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Displacement of L-thyroxine from its binding proteins in human, dog, and rat plasma by α-(*p*-chlorophenoxy)isobutyric acid

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THE HYPOCHOLESTEREMIC and hypolipemic effects of ethyl α-(*p*-chlorophenoxy) – isobutyrate (CPIB), clofibrate, Atromid-S*) have been the subjects of many publications.[1–3] The mechanism of action of this drug is not yet understood. Based on the observations that: (1) thyroidectomy of the rat effectively abolishes the effect of CPIB on serum cholesterol;[3] (2) the maximal effect of CPIB on rat serum cholesterol corresponds with periods of maximal thyroid and adrenocortical function;[4] (3) CPIB enhances liver retention of thyroxine in the rat[5] and causes an increase in weight, protein concentration, and nonfasting glycogen levels of liver in the monkey, dog, and rat;[3] it was postulated that these effects of CPIB are primarily due to the displacement of thyroxine from its binding proteins in the plasma and subsequent redistribution of the hormone between the plasma and the liver.[3,6] There is no direct evidence, however, indicating that thyroxine is displaced from its binding proteins by CPIB. It has been reported that CPIB displaces thyroxine from human serum albumen,[4] but the binding of this hormone to human serum albumin is of minor importance compared with that of thyroxine-binding α-globulin (TBG) or thyroxine-binding prealbumin (TBPA).[7] Furthermore, it is unlikely that thyroxine binding to albumin normally plays a significant role in thyroxine metabolism, since the PBI of analbuminemic patients is normal.[8] To investigate the contention that displacement of thyroxine from plasma-binding proteins is involved in the action of CPIB, we studied the binding between thyroxine and proteins in human, dog, and rat plasma and determined the effect of CPIB on the binding systems.

MATERIALS AND METHODS

Paper electrophoresis was carried out with Tris-maleate buffer (pH 8·6) in an electrophoresis apparatus (LKB model 3276B) at constant voltage gradient of 60 V, at room temperature for 19 hr by the reverse-flow technique of Wolff *et al.*[9] The concentration of thyroxine-^{131}I (sp. act. 40–50 mc/mg, Abbott Laboratories) in the plasma samples† was 0·20 μg/ml. The radioactivity on paper strips was determined by: (1) scanning in a continuous recording chromatogram scanner (Scannogram II, model RSC–160, Atomic Accessories Inc.) with an analytical count ratemeter (model 1620B, Nuclear–Chicago); and (2) radioautography using Blue Brand X-ray film. The exposure time was five days. The paper strip was then sprayed with bromophenol blue to show the positions of various proteins. To determine the effect of a compound on L-thyroxine binding to plasma proteins, an appropriate amount was dissolved in the buffer.

RESULTS AND DISCUSSION

The total thyroxine concentration in normal human serum is approximately 1×10^{-7}M, of which only 6 to 13×10^{-11}M$^{10-12}$ (or 0·06–0·13 per cent) is believed to be in the unbound form. The rest is bound to three serum proteins: TBG, prealbumin, and albumin. The association constants of TBG-thyroxine and albumin-thyroxine complexes have been reported to be $7{\cdot}9 \times 10^{-9}$M^{12,13} and 1×10^{-5}M[11], respectively. These values, however, are based on a number of assumptions which are yet to be vertified and therefore must be considered as rough approximations. Similarly, the binding capacity of TBG and prealbumin has been estimated to be $2{\cdot}6 \times 10^{-7}$M[12] and $1{\cdot}3 \times 10^{-6}$M[14], respectively. The binding capacity of albumin is exceedingly high, but the true value has not been measured.

* I.C.I. trade marks.

† Human plasma was obtained from a Red Cross blood bank (outdated blood). Rat plasma was obtained from heparinized rat blood drawn from the abdominal aorta. Dog plasma was obtained from heparinized dog blood taken from the jugular vein.

It has become increasingly evident that the interaction between thyroid hormones and serum proteins has an important influence on the distribution, degradation, excretion, and action of these hormones.[10, 12] These interactions retard transfer of hormones to extravascular sites more or less impermeable to macromolecules. Ultimately, the hormones must enter into tissue cells or, at least, become intimately associated with cell surfaces. A body of evidence is available which suggests that both the effect and the rate of degradation of thyroxine are functions of the concentration of unbound thyroxine.[10] Although the true values of association constant and binding capacity for TBG, prealbumin, and albumin are not known, there can be little doubt that the concentration of free thyroxine in human blood is extremely low, that more than 99·8 per cent of total thyroxine in blood is bound to proteins, and that any compound which depresses protein binding of thyroxine, either by changing the affinity constant or by reducing the concentration of available unoccupied binding sites, should potentiate the action of endogenous thyroxine and alter its rate of metabolism.

Since CPIB is rapidly hydrolyzed by tissue and serum esterases *in vivo* to α-(*p*-chlorophenoxy) isobutyric acid[15], the latter compound was used in this study. The serum concentrations of α-(*p*-chlorophenoxy) isobutyric acid in man at 1·5 hr after oral administration of 2 g of CPIB containing 2% androsterone were in the neighbourhood of 200 μg/ml.[16] At the concentration of 6×10^{-3}M, α-(*p*-chlorophenoxy) isobutyric acid was found to depress thyroxine binding to prealbumin in human plasma (Fig. 1 and 2). At a very high concentration (3×10^{-2}M), it also depressed albumin binding of thyroxine (Fig. 3). The binding of thyroxine to TBG was not depressed even at a very high concentration (Fig. 3). Each of the above experiments was repeated at least four times with plasma from different subjects and the results were always consistent. Prealbumin binding of thyroxine has been shown to be quantitatively significant under physiological conditions.[7, 17, 18] As much as 30 per cent of total serum thyroxine is believed to be associated with serum prealbumin. In view of the fact that less than 0·2 per cent of thyroxine in blood is in the unbound form, it appears that any interference with the binding of thyroxine by prealbumin would significantly increase the concentration of free thyroxine in the blood circulation.

In the dog, thyroxine is bound to three serum proteins: an α-globulin, a β-globulin, and albumin, among which the binding to the α-globulin was most important[19] (Fig. 1). α-(*p*-chlorophenoxy) isobutyric acid inhibited the α-globulin binding of thyroxine and also interfered with the albumin binding (Fig. 2). These interferences would be expected to have a significant effect on the concentration of free thyroxine in the blood.

In the rat, thyroxine is bound to an α-globulin and albumin[19] (Fig. 1). α-(*p*-chlorophenoxy) isobutyric acid depressed only the less important albumin binding of thyroxine (Fig. 2). Since the importance of albumin binding of thyroxine in the rat is still open to dispute[19, 20] and quantitative information concerning this interaction is lacking, no conclusion can be drawn at this time. It may be said, however, that the effect of CPIB on free thyroxine concentration in the circulating blood is much less in the rat than in man or dog.

There is a substantial body of indirect evidence which implicates thyroxine in the action of CPIB. This evidence has been summarized in two recent reports by Platt *et al.*[3, 21] Our findings support the contention that displacement of thyroxine from its plasma binding proteins is involved in the mechanism of action of CPIB. They do not exclude, of course, other possible mechanisms such as depression of cholesterol biosynthesis.[22] The observed hypocholesteremic and hypolipemic effects of CPIB may well be the combined results of multiple actions.

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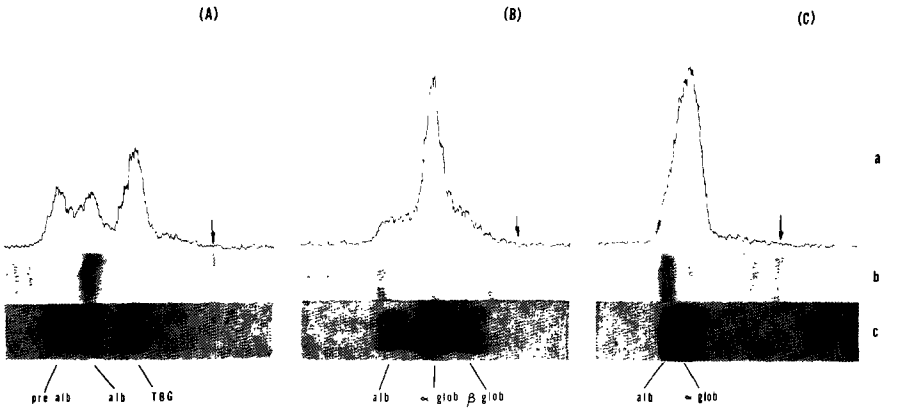

FIG. 1. Reverse-flow paper electrophoresis in *Tris*-maleate buffer at pH 8·6 of L-thyroxine-^{131} I–human plasma mixture (A), L-thyroxine-^{131} I–dog plasma (B), and L-thyroxine-^{131} I rat plasma mixture (C). The paper chromatogram (b), stained for protein, is aligned with radioactive tracings (a) and radioautographs (c). The arrow indicates the point at which plasma was applied. The concentration of exogenous L-thyroxine-^{131} I in the plasma sample was 0·23 μg/ml.

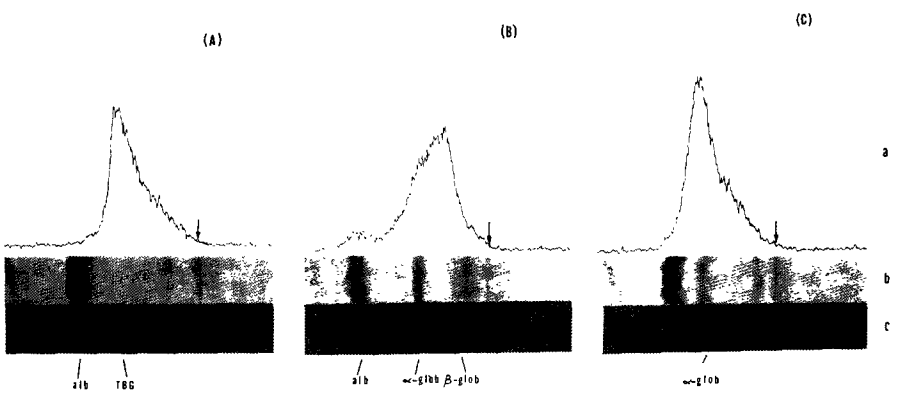

FIG. 2. Reverse-flow paper electrophoresis in *Tris*-maleate buffer at pH 8·6 of L-thyroxine-^{131} I–human plasma (A), L-thyroxine-^{131}I–dog plasma (B), and L-thyroxine-^{131}I–rat plasma (C) mixtures. The buffer contained *p*-chlorophenoxyisobutyric acid (6 10^{-3}M). The paper chromatograms (b) are aligned with radioactive tracings (a) and radioautographs (c). The concentration of L-thyroxine-^{131}I in the plasma sample was 0·23 μg/ml.

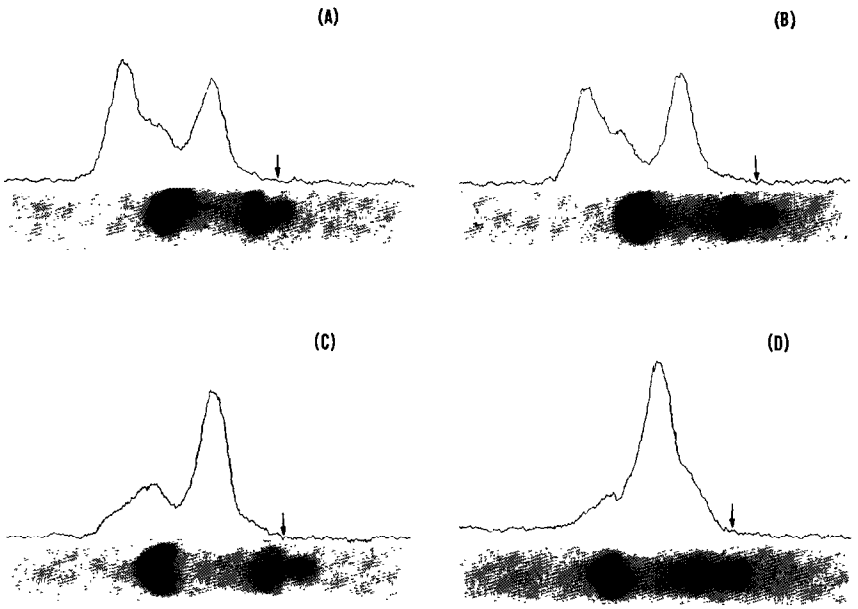

FIG. 3. Effect of CPIB at 6×10^{-4}M (B), 6×10^{-3}M (C), and 3×10^{-2}M (D) on L-thyroxine-^{131}I–human plasma protein association. (A) is a control.

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Comparison of cycloheximide effect on hepatic protein synthesis at the microsomal level *in vivo* and *in vitro*

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CYCLOHEXIMIDE is an inhibitor of protein synthesis *in vivo*[1, 2] and *in vitro*.[3] However, in our previous studies[4–7] we were able to show that cycloheximide administration to rats *in vivo* stimulated the L-^{14}C-phenylalanine incorporation *in vitro* in an incubation system with hepatic microsomes isolated from treated animals. We established that this cycloheximide-induced stimulation on the system *in vitro* was localized in the microsomal fraction, was independent of changes of cofactor requirements, unrelated to the stabilization of endogenous RNA, and probably dependent on the mediation of the adrenal glands.

There were reports in the literature[2, 3, 8–10] that the aggregation of the ribosomes in the microsomal fraction might be affected by cycloheximide and that such a shift in the degree of aggregation could account for the influence of cycloheximide on protein synthesis.

We have now investigated some of the differences between cycloheximide-induced effects on protein synthesis *in vivo* and *in vitro*. We find that the degree of aggregation of the microsomal fraction is not altered by cycloheximide treatment *in vivo* or *in vitro* under our experimental conditions. We find that cycloheximide pretreatment *in vivo* inhibits amino acid incorporation into protein *in vivo* (except in adrenalectomized animals), but stimulates protein synthesis *in vitro*. The addition of cycloheximide to the incorporation system *in vitro* reverses the stimulation of protein synthesis brought about by prior injection of cycloheximide *in vivo*.

MATERIALS AND METHODS

Cycloheximide was obtained from the Cancer Chemotherapy National Service Centre (thanks to Dr. J. A. R. Mead). Animals used were female Sprague–Dawley derived rats weighing 160 g each,