and efficacy of the β-adrenergic agonist. The results are compatible with the view that thyroid hormone increases the density of functional β-adrenoceptors in cardiac pacemaker tissue.

Several recent reports have shown that thyroid hormone increases the density of specific β-adrenergic binding sites in cardiac muscle membranes by about 100%[2-4], whereas hypothyroidism decreases the number of binding sites by about one third[5]. Since the change in binding site density was not accompanied by altered binding constants, a single homogeneous population of sites seemed to prevail under all conditions. It has been suggested that clinical signs of changed myocardial reactivity to catecholamines in states of thyroid dysfunction[6,7] may be causally related to a hormone-induced alteration of β-adrenoceptor density.

On the other hand, some evidence suggests that the physiological response of mammalian cardiac tissue to β-adrenergic stimulation is not necessarily proportional to the occupation of specific β-adrenergic binding sites. In some species maximal positive chronotropic or inotropic responses were observed with catecholamine concentrations that would occupy only about 10% of the total number of high affinity binding sites detected in binding studies[8,9]. If thyroid hormones were to alter the number of cardiac β-adrenergic binding sites without changing concomitantly the density of functional β-adrenoceptors, then thyroid dysfunction would affect the apparent potency without affecting the efficacy of a β-adrenergic agonist[10,11]. By contrast, the K_D value for an antagonist estimated from a Schild plot[12] would remain unchanged. Several authors have indeed reported that increased or decreased potencies for catecholamines were associated with hyper- or hypothyroidism respectively[7,13-15]. However, the effects of thyroid status on the chronotropic and inotropic efficacy of β-adrenergic agonists are controversial and are not well documented. For example, an increase[16] or no change of efficacy[13] have both been reported. We have, therefore, studied the potency and efficacy of the β-agonist isoprenaline in the presence and absence of an antagonist in atria from hyperthyroid, normothyroid and hypothyroid rats. The results suggest that the density of functional β-adrenoceptors in rat cardiac pacemaker tissue is sensitive to thyroid hormones.

Materials and methods. Female rats of Wistar and SIV 50 strains were made hyperthyroid by daily injection of 250 µg/kg i.m. triiodothyronine (T_3) for 3 consecutive days. Atria were assayed on the 4th day. Hypothyroidism was induced by adding the thyrostatic compound thiamazole to the drinking water (40 mg/l). Simultaneously the rats were fed an iodine deficient diet (Altromin C 1042, Altrogge Spezialfutterwerke, Lage, FRG). The mean uptake of thiamazole was 3.7 mg/animal/day for 35 days. At the end of this period the mean plasma thyroxine levels had fallen from 36 to 12 nmole/l. The hypothyroid state was reversed by daily injections of 250 µ/kg T_3 for the last 3 days of a 35-day thiamazole regime. The animals were decapitated whilst under ether anaesthesia and the hearts were quickly removed. Left and right atria respectively were used to test the inotropic or chronotropic response. The muscles were mounted between 2 silver electrodes and connected to a force-displacement transducer (Grass FT 03). The resting tension was set to 0.5 g. The left atrium was stimulated with 2 Hz by 2-msec square wave pulses of twice threshold strength. Isometric contractile force was registered on a pen-recorder. The right atria contracted spontaneously. Beat frequencies were counted from recorder tracings. Tyrode's solution containing (mM) 137 NaCl, 11.9 $NaHCO_3$, 0.4 NaH_2PO_4, 5.4 KCl, 3.6 $CaCl_2$, 2.1 $MgCl_2$, and 5.5 D-glucose, bubbled with 5% CO_2/95% O_2 and kept at 35 °C, was used as the bathing medium. After an initial 45-min equilibration period we obtained cumulative concentration-response (C-R) curves for (±)isoprenaline in the presence and absence of (±)propranolol. The 10 mM isoprenaline stock solution contained 50 mM ascorbic acid as an antioxidant. The preparations were equilibrated for 60 min with each concentration of antagonist (0.05–1 µM) before a new isoprenaline C-R curve was determined. Graphical methods (Schild plot, Hill plot) and computerized non-linear least square fitting procedures[12] were both concomitantly used to obtain the following parameters: Hill coefficient, half maximally effective concentration of isoprenaline (EC 50), maximal effect of isoprenaline (E max), apparent dissociation constant (K_D) for propranolol. The results of graphical analysis did not differ significantly from computer calculated values. Student's t-test was used for the statistical analysis of the data.

Effects of thyroid state on the response of isolated spontaneously beating right atria of rats to β-adrenergic stimulation and inhibition

Condition	Maximal frequency (beats/min)		Basal frequency (beats/min)		EC50 (±)isoprenaline (nM)	K_D (±)propranolol (nM)
	No Propranolol	Propranolol[a]	No Propranolol	Propranolol[a]		
Control	433±9 (11)	428±11 (10)	260±12 (10)	227±7 (10)	4.51±1.05 (12)	7.6±1.45 (10)
T_3[b]	477±9* (7)	472±17* (4)	334±21* (9)	228 (2)	0.65±0.09* (3)	6.6±1.9 (5)
Thiamazole[c]	399±15 (10)	397±13 (10)	218±7* (10)	200±5 (10)	10.5±2.1* (10)	6.9±2.45 (8)
Thiamazole[c] + T_3[b]	469±8* (5)	474±11* (4)	316±31** (4)	226±4 (5)	3.73±1.53** (5)	5.6±1.16 (4)

[a] (±) Propranolol 5×10^{-8} M; [b] triiodothyronine 250 µg/kg daily i.m. for 3 days prior to the experiment; [c] mean dosage: 3.7 mg/day for 35 days; * significantly different from controls ($p < 0.05$); ** significantly different from atria of thiamazole-treated rats. Number of experiments in brackets.

Results and discussion. 1. Inotropic effects. In T_3-treated normal- or hypothyroid rats, the contractile force of left atria after maximal stimulation with isoprenaline (471 ± 40 mN/mg wet weight, $n = 5-6$) was significantly lower than in controls (572 ± 37, $n = 10$) and in atria from hypothyroid animals (591 ± 83, $n = 10$).

The K_D values for propranolol (7.8 ± 1.6 nM, $n = 10$) and the EC 50 values for isoprenaline (7.5 ± 0.8 nM, $N = 10$) remained essentially unchanged under all conditions. The Hill coefficient for the contractile response to isoprenaline was always significantly > 1 (1.36 ± 0.06, $n = 10$) irrespective of the thyroid hormone level.

These results cannot be accounted for by changes in β-adrenoceptor numbers and suggest that thyroid hormone-induced effects on myocardial contractility are not mediated by β-adrenergic mechanisms.

2. Chronotropic effects. Figure 1 compares the frequency response to isoprenaline of atria from thiamazole-treated, hypothyroid animals with the response of atria after T_3-induced reversal of hypothyroidism. After T_3 treatment we observed an increase in the maximal response and a shift of the C-R curves for isoprenaline to the left. Both effects were statistically significant (table). By contrast, the K_D value for propranolol, calculated from the antagonist-induced shift of the curves, remained constant. The Hill coefficient was close to 1 under all conditions (0.95 ± 0.035, $n = 10$). T_3 treatment of normal or hypothyroid rats caused a large increase in basal frequency of right atria which was suppressed by 50 nM propranolol (see table).

Stimulation of cardiac β-receptors by endogenous catecholamines in the hyperthyroid state, resulting in elevated basal frequencies (figure 1, table), can result in an overestimation of isoprenaline EC 50 values. Therefore, in figure 2 we have used non-normalized C-R curves for isoprenaline, all obtained in the presence of 50 nM propranolol, in order to compare the effects of T_3 on the potency and efficacy of isoprenaline with the thiamazole results without the complication of different resting frequencies. These curves were obtained in the same series of experiments as those of figure 1. For reference purposes the curve for thiamazole + T_3 in figure 2 (symbol ○) is identical with the corresponding curve (symbol ■) in figure 1. T_3 treatment decreased the mean EC 50 value for (±)isoprenaline from 47 ± 7.6 to 13.5 ± 3.4 nM and enhanced the maximal change in frequency that could be obtained with isoprenaline (table). Thiamazole, on the other hand, increased the EC 50

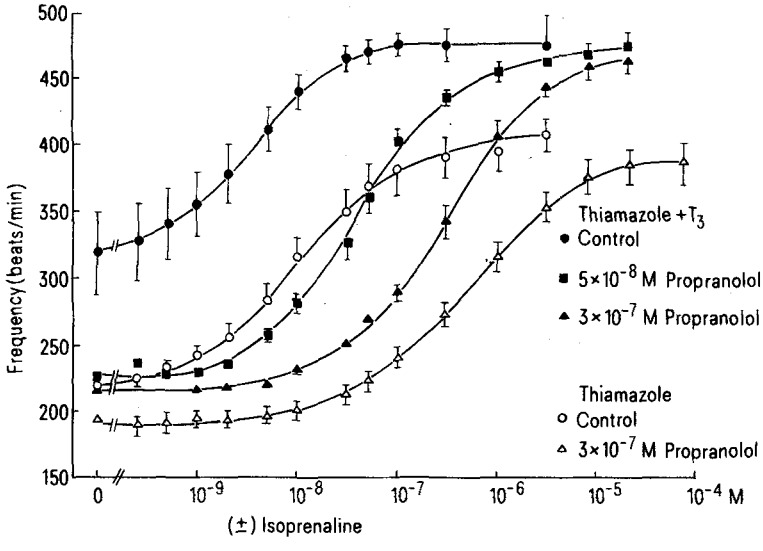

Fig. 1. Effect of thyroid state on the positive chronotropic effect of isoprenaline in isolated, spontaneously beating right atria from rats in the absence and presence of the β-adrenergic antagonist (±)propranolol. Open symbols (○, △): atria from thiamazole-treated hypothyroid animals ($n = 10$). Closed symbols (●, ■, ▲): atria from thiamazole-treated animals given in addition 250 µg/kg i.m. triiodothyronine (T_3) daily for 3 days prior to the experiment ($n = 5$). Bars give ± SEM. Note significant increase by T_3 of maximal frequency response to isoprenaline.

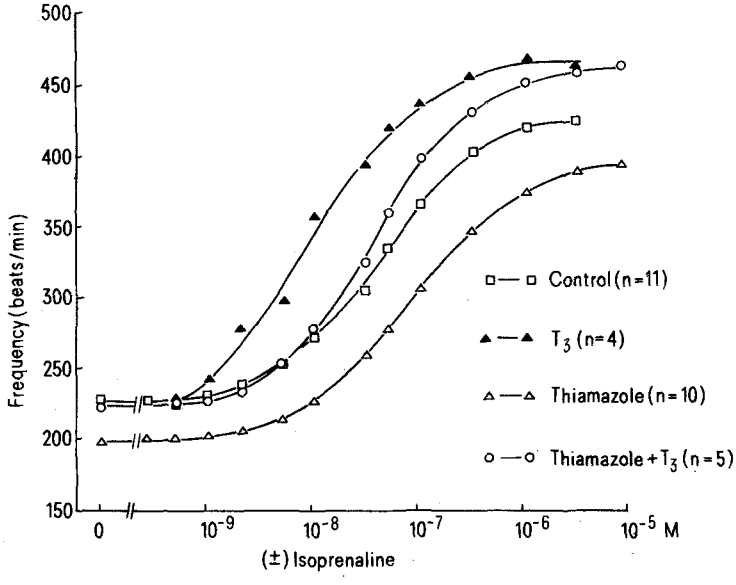

Fig. 2. Effect of thyroid state on concentration response curves for the positive chronotropic responses to isoprenaline. Spontaneously beating right atria from untreated rats (□) are compared to atria from animals treated with T_3 (▲), thiamazole (△) or thiamazole + T_3 (○). All curves were obtained in the presence of 50 nM propranolol in order to obtain similar basal frequencies. Note that the curve for thiamizole + T_3 (○) is identical to the corresponding curve (symbol ■) in Fig. 1. Experimental points represent mean values from 4–11 atria. Note the shift in EC 50 values associated with hormone stimulation.

value to 92 ± 16 nM. Compared to controls, the maximal frequency response was somewhat reduced but this effect was not statistically significant. A lack of effect of hypothyroidism on the efficacy of isoprenaline has also been reported for thyroidectomized rats[17]. Figure 2 also shows that the effects of thiamazole could be reversed when T_3 was injected daily for 3 days prior to the experiment.

The results of this study confirm that an increase in plasma T_3 level in previously normo- or hypothyroid animals enhances the potency of isoprenaline with respect to its chronotropic effect in isolated cardiac preparations. In addition, we have consistently observed a significantly higher efficacy. This observation is compatible with the view that the hormone treatment, at least in the rat, increases both the density of β-adrenergic bindings sites[2-4] and the number of functional receptors that are coupled to the physiological response. However, alternative explanations including the effects of T_3 at reaction steps beyond the receptor level cannot be excluded on the basis of our data. An inhibitory effect of T_3 on the extraneuronal uptake ($uptake_2$) of isoprenaline as a reason for the potency shift of this agonist is unlikely but cannot be excluded definitely. However, on the basis of known properties of $uptake_2$ in rat hearts[18] there is no way of explaining the increase in the chronotropic efficacy of isoprenaline by an inhibition of this uptake mechanism. Models of β-adrenoceptor function which assume that the number of functional receptors determines the rate at which the physiological response is approached rather than the absolute size of the response[19] are not applicable to our data without additional assumptions.

Clinical studies have suggested that the potency of isoprenaline is not consistently affected in hypo- or hyperthyroid patients[20]. If β-receptor density in humans is changed at all by thyroid hormone, then these findings could indicate that the discrepancy between the number of β-adrenergic binding sites and the density of functional receptors in humans is smaller than in the rat.

1 This study was supported by the Swiss National Science Foundation grant No. 3.374.-0.78. We thank Dr Bachmann from the Central Laboratory of the University Hospital Bern for the estimation of plasma thyroxine levels.
2 S. Kempson, G.V. Marinetti and A. Shaw, Biochim. biophys. Acta *540*, 320 (1978).
3 L.T. Williams, R.J. Lefkowitz, A.M. Watanabe, D.R. Hathaway and H.R. Besch, Jr, J. biol. Chem. *252*, 2787 (1977).
4 J.S. Tsai and A. Chen, Nature *275*, 138 (1978).
5 S.P. Banerjee and L.S. Kung, Eur. J. Pharmac. *43*, 207 (1977).
6 G.S. Levey, Am. J. Med. *50*, 413 (1971).
7 K. Wildenthal, J. Pharmac. exp. Ther. *190*, 272 (1974).
8 A.J. Kaumann, Naunyn-Schmiedebergs Arch. Pharmak. *305*, 97 (1978).
9 J.C. Venter, Molec. Pharmac. *16*, 429 (1979).
10 R.P. Stephenson, Br. J. Pharmac. *11*, 379 (1956).
11 L.T. Williams and R.J. Lefkowitz, in: Receptor Binding Studies in Adrenergic Pharmacology, p. 127. Raven Press, New York 1978.
12 D.R. Waud, in: Advances in General and Cellular Pharmacology, vol. I, p. 145. Ed. T. Narahashi and C.P. Bianchi. Plenum Press, New York 1976.
13 G. Kunos, Br. J. Pharmac. *59*, 177 (1977).
14 H. Hashimoto and M. Nakashima, Eur. J. Pharmac. *50*, 337 (1978).
15 H.J. Schümann, E. Borowski and G. Gross, Eur. J. Pharmac. *56*, 145 (1979).
16 P.A. Longhurst and J.H. McNeill, Proc. west. Pharmac. Soc. *22*, 415 (1979).
17 J.H. McNeill and W.W. Simpson, Proc. west. Pharmac. Soc. *22*, 407 (1979).
18 U. Trendelenburg, Life Sci. *22*, 1217 (1978).
19 A.M. Tolkovsky and A. Levitzki, Biochemistry *17*, 3795 (1978).
20 D.G. McDevitt, J.G. Riddell, D.R. Hadden and D.A.D. Montgomery, Br. J. clin. Pharmac. *6*, 297 (1978).

Effect of seasonal variation on the acute toxicity of cyclophosphamide in the Chinese hamster *(Cricetulus griseus)* and the mouse under laboratory conditions

C. Pericin[1]

Ciba-Geigy Ltd, Pharma Toxicological Laboratories, CH-4002 Basel (Switzerland), 11 April 1980

Summary. The acute toxicity of cyclophosphamide, studied orally in chinese hamsters and intravenously in Tif: MAGf (SPF) mice, showed seasonal variation in Chinese hamsters but not in mice.

In an internal biological test in chinese hamsters and mice tolerance variations were observed in response to a similar dose of cyclophosphamide (Endoxan-Astra®) when given at different periods of the year. The dependence of some drug effects upon several biological rhythms has been reported in experimental animals[2-4]. It was decided therefore to test the occurrence of possible seasonal variation in the acute toxicity of cyclophosphamide in Chinese hamster and mouse under consistent laboratory conditions.

Methods. 14 parallel acute toxicity studies were done in Chinese hamsters *(Cricetulus griseus)* obtained from Chick-Line, Vineland, N.J., USA) and in Tif: MAGf (SPF) mice within a period of 3 years.

The tests were performed at day 17 of each of the following months: January, May, July, September and November. The Chinese hamsters, which had an average weight of 28 g and the mice (23 g) were caged in Macrolon® boxes (5 animals/box/sex/species) in a room maintained at a constant temperature of 22 ± 1 °C and a relative humidity of about. 55% with a 14-h light cycle/day. Water and pellet food (No. 890 for mice and No. 924 for Chinese hamster, Nafag, Gossau SG, CH) were given ad libitum. The animals were fasted overnight before treatment.

Freshly prepared solutions of cyclophosphamide (20 mg/ml aqua dest.) were administered to the Chinese hamsters by gavage and to the mice by i.v. injection (duration: 10 sec) into the tail vein. Groups of 5 males and 5 females were used for each dose level. All administrations were given in the morning, beginning at 08.00 h. The amount of cyclophosphamide administered was 600, 1000, 1300, 1600, 2000 mg/kg b.wt for Chinese hamsters and 100, 200, 300, 400, 500, 600, 700 mg/kg b.wt for mice.

Acute toxicity determinations were based on deaths occurring during a 15-day period. LD_{50} values and confidence limits were calculated by the probit analysis method[5]. The data were subjected to analysis of covariance which compared the monthly means and tested whether there was a linear trend over the $2\frac{1}{2}$ year period[6].