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PLASMA PROTEIN BINDING OF PROPRANOLOL AND ISOPRENALINE IN HYPERTHYROIDISM AND HYPOTHYROIDISM

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1 The possibility that thyroid disease might result in alterations in the plasma protein binding of drugs has been investigated by studying the binding of propranolol and isoprenaline in patients with hyperthyroidism and hypothyroidism.

2 Plasma protein binding of propranolol and isoprenaline has been measured in seven hyperthyroid patients and ten hypothyroid patients. Plasma binding was estimated by equilibrium dialysis at 37°C using tritiated propranolol and isoprenaline, both when the patients had thyroid dysfunction and again when they were euthyroid.

3 In the hyperthyroid group, mean propranolol binding varied from $86 \pm 1.7\%$ when hyperthyroid to $88.4 \pm 0.8\%$ when euthyroid. The comparable isoprenaline figures were $65.1 \pm 3.2\%$ and $68.1 \pm 1.4\%$ respectively. Neither difference was significant.

4 Isoprenaline binding was significantly lower ($64.3 \pm 1.6\%$) when patients were hypothyroid than when they became euthyroid ($68.8 \pm 1.2\%$). Propranolol binding was not altered by hypothyroidism.

5 It is concluded that clinically important alterations in free drug concentrations of propranolol or isoprenaline do not occur in hyperthyroidism or hypothyroidism.

Introduction

Drug-protein binding can be altered by disease processes such as renal and hepatic disease (Reidenberg & Affine, 1973). The effect of thyroid disease on plasma protein binding of drugs has, however, not been investigated.

Such changes could be of clinical importance, particularly for β-adrenoceptor antagonists which, are now widely used in the management of hyperthyroidism (McDevitt, 1977). Propranolol is highly bound to plasma proteins and the degree of this binding varies little among normal subjects (Evans & Shand, 1973), so that small alterations in propranolol binding could result in large changes in free drug concentration. Recently plasma protein binding of propranolol has been shown to be affected by renal failure, Crohn's disease, inflammatory arthritis and cirrhosis (Borgå, Ödar-Cederlof, Piafsky & Sjoqvist, 1977; Branch, Kornhauser, Shand, Wilkinson & Wood, 1977).

We report here a study investigating the plasma protein binding of propranolol and its agonist, isoprenaline, in patients with hyperthyroidism and hypothyroidism.

Methods

Seven hyperthyroid patients, six female and one male, aged 28-55 years and ten hypothyroid patients, eight female and two male, aged 36-53 years were studied. Table 1 shows detail of the two groups and includes mean results of thyroid function tests.

Patients were seen on two occasions, once after diagnosis and before treatment and again when euthyroid. The average time between the two events was 5-7 months with a range of 2-18 months. On the first occasion patients were not taking any drugs. On the second occasion, the formerly hypothyroid patients were taking only thyroxine. Two formerly hyperthyroid patients, being treated with carbimazole, had stopped treatment for 48 hours before sampling. No other drugs were being taken. On each occasion 20 ml of venous blood were removed for protein-binding studies and the plasma was separated immediately by centrifugation.

Binding of tritiated propranolol and isoprenaline to plasma proteins was estimated by equilibrium dialysis at 37°C. Portions of plasma (1 ml) containing either propranolol or isoprenaline were placed in dialysis tubing and the tubing tied at both ends. The plasma was dialysed for 18 h against 0.067 M phosphate buffer, pH 7.4. In the case of isoprenaline all solutions were flushed with nitrogen to avoid oxidation. After dialysis, concentrations of propranolol and isoprenaline were measured by liquid scintillation spectrometry. Four concentrations each of propranolol and isoprenaline were chosen so that final

concentrations in plasma were in the clinically encountered range (Table 2). Percentage protein binding was calculated by the relationship:

$$\text{Percent protein bound} = \frac{\text{Final concentration in plasma} - \text{concentration in buffer}}{\text{Final concentration in plasma}} \times 100$$

Isoprenaline, before and after equilibrium dialysis for 18 h, was subjected to thin layer chromatography on aluminium sheets pretreated with silica gel 60 (E. Merck, Damstadt). The developing solvent was n-butanol : acetic acid : water (40 : 40 : 20 by volume).

Statistical comparison of means was performed using Student's paired *t*-test.

Results

After dialysis, plasma concentrations of propranolol and isoprenaline for all patients were in the ranges shown in Table 2. There was no variation in plasma protein binding of either drug in these ranges.

Figure 1 shows the percentage of propranolol and isoprenaline bound to protein plotted against their final concentrations in plasma for all ten hypothyroid patients before treatment and reflects the results for the other groups. Plasma protein binding of propranolol was generally in the range of 82-90% and of isoprenaline was generally in the range 60-70%.

Table 3 shows the percentage protein binding of propranolol and isoprenaline in the seven hyperthyroid patients before and after treatment. Each value is the mean of the individual results for the four concentrations studied. For both drugs an increase in

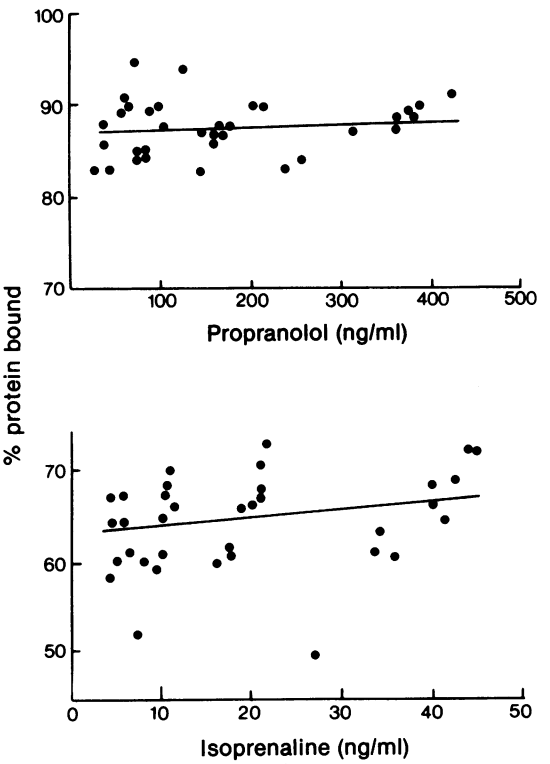

Figure 1 Percentage plasma protein binding of propranolol and isoprenaline versus their final concentrations in plasma in ten hypothyroid patients before treatment.

Table 1 Details (mean ± s.e. mean) of the seven hyperthyroid and ten hypothyroid patients

Thyroid status	*Sex*	*Age (years)*	*T_3 (% uptake)*	*T_4 (nmol/l)*	*F.T.I.*	*T.S.H. (mu/l)*
Hyperthyroid	6F, 1M	28–55	85.8 ± 2.3	199.3 ± 15.0	232.7 ± 23.2	<0.5
Hypothyroid	8F, 2M	36–53	126.2 ± 2.8	11.2 ± 4.7	10.0 ± 3.4	71.4 ± 9.3

Normal ranges T_3 95-122% uptake; T_4 54.1-144.1 nmol/l; FTI, 51.5-141.6; TSH 1.5-7.2 mu/l.

Table 2 Initial plasma concentrations and final plasma concentration ranges for propranolol and isoprenaline

	Initial plasma concentrations (ng/ml)	*Final plasma concentrations (ng/ml)*
Propranolol	100, 200, 400, 800	27–425
Isoprenaline	25, 50, 100, 200	3.5–56

mean percentage protein binding occurred when patients became euthyroid. These differences were small, 2.3% for propranolol and 3% for isoprenaline and were not significant.

Results for the ten hypothyroid patients are shown in Table 4. Results are similar to the results for the hyperthyroid patients in that an increase in mean percentage protein binding occurred for each drug when patients became euthyroid. Again the differences were small; 0.9% for propranolol and 4.5% for isoprenaline. In these patients the differences for isoprenaline was statistically significant ($P < 0.001$).

Thin layer chromatography of isoprenaline demonstrated the presence of a single spot on each occasion, the RF was 0.26 at 0 h and 0.27 at 18 h. There were no other spots present.

Discussion

The results of this present study indicate that the plasma protein binding of neither propranolol nor isoprenaline is significantly altered in hyperthyroidism. With propranolol, only two of six hyperthyroid patients showed variations of greater than 1.5% between the two occasions on which they were tested. The mean binding of isoprenaline in the subjects when euthyroid was of the order of 68% (range 62.0–74.0).

Table 3 Percentage plasma protein binding of propranolol and isoprenaline in seven hyperthyroid patients

Patients	*Treatment*	*Propranolol plasma binding (%)*		*Isoprenaline plasma binding (%)*	
		Pre-treatment	*Post-treatment*	*Pre-treatment*	*Post-treatment*
1	R	77.7	86.4	52.7	65.2
2	R	88.2	88.8	74.9	70.3
3	R	—	—	58.7	62.0
4	R	88.1	89.6	71.6	68.1
5	D	88.8	88.4	66.1	67.4
6	D	87.8	91.2	72.5	74.0
7	S	85.6	85.9	59.5	69.4
Mean		86.1	88.4	65.1	68.1
s.e. mean		1.7	0.8	3.2	1.4

R = Radioactive iodine; D = drug; S = surgical
Propranolol pre-*v* post-treatment; $t = 1.7052$; $n = 5$; $0.2 > P > 0.1$
Isoprenaline pre-*v* post-treatment; $t = 1.2114$; $n = 6$; $0.3 > P > 0.2$

Table 4 Percentage plasma protein binding of propranolol and isoprenaline in the ten hypothyroid patients

Patients	*Propranolol plasma binding (%)*		*Isoprenaline plasma binding (%)*	
	Pre-treatment	*Post-treatment*	*Pre-treatment*	*Post-treatment*
1	85.2	89.9	61.6	66.1
2	86.3	88.2	59.3	62.5
3	90.5	88.4	53.5	64.6
4	86.5	89.5	67.4	72.4
5	85.3	88.7	67.0	69.6
6	86.2	89.8	63.3	66.8
7	89.7	89.8	66.6	73.6
8	92.1	83.4	67.1	71.1
9	88.6	89.7	65.7	67.1
10	86.8	88.9	71.6	73.9
Mean	87.7	88.6	64.3	68.8
s.e. mean	0.8	0.6	1.6	1.2

Propranolol pre-*v* post-treatment; $t = 0.7384$; $n = 9$; $P > 0.4$
Isoprenaline pre-*v* post-treatment; $t = 5.0198$; $n = 9$; $P > 0.001$

With binding of this magnitude, a substantial alteration would be required to produce large enough proportionate increases in free drug concentrations to be clinically important. In these subjects, the greatest increase in free isoprenaline concentration occurring with hyperthyroidism was from 34.8 to 47.3% (Patient 1)—an increase of about one-third—but in most others the changes were much smaller and in some (Patients 2 and 4), free isoprenaline concentration fell during the hyperthyroid state. The mean decrease of isoprenaline binding in hyperthyroidism was only 3% and was not significant.

In hypothyroidism, the mean results were similar to those obtained in the hyperthyroid study. In this group, however, the binding of isoprenaline was significantly lower in the disease than the euthyroid state: no explanation for this can be offered. Propranolol binding was unchanged.

The results for plasma protein binding of propranolol (88-89%) are lower than those previously reported in man by Evans, Nies & Shand, (1973), who found propranolol binding to be of the order of 93%. However, their studies were carried out at room temperature, whilst the present results were obtained at 37°C. Ilett, Hughes & Jellett (1975), have shown a significant reduction in plasma protein binding of propranolol between 4°C and 37°C, and this may explain the difference. Borgå *et al.* (1977) have also recently reported mean propranolol binding of 87.7% in 8 normal subjects. It would appear that neither hyperthyroidism nor hypothyroidism result in alteration of plasma protein binding of propranolol.

There is a lack of published information concerning plasma protein binding of isoprenaline and it was not possible to compare the results obtained in this study with any other. However, the mean results in the two groups when euthyroid were almost identical, 68.1 and 68.8%. In addition, the standardized within-patient method should have ensured that deficiencies of technique or deterioration of isoprenaline were not attributed to disease process. Thin layer chromatography did not suggest that deterioration was likely to be a substantial factor.

Alterations in the plasma binding of catecholamines, if they occurred, might be of relevance to the pathogenesis of hyperthyroidism. Many of the clinical manifestations of this disease resemble the effects of stimulation of the sympathetic nervous system, but the reasons for this have never been satisfactorily explained. Increased sensitivity to catecholamines has been said to occur in hyperthyroidism, but catecholamine concentration-response curves have not been found to alter in patients when they were hyperthyroid compared to when they were euthyroid (Aoki, Wilson & Theilin, 1972; McDevitt, Riddell, Hadden & Montgomery, 1978). Another possible explanation involves alterations in protein binding: if the fraction of catecholamines bound to plasma proteins was substantially reduced (and, conversely, the free fraction increased) then an apparent increase in sensitivity to catecholamines might be seen. The endogenous catecholamine of principal relevance in this situation is undoubtedly adrenaline because of its capacity to stimulate β-adrenoceptors.

The results of this present study with isoprenaline, a catecholamine which is not endogenous, are not strictly comparable. However, the binding of adrenaline in this situation cannot easily be measured not only because adrenaline is even more unstable than isoprenaline but also because plasma protein systems inevitably contain endogenous circulating adrenaline. Isoprenaline binding may represent the best opportunity for assessing catecholamine binding in thyroid disease. In addition Danon & Sapira (1972) have shown that the affinity of catecholamines for binding to human serum albumin is dependent more on the hydroxyl groups than on either the nature or even the presence of the alkyl side chain. In their study, the relative affinities of adrenaline and isoprenaline were almost identical. Overall, then, if the plasma protein binding of isoprenaline is in any way representative of endogenous catecholamine binding, it would appear unlikely that increases in free catecholamine concentrations in patients with hyperthyroidism could explain the clinical manifestations of the disease.

In conclusion, observed changes in protein binding of propranolol and isoprenaline in thyroid dysfunction were similar in hyperthyroid and hypothyroid patients, namely small negative differences, which were only significant in the case of isoprenaline in hypothyroidism. Such small changes would be unlikely to result in clinically important changes in free drug concentrations.

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