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ABSENCE OF CHANGES IN DRUG DISPOSITION AND CATECHOLAMINE SENSITIVITY IN THE HYPERTHYROID DOG

TAKASHI ISHIZAKI & KAZUJI TAWARA*

Division of Clinical Pharmacology, Clinical Research Institute, National Medical Center Hospital, Tokyo, and *Section of Clinical Pharmacology, Department of Pharmacology, Jichi Medical College, Tochigi, Japan

1 In order to study the relative contribution of hepatic drug metabolizing enzymes and hepatic blood flow to the clearance of drugs in the hyperthyroid state, the disposition kinetics of two model compounds (antipyrine and propranolol) were examined in thyroid-fed dogs as compared to euthyroid and phenobarbitone-pretreated animals.

2 In hyperthyroid dogs, the possibility of catecholamine hypersensitivity was evaluated by assessing the chronotropic response to isoprenaline and by constructing a drug concentration-effect (β-blockade) relationship.

3 The plasma propranolol half-life (0.97 ± 0.12 h) of the hyperthyroid animals did not differ significantly from either the euthyroid group or the phenobarbitone-pretreated group. This was observed with no significant change in the apparent volume of distribution among the three experimental groups.

4 Phenobarbitone pretreatment accelerated significantly the elimination of antipyrine (half-life, 1.09 ± 0.15 h, $P < 0.01$) as compared to the euthyroid (2.84 ± 0.35 h) and the hyperthyroid groups (2.58 ± 0.13 h), respectively, without any changes in the apparent volume of distribution in any group.

5 Neither the chronotropic responses to exogenously administered catecholamine, nor the antagonist concentration-effect relationships support the concept that the hyperthyroid state potentiates sensitivity of the receptor-effect system of the heart.

6 The data obtained from the present study fit best with the view that thyroid hormone excess alters neither the disposition of the model compounds used nor the catecholamine-sensitivity examined.

Introduction

Studies in laboratory animals (Kato & Gillette, 1965; Kato, 1977; Rumbaugh, Kramer & Colby, 1978) have shown that thyroid hormones are among the many endocrine factors which influence the activity of hepatic microsomal drug-metabolizing enzymes. Furthermore, in a number of clinical investigations (Eichelbaum, Bodem, Gugler, Schneider-Deters & Dengler, 1974; Vesell, Shapiro, Passananti, Jorgensen & Shively, 1975; Saenger, Rifkind & New, 1976), hyperthyroidism has been shown to accelerate drug clearance whereas hypothyroidism increases the plasma half-life of drugs. In an extensive review concerning drug metabolism in thyroid disease (Eichelbaum, 1976) it was recently concluded that there are not sufficient data available to allow any general prediction as to how thyroid disease could alter drug metabolism.

Another matter of long-lasting controversy in the hyperthyroid state has been that the relationship between the sympathetic nervous system and thyroid hormones and the clinical features of hyperthyroidism have been attributed to the potentiation of catecholamines by thyroxine (Harrison, 1964). Adequately designed studies in both animals (Van der Shoot & Moran, 1965; Margolius & Gaffney, 1965; Cairoli & Crout, 1967) and man (Wilson, Theilen, Hege & Valenca, 1966; Aoki, Wilson, Theilen, Lukensmeyer & Leaverton, 1967) of catecholamine responsiveness in artificially induced hyperthyroidism have not demonstrated such hypersensitivity. However, all of these studies have failed to examine drug concentration-response relationships, which appear to be essential in drawing conclusions regarding such hypersensitivity.

We have studied, therefore, the kinetic disposition of two drugs, antipyrine and propranolol, both administered simultaneously to euthyroid, thyroid-fed and phenobarbitone-pretreated dogs. Chronotropic

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response to isoprenaline and β-blockade effectiveness were evaluated in relation to plasma concentration of the β-antagonist. These two drugs were chosen as model compounds for the present study because an estimate has already been obtained of the relative contributions of hepatic enzyme (microsomal mixed-function oxidases) activity and liver blood flow to the clearance of the two drugs, namely, that antipyrine exhibited a low hepatic extraction ratio (clearance dependent on the activity of drug-metabolizing enzymes) and propranolol exhibited a higher hepatic extraction ratio (clearance dependent on hepatic blood flow) (Branch, Shand, Wilkinson & Nies, 1974; Nies, Shand & Wilkinson, 1976).

Methods

Animal preparations

Twenty-three mongrel dogs of either sex with initial body weights ranging from 8.5 to 18.0 kg were randomly divided into three major experimental groups; eight control (euthyroid), eight hyperthyroid and seven phenobarbitone-treated dogs. To reduce the influence of variations due to age and environment of the dogs, the experiments were randomized as much as possible. Thus the experiments were scheduled so that three dogs of each of the three experimental groups were allocated as matched members and used alternately in each series. Hyperthyroidism was induced by including thyroid powder (0.8 g per kg of body weight per day) in the diet for 21 to 30 days. This regimen has been shown to produce the classical haemodynamic and metabolic manifestations of hyperthyroidism in dogs (Margolius & Gaffney, 1965). Heart rates in conscious animals, which were counted directly by palpation through the chest wall, and body weights were measured daily throughout the period of thyroid feeding. On the day of the experiment thyroid status was assessed by comparing the concentrations of thyroxine (T_4), protein-bound serum iodine (PBI), tri-iodothyronine (T_3) uptake of resin (T_3-uptake) and serum cholesterol in the thyroid-fed dogs with those of control dogs. These measurements were performed by a commercial medical laboratory (Sapporo Medical Laboratory Center, Sapporo). Other measurements to evaluate thyroid status included heart rates (conscious and anaesthetized), weight loss, systemic blood and pulse pressures, rectal temperature and clinical appearances.

The ability of phenobarbitone to increase the rate of elimination of a large number of drugs is generally attributed to an increase in the activity of the drug-metabolizing enzymes (Conney, 1969). According to the method of Cucinell, Koster, Conney & Burns (1963), phenobarbitone (16 mg per kg of body weight per day) was included in the diet for 28 to 42 days. This dose regime has been shown to cause a marked increase in the elimination rate of various drugs, including antipyrine in dogs (Cucinell *et al.*, 1963; Cucinell, Conney, Sansur & Burns, 1965; Conney, 1969).

Experimental procedures

Treatment with thyroid powder and phenobarbitone was stopped one day before the experiment. Dogs were anaesthetized with pentobarbitone sodium (30 mg/kg i.v.) and maintained at a light level of surgical anaesthesia. Additional doses of the anaesthetic were given when considered necessary. Ventilation via an endotracheal tube was artificially maintained with room air using a respirator (Natsume KN-50). All dogs were bilaterally vagotomized. Rectal temperatures, monitored throughout each experiment with a mercury thermometer, were measured and maintained with electric heating pads at the level observed at the outset of the experiments. Femoral arterial pressure was recorded with a transducer (Nihon Kohden MPU-0.5) and heart rate was measured by a cardiotachometer (San-ei Model 2140) driven by the R wave of a lead II electrocardiogram. All measurements were recorded continuously on an eight-channel ink-writing polygraph (San-ei Model 142-8). After the above preparations were completed, a 30 min rest period was allowed.

The control heart rate response to rapid (1 to 2 s) intravenous injection of isoprenaline at doses of 0.1 and 0.3 μg/kg through a catheter inserted into the femoral vein was recorded. These two intravenous doses of isoprenaline were chosen because previous studies (Kaplan & Commarato, 1973; Ishizaki & Tawara, 1979) have indicated that these dose levels can be used adequately to assess the β-blockade effect in anaesthetized vagotomized dogs. Following control observations on β-agonist challenges, intravenous doses of antipyrine (75 mg/kg) (Vesell, Lee, Passananti & Shiveley, 1973) and propranolol (0.3 mg/kg) (George, Orme, Buranapong, Macerlean, Breckenridge & Dollery, 1976) were simultaneously administered via the right and left femoral vein, respectively, and a period of 3 to 5 min was allowed at the end of the administration. The heart rate responses (beats/min, and percentage increases from the base heart rate) to the same doses of isoprenaline as in the control period were determined at 1, 2, 3 and 4 h after the β-antagonist was administered (Margolius & Gaffney, 1965; Van der Schoot & Moran, 1965; Cairoli & Crout, 1967).

For β-blockade assessment, both the absolute reduction (R) in beats/min of isoprenaline-induced tachycardia (Kaplan & Commarato, 1973) and the percentage reduction (%R) from the control (Black,

Duncan & Shanks, 1965; Dunlop & Shanks, 1969) were calculated at the same time schedule as the chronotropic response after the drug injection. The absolute reduction (R) is the difference between the isoprenaline-induced increase of the heart rate before and after the drug. The percentage reduction (%R) is the absolute reduction (R) expressed as a percentage of the control heart rate increase induced by isoprenaline (Ishizaki & Tawara, 1979).

Blood samples (7 ml each) for drug assay were withdrawn from each femoral vein, opposite to where the two drugs were simultaneously administered, at 0.5, 1, 2, 3, 4 and 5 h after completion of the injection. In order to compensate for blood loss throughout each experiment, the blood cell mass in the same volume of 0.9% w/v NaCl solution (saline) as that of whole blood taken for the drug assay was returned back to the dog after the plasma had been separated.

Plasma antipyrine concentrations were determined by the spectrophotometric method of Brodie, Axelrod, Soberman & Levy (1949). Propranolol was assayed by the method of Shand, Nuckoll & Oates (1970). The presence of propranolol in plasma did not interfere with the assay for antipyrine and vice versa as indicated by Greenblatt, Franke & Huffman (1978).

Pharmacokinetic and statistical analyses

Plasma elimination half-life of drugs ($T_{\frac{1}{2}}$) was calculated from the least-squares regression slope of the terminal (β-phase) log-linear plasma data points. The overall elimination rate constant (β) was calculated as $\beta = 0.693/T_{\frac{1}{2}}$. C_0, the concentration at zero time, was estimated by extrapolation of the β slope back until it crossed the ordinate (zero time). The apparent volume of distribution (Vd) was calculated from the relationship, Vd = dose (i.v.)/C_0. The area under the plasma concentration-time curves $[AUC]_0^\infty$ was calculated by the trapezoidal rule: $[AUC]_0^\infty = [AUC]_0^T + C_p^T/\beta$, where $[AUC]_0^T$ is the area under the curve from 0 to the last measured time of plasma concentrations of drugs and C_p^T is the last recorded plasma concentration of the experiments. The total plasma clearance (Cl_{tot}) was calculated from Cl_{tot} = dose (i.v.)/$[AUC]_0^\infty$.

The paired or unpaired Student's t test was used to test the probability that a significant difference existed between the means of the three experimental groups. The regression line was calculated by the least-squares analysis and tested for coefficient correlations. A P value less than 0.05 was considered statistically significant.

Drugs

Thyroid powder, U.S.P., phenobarbitone sodium, (±)-propranolol hydrochloride, antipyrine, isoprenaline hydrochloride and pentobarbitone sodium were obtained from commercial sources. Except for thyroid powder, phenobarbitone sodium and pentobarbitone, drugs were dissolved freshly in sterile saline at the required concentration; doses are expressed in terms of the base.

Results

Effects of thyroid or phenobarbitone treatment.

Daily feeding of thyroid powder for a period of 3 to 4 weeks made the dogs hyperactive and some of the animals also developed diarrhoea. The thyroid-fed dogs showed distinct increases in plasma T_4, T_3-uptake, PBI, conscious and anaesthetized heart rates and rectal temperature (Figure 1). However, the magnitude of the hyperthyroid state produced was variable among different dogs such that T_4 ranged from 3.6 to 25.0 μg/100 ml (control, 1.4 to 3.3 μg/100 ml), T_3-uptake from 49.6 to 63.4 percent (control, 45.7 to 55.5%) and PBI from 3.1 to 18.2 μg/100 ml (control, 0.9 to 2.0 μg/100 ml). The correlation coefficient values calculated for the thyroid function tests of the thyroid-fed dogs were: T_4 vs. PBI ($r = 0.996$, $P < 0.01$); T_4 vs. T_3-uptake ($r = 0.651$, $P < 0.05$) and T_3-uptake vs. PBI ($r = 0.653$, $P < 0.05$). In addition, the thyroid-fed animals lost weight (from 12.9 ± 1.0 kg at the allocation period to 10.8 ± 0.6 kg on the day of experiment, $P < 0.025$). In the other two groups, no significant change in body weight was seen. Although the serum cholesterol of the hyperthyroid group (87.4 ± 14.1 mg/100 ml) was less than that of the control group (100.4 ± 9.5 mg/100 ml), the value did not reach statistical significance ($P < 0.1$).

The phenobarbitone-treated dogs were lethargic and hypokinetic, particularly for the first week after the start of drug ingestion. Some dogs showed ataxic gait. However, these manifestations became gradually less marked and disappeared within 2 weeks. The total dose of pentobarbitone sodium required to maintain an appropriate level of anaesthesia throughout the experiment was 91.8 ± 9.8 mg/kg in the phenobarbitone-treated group, which was significantly greater ($P < 0.01$) than either the control (39.1 ± 9.1 mg/kg) or the hyperthyroid groups (47.9 ± 6.2 mg/kg).

The data for basal heart rate, systemic blood and pulse pressures and heart rate response to isoprenaline observed at the control period are summarized in Table 1. The basal heart rate of the control and phenobarbitone-treated dogs was significantly less than that of the hyperthyroid dogs (Table 1). On the other hand, the absolute (beats/min) and % increases in heart rate produced by isoprenaline in the hyperthyroid dogs were not greater than in the control dogs and the responsiveness was significantly blunted

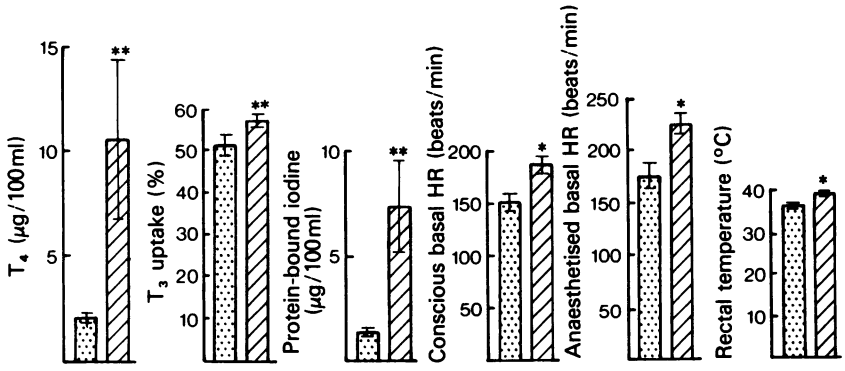

Figure 1 Effects of thyroid feeding on thyroid function tests, basal heart rates and rectal temperature in control (hatched columns) and hyperthyroid dogs (stippled columns); $n = 8$ for both groups. Vertical lines show s.e. mean. Significantly different from control: $^*P < 0.01$; $^{**}P < 0.025$. Thyroid function measurements were performed by a commercial medical laboratory (Sapporo Medical Laboratory Center, Sapporo, Japan). The normal ranges in human reported from this Laboratory are: 5.0 to 13.5 μg/100 ml for T_4; 26 to 36% for T_3 uptake; and 4.0 to 8.0 μg/100 ml for PBI.

in the hyperthyroid group as compared to the other two groups ($P < 0.05$ to 0.01, Table 1).

Disposition kinetics of model drugs

The average plasma concentration-time curves of antipyrine and propranolol in the three experimental groups of dogs are illustrated in Figure 2 and the pharmacokinetic parameters derived are given in Table 2. Since blood samples were not obtained earlier than 30 min after bolus dosing, a brief α or distribution phase may have been present. This was not investigated in this study because the objective of the study was to estimate the elimination kinetics of

Table 1 Heart rate, blood pressure, pulse pressure and heart rate response to isoprenaline observed at control period of three experimental groups of dogs*

	Control ($n = 8$)	*Hyperthyroid* ($n = 8$)	*Phenobarbitone-treated* ($n = 7$)
Basal heart rate (beats/min) conscious	153.3 ± 6.0	187.5 ± 8.6[a,b]	142.0 ± 5.2
anaesthetized	176.3 ± 11.8	227.1 ± 9.3[a,b]	148.0 ± 10.7
Systolic blood pressure (mmHg)	147.5 ± 11.7	164.4 ± 12.0	140.8 ± 13.8
Mean blood pressure (mmHg)	108.6 ± 3.8	110.6 ± 10.5	98.1 ± 3.4
Diastolic blood pressure (mmHg)	87.5 ± 7.9	83.7 ± 6.7	77.8 ± 6.9
Pulse pressure (mmHg)	60.7 ± 5.2	80.6 ± 7.1	65.0 ± 8.2
Heart rate response to isoprenaline			
beats/min increase in response to 0.1 μg/kg	69.0 ± 4.6	43.1 ± 5.2[a]	60.0 ± 6.5
% increase in response to 0.1 μg/kg	39.5 ± 2.4	19.7 ± 2.1[a,b]	41.4 ± 5.7
beats/min increase in response to 0.3 μg/kg	94.5 ± 5.1[d]	66.3 ± 6.9[a,c,d]	87.7 ± 5.8[d]
% increase in response to 0.3 μg/kg	54.4 ± 6.0[e]	30.0 ± 4.0[a,b,e]	60.3 ± 7.5

* All values are mean ± s.e. Numbers in parentheses indicate the number of animals. Haemodynamic values except for conscious basal heart rate were obtained at the outset of experiments under anaesthetic.
[a] Significantly different from control group, $P < 0.01$.
[b] Significantly different from phenobarbitone-treated group, $P < 0.01$.
[c] Significantly different from phenobarbitone-treated group, $P < 0.05$.
[d] Significantly different from a challenging dose of isoprenaline 0.1 μg/kg, $P < 0.025$.
[e] Significantly different from a challenging dose of isoprenaline 0.1 μg/kg, $P < 0.05$.

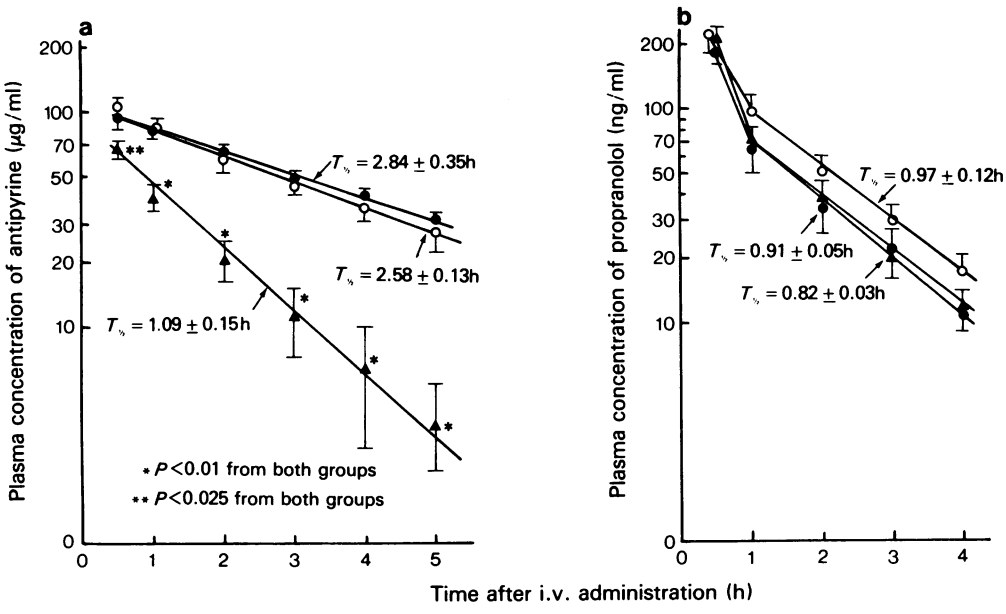

Figure 2 Average plasma concentration-time curves of antipyrine (a) and propranolol (b) in three experimental groups of dogs: (●) controls (n = 8); (○) hyperthyroid group (n = 8); (▲) phenobarbitone-treated group (n = 7). The data are shown as mean values; vertical lines show s.e. mean. In (a) $T_{\frac{1}{2}}$ of phenobarbitone-treated group was significantly shorter ($P < 0.01$) than that of other two groups.

two drugs and a one-compartment kinetic model would therefore be considered adequate to describe the elimination of both drugs. As seen in Figure 2, the plasma levels of propranolol observed at 30 min after the bolus injection did not fit the log-linear terminal plots of the concentration-time decay in some dogs. Except for those cases, the pharmacokinetic values given in Table 2 were calculated from all measured linear log concentration-time plots.

There was no significant difference between the pharmacokinetic parameters of both drugs in the hyperthyroid and control dogs, and with propranolol, there was no significant difference among all three experimental groups. However, the values of β and $[AUC]_0^\infty$ for antipyrine obtained from the phenobarbitone-treated group were significantly different ($P < 0.01$) from those of the other two groups, and these differences were observed without any change in the Vd among the three experimental groups. Thus, the same trends were found for the total plasma clearance (Cl_{tot}) of the two test compounds, and the mean respective values of Cl_{tot} for propranolol and antipyrine were; 30.0 ± 4.9 and 3.36 ± 0.35 ml min^{-1} kg^{-1} in the euthyroid (control) group; 20.5 ± 1.6 and 3.75 ± 0.29 ml min^{-1} kg^{-1} in the hyperthyroid group and 26.7 ± 4.6 and 6.69 ± 0.89 ml min^{-1} kg^{-1} ($P < 0.01$ from the other two groups) in the phenobarbitone-treated group. Although we analyzed all probabilities of the relationships between thyroid function tests and pharmacokinetic data of the two drugs examined in the thyroid-fed dogs, no significant correlation was found. Neither was there a significant correlation between each pharmacokinetic parameter of the two drugs in any of the experimental groups.

Time-course of chronotropic response to isoprenaline

The whole course of the basal heart rate and heart rate response to isoprenaline expressed as both beats/min and % changes observed during the experiments is illustrated in Figure 3. The basal heart rates of the hyperthyroid group before and after propranolol were always significantly greater ($P < 0.01$) than those of other two groups. Although the absolute increase in heart rates in response to two doses of isoprenaline was not significantly different between the three groups after the intravenous administration of propranolol, the % increase in the hyperthyroid dogs was less than that in the control or phenobarbitone-treated animals at certain time points. This difference in the hyperthyroid dogs was considered due to the

Table 2 Kinetic parameters for antipyrine and propranolol estimated in three experimental groups of dogs

Drugs	*Parameters*		*Units*	*Control* ($n = 8$)	*Hyperthyroid* ($n = 8$)	*Phenobarbitone-treated* ($n = 7$)
Antipyrine	Elimination rate constants	(β)	h^{-1}	0.267 ± 0.029	0.274 ± 0.025	0.699 ± 0.072[a]
	Area under the curve from zero to infinity of β-phase	($[AUC]_0^\infty$)	μg/ml·h	416.7 ± 46.6	385.5 ± 40.3	148.5 ± 13.6[a]
	Apparent volume of distribution	(Vd)	l/kg	0.76 ± 0.05	0.79 ± 0.04	0.75 ± 0.08
Propranolol	Elimination rate constants	(β)	h^{-1}	0.765 ± 0.043	0.718 ± 0.072	0.842 ± 0.027
	Area under the curve from zero to infinity of β-phase	($[AUC]_0^\infty$)	μg/ml·h	197.8 ± 30.0	290.1 ± 43.3	220.0 ± 33.5
	Apparent volume of distribution	(Vd)	l/kg	1.71 ± 0.16	1.55 ± 0.34	1.81 ± 0.21

All values are mean ± s.e. Numbers in parentheses indicate the number of animals.
[a] Significantly different from both control and hyperthyroid groups, $P < 0.01$.

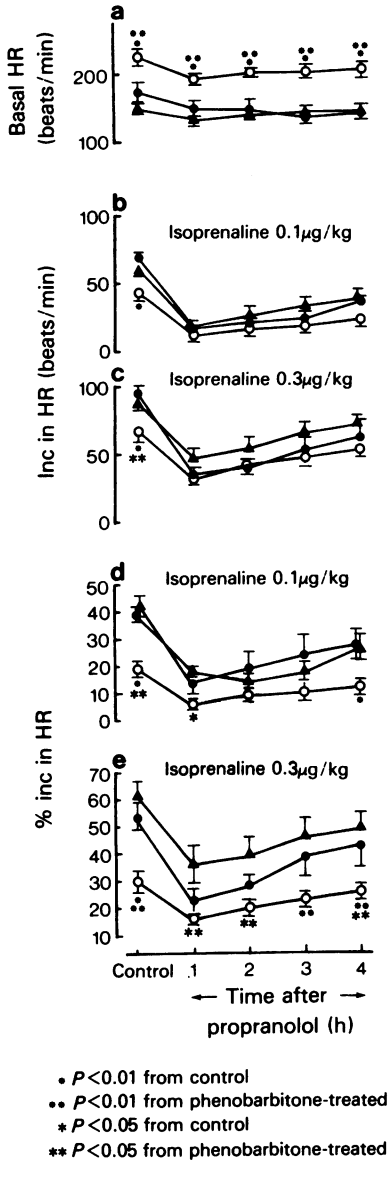

Figure 3 Basal heart rate (HR) in (a) and chronotropic responses to isoprenaline ((b) and (d) 0.1 μg/kg; (c) and (e) 0.3 μg/kg) measured as absolute increases (beats/min) in (b) and (c) and percentage (%) increases in heart rate in (d) and (e) in three experimental groups of dogs before and after propranolol: (●) controls; (○) hyperthyroid group; (▲) phenobarbitone-treated group. $*P < 0.01$ compared with control; $**P < 0.01$ compared with phenobarbitone-treated. $*P < 0.05$ compared with control; $**P < 0.05$ compared with phenobarbitone-treated.

markedly elevated basal heart rates in these dogs (Figure 3a).

Concentration-effect (β-blockade) relationship

We studied the relationship between the logarithm of plasma propranolol concentration (log C) and the β-blockade effects (beats/min reduction, R, or % reduction, %R) observed in each dog of the three experimental groups. The plot of effect (E) vs. log C may be represented by the equation: $E = m.\log C + r$, where m is the slope of this relationship and is generally linear over the range of 20 to 80% of maximum response and r is a constant (Levy, 1966). Figure 4 gives an example of the concentration-effect relationships (concentration-response curves) obtained from each dog of the control and hyperthyroid groups. The beats/min reduction (R) and % reduction (%R) in response to isoprenaline (0.1 μg/kg) as the β-blockade assessment we used are shown. These concentration-response curves were calculated by the least-squares regression analysis. When one applies the equation, $E = m.\log C + r$, for the concentration-effect relationship in each dog as cited in Figure 4 and substitutes a given concentration on the X-axis for log C in this equation, a given β-blockade effect can be estimated on the Y-axis. We estimated the β-blockade effect corresponding to a given level of propranolol (50 ng/ml) in plasma which was approximately at the midpoint concentration observed for the majority of the concentration-effect relationships (Figure 4) and the summarized data obtained from all groups are given in Table 3. It was noted that a given degree of β-blockade estimated at a given plasma propranolol level of 50 ng/ml was less ($P < 0.05$ to 0.01) in the hyperthyroid and phenobarbitone-treated groups than in the control group. We also analyzed the data of the slopes (m-values) of the concentration-effect relationships, the ranges of the β-blockade effects estimated at a plasma propranolol concentration of 50 ng/ml and the variation calculated by the ratio of maximum to minimum β-blockade effects which were observed within the three experimental groups. Although the m-values were variable to a certain extent among different dogs in all groups, the mean values of hyperthyroid animals tended to be larger (a steeper slope of concentration-response curves) than those of the other two groups, but did not reach statistical significance. In addition, there was a trend towards the observation suggesting that there was more variation in β-adrenoceptor affinity for the antagonist among different animals observed within the hyperthyroid group, since the ratio of maximum to minimum range of a given β-blockade effect estimated at a given concentration of propranolol (50 ng/ml) in plasma was found to be larger than that of the other two groups of dogs (Table 3).

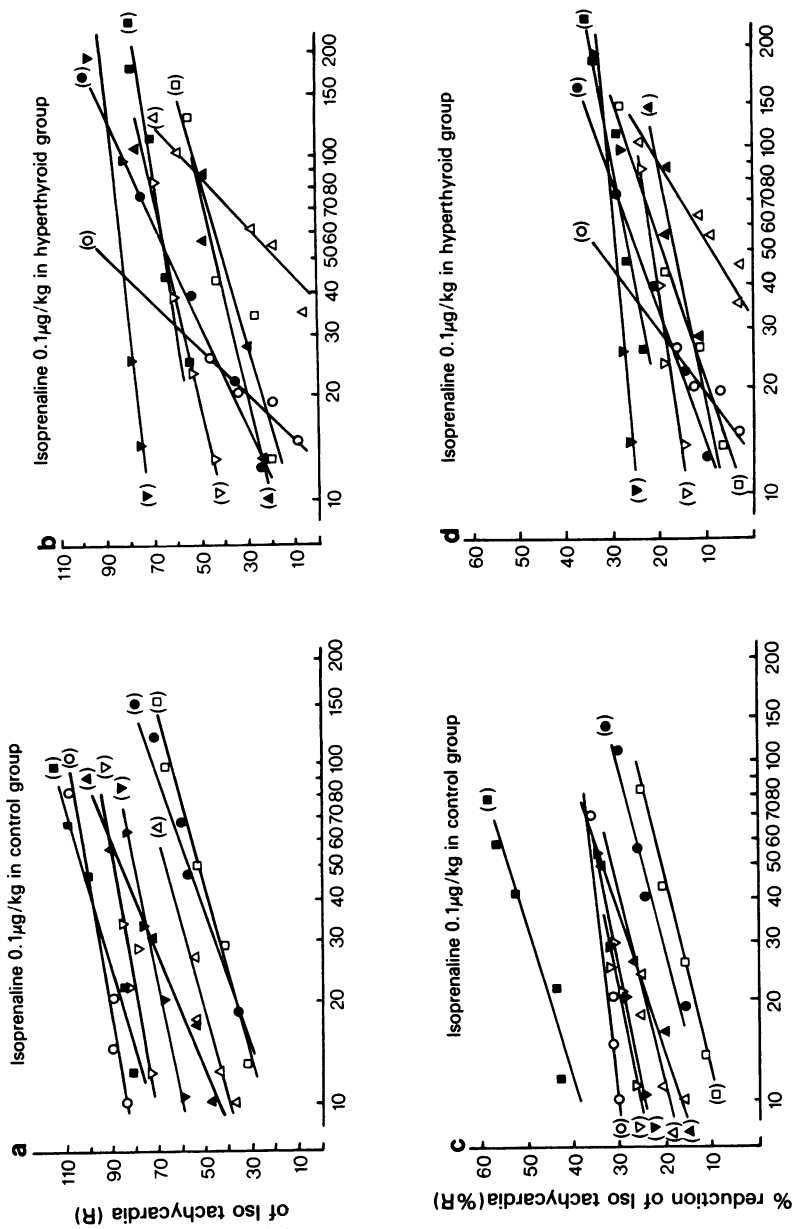

Figure 4 Concentration-effect relationships (concentration-response curves) in response to isoprenaline 0.1 μg/kg in each of the control (a and c) and hyperthyroid dogs (b and d). Each symbol indicates the same dog in each group. The effects (E) of β-blockade are expressed as absolute reduction (beats/min) (R) in (a) and (b) and percentage reduction (%R) in (c) and (d) of heart rate. The slope (m-value) of the concentration-effect relationship can be calculated from the equation, $E = m.\log C + r$ (Lévy, 1966) and a given β-blockade associated with a propranolol concentration of 50 ng/ml in plasma was estimated from individual concentration-response curves. The summarized data for these values in each group are given in Table 3.

Table 3 Summarized data of the slope (m-value) of concentration-effect relationship, β-blockade effect estimated at a plasma propranolol concentration of 50 ng/ml in response to isoprenaline and its variation in three experimental groups of dogs

Measurements	*β-blockade assessment*	*Control*	*Hyperthyroid*	*Phenobarbitone-treated*
m*	R in response to isoprenaline 0.1 μg/kg	36.1 ± 3.7	60.7 ± 17.9	41.6 ± 7.2
	%R in response to isoprenaline 0.1 μg/kg	14.4 ± 1.7	22.7 ± 6.6	21.7 ± 3.8
	R in response to isoprenaline 0.3 μg/kg	44.0 ± 11.2	56.1 ± 17.0	38.6 ± 5.6
	%R in response to isoprenaline 0.3 μg/kg	22.4 ± 8.8	19.5 ± 5.6	17.3 ± 2.8
β-Blockade estimated at a propranolol concentration of 50 ng/ml*	R in response to isoprenaline 0.1 μg/kg	76.6 ± 7.4 (53–102)	60.7 ± 17.9 (18–91)	51.7 ± 3.9[b] (38–66)
	%R in response to isoprenaline 0.1 μg/kg	32.4 ± 3.5 (20–35)	23.3 ± 2.9[c] (7–32)	24.4 ± 0.7[c] (20–26)
	R in response to isoprenaline 0.3 μg/kg	83.2 ± 10.3 (35–118)	50.8 ± 7.4[c] (23–80)	45.9 ± 5.7[a] (28–63)
	%R in response to isoprenaline 0.3 μg/kg	34.6 ± 5.1 (15–42)	17.4 ± 2.6[a] (8–26)	19.7 ± 2.5[c] (11–27)
Variation in β-blockade calculated as maximum/minimum ratio within group	R in response to isoprenaline 0.1 μg/kg	1.9	5.1	1.7
	%R in response to isoprenaline 0.1 μg/kg	1.8	4.6	1.3
	R in response to isoprenaline 0.3 μg/kg	3.4	3.5	2.3
	%R in response to isoprenaline 0.3 μg/kg	2.8	3.3	2.5

* Calculated from the concentration-effect relationship obtained in each dog as cited in Figure 4 and the values of mean ± s.e. are given. Values in parentheses indicate the range (minimum to maximum) estimated from concentration-effect relationship of each dog within the same group.
[a] Significantly different from control group, $P < 0.01$.
[b] Significantly different from control group, $P < 0.025$.
[c] Significantly different from control group, $P < 0.05$.

Discussion

Drug disposition in the hyperthyroid stage

Having a high intrinsic clearance, the hepatic clearance of propranolol has been shown to be dependent on liver blood flow, whereas antipyrine has a low intrinsic clearance and its hepatic clearance is independent of liver blood flow and reflects only the activity of the drug-metabolizing enzymes involved (Branch *et al.*, 1974; Nies *et al.*, 1976). If thyroid hormone excess could influence the mechanisms of drug elimination processes in the liver, it could be assumed that any change(s) in the elimination parameters of drug disposition would emerge. In the present study, no significant changes in the pharmacokinetics of the two model compounds were observed, despite the fact that our thyroid-fed dogs exhibited clear-cut evidence of hyperthyroidism (Figure 1). On the other hand, our results clearly indicate that the elimination of antipyrine which is known to be due to the hepatic mixed-function oxidases (Branch *et al.*, 1974; Conney, 1969; Kato, 1977), is accelerated by phenobarbitone treatment.

The phenobarbitone-related changes in antipyrine kinetics observed are consistent with those obtained from animal studies (Cucinell *et al.*, 1965; Branch *et al.*, 1974). Since the phenobarbitone dosing regimens used in our experiments have been shown to induce the metabolism of various drugs including antipyrine in dogs (Cucinell *et al.*, 1963; 1965) and the phenobarbitone-treated group required a higher dose of pentobarbitone to maintain an adequate level of anaesthesia throughout the experiments, we assumed that this alteration is due to the induction of hepatic microsomal mixed-function oxidases. In previous studies (Cooper & Brodie, 1954; Kato & Chiesara, 1962), pentobarbitone has been chosen as the model compound for testing hepatic drug-metabolizing capacity since its duration of action is primarily dependent upon the rate of its hepatic metabolism. More recently, Means, Schnell, Miya & Bousquet (1978) have clearly shown that the plasma level decline of pentobarbitone is the index of hepatic drug metabolism which best reflects the modification of responses to pentobarbitone following phenobarbitone pretreatment of the rat.

The observations that the artificially induced hyperthyroid state in dogs did not influence the antipyrine kinetics are inconsistent with observations with the same drug in spontaneously occurring thyrotoxicosis in man (Eichelbaum *et al.*, 1974; Vesell *et al.*, 1975; Saenger *et al.*, 1976), but not with those reported for dipyrone (Brunk, Combs, Miller, Delle & Wilson, 1974). Although the reason(s) for this difference cannot be given with certainty, possible explanations may exist in the difference of antipyrine metabolism among different species, the differing effect(s) of endogenously elevated and exogenously administered thyroid hormones or of the different magnitudes of thyrotoxicosis spontaneously occurring and artificially produced on the hepatic mixed-function oxidases. Previous experiments have suggested that species differences in antipyrine metabolism between laboratory animals and man exist (Vesell & Page, 1969; Vesell *et al.*, 1973; Branch *et al.*, 1974; Statland, Astrup, Black & Oxholm, 1973). In the dog, Vesell *et al.* (1973) found a good inverse correlation between antipyrine $T_{\frac{1}{2}}$ and the activity of hepatic microsomal aniline hydroxylase and ethylmorphine *N*-demethylase whereas in man Davies, Thorgeirsson, Breckenridge & Orme (1973) were unable to demonstrate a correlation between antipyrine clearance and the rate of *N*-demethylation of the ethylmorphine *in vitro*. Furthermore, *in vivo* and *in vitro* studies in laboratory animals suggest that thyroid hormone may inhibit the *N*-demethylation of aminopyrine (Kato & Gillette, 1965; Kato, 1977). Brunk *et al.* (1974) found no change in dipyrone metabolism when liothyronine was given to eight normal subjects and observed that in the hyperthyroid subject, plasma dipyrone levels were normal and the urinary excretion of 4-aminoantipyrine was decreased. These findings suggest that an excess of thyroid hormone may decrease the *N*-demethylation of dipyrone in the liver. Assuming that the predominating pathway of antipyrine metabolism in the dog liver exists in its *N*-demethylation, and that the exogenously administered thyroid hormone does not significantly alter it, then no change in antipyrine kinetics would emerge. However, this remains speculative since the metabolic pathway of antipyrine has not been investigated in the dog.

Rumbaugh *et al.* (1978) have demonstrated that administration of small amounts of T_4 (2.4 to 5 μg/100 g body weight, daily) to hypophysectomized rats increased the hepatic mixed-function oxidases and larger amounts of T_4 (12.5 to 50 μg) reversed the stimulatory effects of the smaller doses. These results indicate that physiological amounts of T_4 uniformly stimulate hepatic drug metabolism while supraphysiological amounts inhibit the metabolism of some substrates as suggested by other investigators (Kato, Tanaka & Takahashi, 1970). The thyroid-fed dogs in our study did show a rather high and variable degree of thyrotoxicosis. Furthermore, there was no significant correlation between the thyroid function tests examined and the pharmacokinetic parameters for antipyrine while there was a significant correlation ($P < 0.05$ to $P < 0.01$) among the various thyroid function tests. Although an exact explanation cannot be offered for our observations obtained from the dog, the possibility exists that thyroid hormone excess could affect the drug-metabolizing enzyme system in different ways in different species. Therefore, it is

likely that the finding that the hyperthyroid state in adult man accelerates the metabolic clearance of antipyrine (Eichelbaum *et al.*, 1974; Vesell *et al.*, 1975) may reflect the degree of thyrotoxicosis and the extent to which the resulting amounts of thyroxine can stimulate the drug-metabolizing enzyme(s) in the liver.

The disposition data of propranolol obtained from our dog experiments appear to be in agreement with the recent observations of hyperthyroid patients by Bell, Russel, Nelson, Kelly & McDevitt (1977) and Rubenfeld, Silverman, Welch, Mallette & Kohler (1979). An increase in hepatic blood flow in the hyperthyroid state should, in theory, contribute to the changes in propranolol clearance (Branch *et al.*, 1974; Nies *et al.*, 1976), but seems unlikely to account for it, since the only available information in the literature (Myers, Brannon & Holland, 1950) has shown that the hepatic blood flow in human hyperthyroidism is normal or only slightly increased.

Chronotropic response and concentration-effect relationship in hyperthyroid dogs.

Our data in hyperthyroid dogs, when viewed collectively, do not support the concept that the hyperthyroid state potentiates sensitivity of the receptor-effect system of the heart to exogenously-administered catecholamines. This finding is in agreement with the conclusions of similar investigations of artificially-induced hyperthyroidism in dogs (Van der Shoot & Moran, 1965; Margolius & Gaffney, 1965), rats (Cairoli & Crout, 1967) and man (Wilson *et al.*, 1966; Aoki *et al.*, 1967; McDevitt, Riddell, Hadden & Montgomery, 1978). The evidence obtained in our experiments would fit with the view that the intrinsic rate of the sino-atrial node in the hyperthyroid dogs is fundamentally higher than that of the euthyroid dogs since the basal heart rates before and after administration of the β-antagonist in the thyroid-fed animals were always significantly greater ($P < 0.01$). Propranolol did lower the basal heart rate of the thyroid-treated dogs and this effect of propranolol was not qualitatively different from that seen in the euthyroid animals (Figure 3a). These results imply that the intrinsic rate of the cardiac pacemaker in thyroid-treated animals is set not only in part by the adrenergic component but also largely by a direct action of the thyroid hormone (thyroxine) on pacemaker cells, as indicated by Cairoli & Crout (1967) and concluded by Levey (1971).

In terms of the antagonist concentration-effect relationships, the results of our studies in hyperthyroid dogs suggest that the effectiveness of β-blockers is unaltered or is rather blunted as compared to the control animals (Figure 4 and Table 3). Recent reports measuring a number of parameters of sympathetic nerve function (Stoffer, Jiang, Gorman & Pikler, 1973; Nishizawa, Hamada, Fujii, Morii, Okuda & Wada, 1974; Noth & Spaulding, 1974) indicate that, in general, the amount of catecholamines discharged is *decreased* in hyperthyroidism. Thus, the rather reduced β-blockade of our hyperthyroid dogs seems to be compatible with the above concept. Although the slopes (m-values) of the concentration-response curves and a given effect associated with an extrapolated concentration of propranolol (50 ng/ml) in plasma varied among each of the different dogs in different experimental groups, there was a trend towards more variability in the hyperthyroid group than in other groups (Table 3). In this respect, McDevitt, Riddell, Hadden & Montgomery (1978) have recently demonstrated that the dose-response curves of heart rate responsiveness to increasing doses of intravenous isoprenaline are variable (hyposensitivity, no change or hypersensitivity) in their patients with spontaneous hyperthyroidism, and that the slopes of the dose-response curves varied with each patient, which appears to be consistent with our observations in hyperthyroid dogs.

The results obtained from the phenobarbitone-treated animals indicate that the β-blockade effects assessed by both absolute heart rate (R) and percentage heart rate reduction (%R), like the hyperthyroid group, was less than those of the control group (Table 3). Although the exact explanation cannot be offered for these phenomena, we are tempted to assume that these observations are due to some aspects of the pharmacological effects of barbiturates on the circulatory system (Maynert, 1965). These include: (1) barbiturates can produce myocardial depressant actions; (2) barbiturates do not sensitize the heart to adrenaline and (3) cardiac irregularities produced by cyclopropane can be prevented by prior administration of barbitone or amylobarbitone. All these actions could be relevant to a rather reduced β-blockade observed in this experimental group. However, such an explanation remains speculative and whether and to what extent, if any, phenobarbitone can affect the adrenoceptor sensitivity awaits further investigation.

In summary, our data described here may have some clinical implications although obtained from animal experiments. Firstly, it seems reasonable to assume that the propranolol concentration required for adrenoceptor blockade in patients with hyperthyroidism is similar to that needed in those without this disease or in the euthyroid state, although the propranolol levels that will result in an adequate therapeutic response in hyperthyroid patients are not known with certainty. Our results, however, do not exclude the possibility that in man the disposition of propranolol is altered by thyrotoxicosis and there may be thyroid-status-related changes in catecholamine responsive-

ness as well, even though one may adduce fairly compelling evidence to suggest that man resembles the dog in these matters. Secondly, the drugs inhibiting thyroid hormone(s) synthesis will remain the first choice for treating tachycardia of hyperthyroidism with the adjunct therapy of a β-antagonist. Thirdly, since the phenobarbitone treatment did not distort the elimination kinetics of propranolol, a drug interaction between barbiturate and propranolol seems unlikely. Finally, since the available data so far do not allow a general statement about drug disposition and since various pathways and substrates are apparently affected in different ways by thyroid dysfunction (Eichelbaum, 1976; Kato, 1977), each drug must be studied individually in thyroid disorders. Furthermore, the disposition data of certain drugs obtained from artificially-induced hyperthyroidism of an animal species as in this study may not be directly applicable to those in the spontaneously occurring human hyperthyroid state.

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