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THE ROLE OF THE THYROID HORMONE IN THE EFFECT OF *p*-CHLOROPHENOXYISOBUTYRATE IN RATS*

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Abstract—Feeding 0·3% *p*-chlorophenoxyisobutyrate (CPIB) in the diet to weanling male rats increased liver mitochondrial α-glycerophosphate dehydrogenase (GPD) from the basal level of 25 units to about 110 units in 10–14 days, and maintained that level for at least 6 weeks. Liver and adipose tissue malic enzyme were also increased, while the GPD response in other tissues which respond to thyroxine (T_4) was sporadic. The magnitude of this liver GPD response was equivalent to the s.c. administration of 4–8 μg T_4/100 g body wt/day. The daily administration of a 3-μg dose of T_4 along with CPIB had no effect on the rate or magnitude of the response obtained with CPIB alone, but 40 μg T_4 increased both the rate and magnitude. CPIB alone increased liver GPD at a rate which was comparable with that produced by 40 μg T_4 alone. The GPD response to CPIB was inhibited by actinomycin, cycloheximide or ethionine. When new protein synthesis was blocked by ethionine, CPIB did not inhibit the destruction of preformed GPD. CPIB had little or no effect on liver GPD in thyroidectomized (Tx) rats, but the injection of 1 μg T_4 or 0·35 μg T_3 per 100 g body wt/day to Tx rats fed CPIB gave the same liver GPD response obtained in intact rats with CPIB alone. Methimazole (MI) also prevented the CPIB effect on liver GPD. CPIB had no effect on liver GPD in those species which did not respond to T_4. CPIB had no effect on thyroid weight or histology. It increased the Q_{O_2} of liver slices but not the metabolic rate of intact rats. The small stimulus for new protein synthesis normally provided by endogenous thyroid hormone was converted into a large liver GPD response by the CPIB.

CPIB† HAS BEEN studied extensively[1] as a new drug which is capable of decreasing the plasma cholesterol and triglycerides in hyperlipemic patients. It appears to have its primary effect on the triglycerides in the low density (S_f 20–400) lipoprotein fraction.[1–5] CPIB inhibits cholesterol synthesis *in vitro*[7–9] at concentrations which are relatively high when compared with blood and liver levels obtained *in vivo*.[6] However, it has no effect on plasma cholesterol levels when the latter is the only plasma lipid which is elevated[1, 2, 10] or when the elevated cholesterol occurs in the S_f 0–20 lipoprotein fraction[1,3,5]. It exhibits little or no toxicity and no microscopic pathology,[6, 11] although

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† Abbreviations used: CPIB, *p*-chlorophenoxyisobutyrate; GPD, mitochondrial α-glycerophosphate dehydrogenase; T_4, L-thyroxine; Tx, thyroidectomized; MI, methimazole (1 methyl, 2-thiolimidazole); MR, metabolic rate; T_3, L-3,5,3′-triiodothyronine; Triac, 3,5,3′-triiodothyroacetic acid; Tetrac, 3,5,3′,5′-tetraiodothyroacetic acid; Isopropyl-T_2, 3,5-diiodo-3′-isopropyl thyronine; TSH, thyroid stimulating hormone; PBI, protein-bound iodine.

the size and number of mitochondria and lysosome-like bodies are increased in rat liver by the drug.[12,13] Hess *et al.*[13] reported that several enzymes associated with liver mitochondria, including α-glycerophosphate dehydrogenase (L-glycerol-3-phosphate: cytochrome C oxidoreductase, EC 1.1.2.1), are also increased by the drug. The rather marked elevation in liver α-glycerophosphate dehydrogenase (GPD) which was produced by CPIB suggested a thyroidal effect, since previous studies[14] have demonstrated a high degree of specificity for this enzyme response to thyroxine and its analogs.

The studies reported in this paper showed that CPIB had no thyromimetic effect *per se*; it was effective in increasing liver GPD only when some form of endogenous or exogenous thyroid hormone was also present. It did not allow the GPD to accumulate because of some interference with the normal destruction of the enzyme, and there was no indication of increased activity of the thyroid gland. Hence, it enhanced the effectiveness of the normal amount of endogenous thyroid hormone in the liver. The hyperthyroidal effect produced by CPIB was confined largely to the liver, and was characterized by a typical increase in malic enzyme (L-malate: NADPH oxidoreductase, EC 1.1.1.40) and liver slice Q_{O_2} as well as GPD, but was not accompanied by an increased metabolic rate (MR) in the intact rat.

METHODS

Unless specified otherwise, all experiments were conducted with weanling (50 g) male Holtzman rats; the sodium salt of CPIB was added to a purified diet[15,16] and fed *ad libitum*. L-Thyroxine (T_4) or its analogs were dissolved in alkali, diluted with saline, and injected (final pH, 8–9) s.c. daily on a body wt. basis. Tissue GPD[16,17] and malic enzyme[18] concentrations were determined as previously described.

RESULTS

GPD response to CPIB. Table 1 shows the liver GPD response to the feeding of 0·1, 0·2, 0·3 or 0·5% (w/w) CPIB for 14 days. The GPD concentrations per unit weight of liver increased with increasing doses of the CPIB, reaching a 9-fold increase over the basal level with 0·5% CPIB in the diet. Total liver enzyme activity increased

TABLE 1. EFFECTS OF INCREASING AMOUNTS OF DIETARY CPIB ON LIVER GPD, LIVER WEIGHT AND FOOD CONSUMPTION*

		CPIB in diet (%)			
	Basal diet	0·1	0·2	0·3	0·5
Liver GPD (μl O_2/150 mg/10 min)	13 ± 1·5	46 ± 3·6	64 ± 2·8	98 ± 6·6	120 ± 6·3
Total liver GPD	1164 ± 142	4852 ± 436	7410 ± 426	11,621 ± 833	15,085 ± 927
% Body wt. as liver	5·8 ± 0·1	6·9 ± 0·1	7·6 ± 0·2	8·1 ± 0·1	8·3 ± 0·1
Wt. gain/12 days	94 ± 5·7	92 ± 6·8	85 ± 3·2	76 ± 3·4	84 ± 1·9
g food/rat/day	15·1 ± 0·7	13·0 ± 1·7	15·6 ± 0·3	15·3 ± 0·2	15·8 ± 0·3
mg CPIB/rat/day	0	13·0 ± 1·7	31·1 ± 0·5	45·7 ± 0·6	79·3 ± 1·2

* Each value is expressed as mean ± S.E. CPIB = *p*-chlorophenoxyisobutyrate; GPD = mitochondrial α-glycerophosphate dehydrogenase.

even more, due to the increased size (43 per cent) of the liver. (In other experiments the increase in liver weight averaged about 50 per cent.) The CPIB had essentially no effect on the weight of daily food intake.

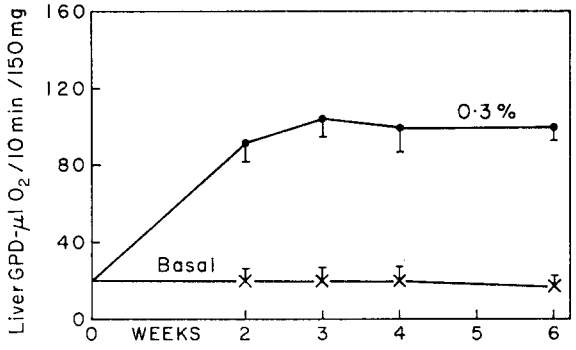

FIG. 1. The liver GPD response in weanling male rats fed 0·3% (w/w) dietary CPIB for 6 weeks. Results with the Na salt and ethyl ester of CPIB were combined. S.E.M. is shown for groups of 8 rats by the one-sided bars which represent both the + and − values.

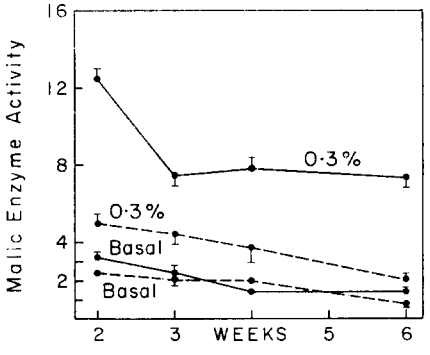

FIG. 2. The liver and adipose malic enzyme response to CPIB (Na salt or ethyl ester) in the same experiment as in Fig. 1. Solid lines = liver; dotted lines = adipose tissue. Malic enzyme activity = μmoles NADPH/min/g fresh tissue.

Fig. 1 shows the liver GPD response to the feeding of 0·3% CPIB for 2–6 weeks. Both the sodium salt and ethyl ester of CPIB were tested, but the results were combined when no difference between them could be detected. In this experiment, the liver GPD reached a plateau at about 100 units between weeks 2 and 6. In other experiments, the 2-week value varied from 84–148 enzyme units (av. = 113). When compared with a previous assay curve,[19] the magnitude of this GPD response was equivalent to the response produced by the administration of approximately 4 μg T_4/100 g body wt/day. When a 50 per cent increase in liver weight in the CPIB-fed rats was also taken into account, the total liver GPD response to CPIB was equivalent to 7 or 8 μg T_4. Since the rat has an endogenous T_4 output of about 1 μg/100 g/day,[16, 19] the CPIB increased either the output or the effectiveness of the endogenous T_4 some 4–8 times. The liver and adipose tissue malic enzyme also responded to T_4,[14,18] and Fig. 2 shows

the increased malic enzyme activity in these tissues which resulted from the feeding of CPIB.

Table 2 shows the effect of feeding CPIB for 2–6 weeks on the GPD and malic enzyme activities in other rat tissues. GPD activities in kidney, skeletal muscle, adipose tissue, heart and pancreas are all increased by T_4 administration, but only heart GPD was increased slightly by CPIB in this experiment; in other experiments, kidney and adipose tissue GPD were sometimes increased slightly. Lung and brain

TABLE 2. AVERAGE TISSUE ENZYME ACTIVITIES DURING THE FEEDING OF CPIB FOR 2–6 WEEKS TO WEANLING MALE RATS*

Enzyme	Tissue	Basal diet	Dietary CPIB (%, w/w) 0·1	0·3 (Na)	0·3 (Et)	0·5
GPD (μl O_2/10 min/ 150 mg)	Kidney	24	28	26	26	32
	Skeletal muscle	70	67	64	65	57
	Adipose tissue	3	2	2	4	3
	Heart	10	15	19	17	22
	Pancreas	4	6	5	4	3
	Lung	6	9	9	7	9
	Brain	34	34	33	39	36
Malic enzyme†	Kidney	0·85	0·80	1·19	1·07	1·33
	Skeletal muscle	0·42	0·38	0·51	0·45	0·46

* The S.E.M. was about 6 per cent of the mean for the kidney, muscle and brain analyses, 8·5 per cent for heart, 14 per cent for lung, 19 per cent for pancreas, and 28 per cent for adipose tissue. There were approximately 11 rats/group for lung and brain, and 16 rats/group for all others. Values for 0·1 and 0·5% CPIB were combined results from Na salt and ethyl ester.

† Malic enzyme = μmoles NADPH/min/g fresh tissue.

GPD, as well as the malic enzyme in kidney and skeletal muscle, do not respond to T_4 and were not increased by CPIB. In general, the thyroxine-like effect of CPIB was largely, but not exclusively, confined to the liver, and the drug had no effect on these enzyme activities in tissues which do not respond to T_4.

Fig. 3 shows the liver GPD response to two different doses of T_4 in the presence and absence of CPIB during the initial 2-week feeding period. These unit-concentration curves show only a relatively small synergism in the response rate when the T_4 and CPIB stimuli are combined. The increased size of the liver, which results from the administration of CPIB,[7,9,13,20,21] and the changes in total liver GPD are shown in Fig. 4. In these weanling male rats, the feeding of 0·3% CPIB in the diet increased the liver weight by about 50 per cent; the injection of 3 or 40 μg T_4 in the presence or absence of CPIB had relatively little effect on liver weight. The total liver GPD response showed that (1) the administration of a small dose of T_4 (3 μg) along with CPIB had no effect on the rate or magnitude of the response obtained with CPIB alone (2) the administration of 40 μg T_4 along with CPIB increased the rate as well as the magnitude of the GPD response and (3) CPIB alone increased liver GPD at a rate which was comparable with that produced by a "maximal" stimulus from 40 μg T_4 alone. Fig. 5 shows a small kidney response to CPIB in this experiment, but the presence or absence of CPIB had no effect on the response of kidney GPD to T_4.

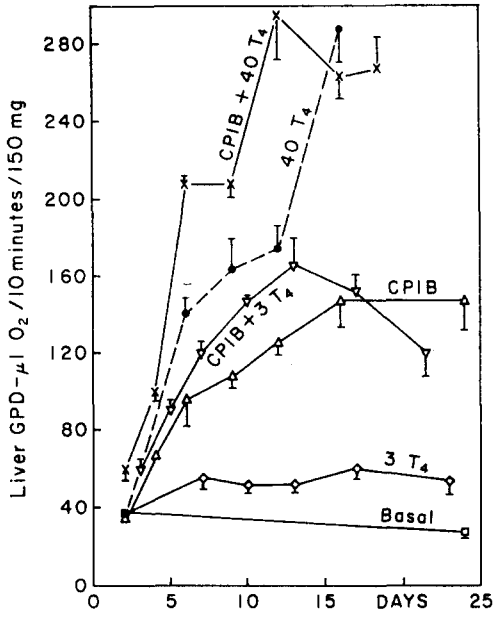

FIG. 3. The liver GPD response in weanling male rats (5–6 rats/group) fed the basal diet ± 0·3% CPIB (w/w) and injected s.c. with 3 or 40 μg L-T_4/100 g body wt/day.

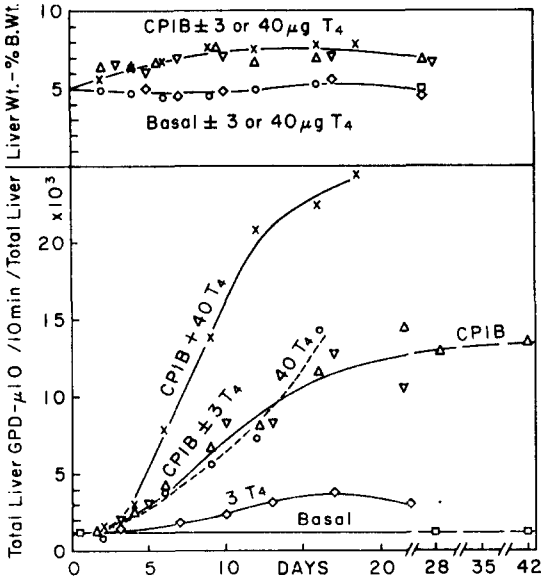

FIG. 4. Same experiment as in Fig. 3. Upper section: change in liver weight (as % of body wt.); lower section: change in total liver GPD (activity per 150 mg × 1000/150 × wt. of liver in g).

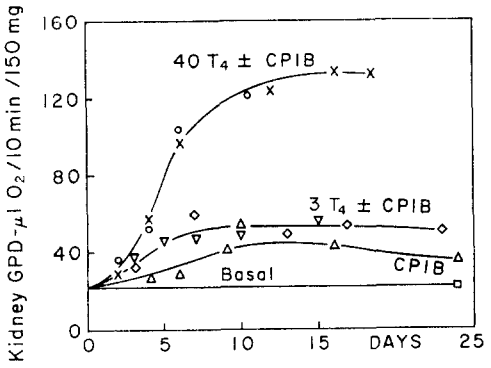

FIG. 5. Same experiment as in Fig. 3, showing the change in kidney GPD.

Protein synthesis and destruction. The increased GPD activity which follows the administration of T_4 is due to new protein synthesis.[18] If the increased GPD which follows the administration of CPIB is a thyroidal effect, it too should be the result of new protein synthesis. Fig. 6 shows the effect of two protein-synthesis inhibitors on

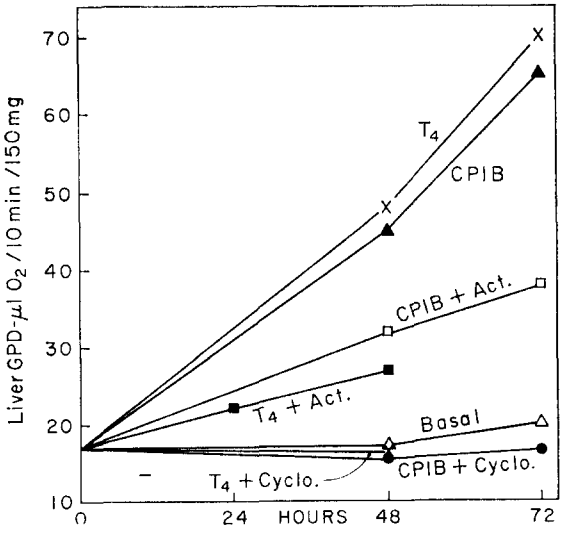

FIG. 6. The inhibition of the liver GPD response to thyroxine (T_4) or CPIB by actinomycin D or cycloheximide. Blue Spruce male rats, 125 g; 40 μg T_4/100 g body wt/day injected s.c.; 50 mg CPIB/100 g body wt. by gavage; 16 μg actinomycin D/100 g body wt. injected i.p. daily; 80 μg cycloheximide/100 g body wt. injected twice daily i.p.; 9 or more rats per group for most points. The difference between CPIB at 48 hr (45·0 ± 3·52) and CPIB plus actinomycin (31·8 ± 1·85) or CPIB + cycloheximide (15·5 ± 0·67) was significant at $P = < 0{\cdot}01$ (14, 16 and 6 rats per group, respectively).

the GPD response to CPIB. Although these inhibitors could not be studied for more than 3 days (for lack of survival), their inhibition of the liver GPD response to CPIB was comparable with their inhibition of the T_4 stimulus.

The possibility that CPIB increased liver GPD by blocking the normal destruction of the enzyme was tested in the following experiment. The GPD activity of the liver was increased to high levels by the injection of 40 μg T_4/100 g body wt/day for 2 weeks while the rats were fed the basal diet. The T_4 stimulus was then stopped, and the rats were fed diets containing CPIB $\pm$ 0·5% ethionine (w/w). Dietary ethionine inhibits the GPD response to T_4[18] and ethionine is also effective against CPIB.[13] The upper portion of Fig. 7 shows the changes in total liver GPD in this experiment; the lower

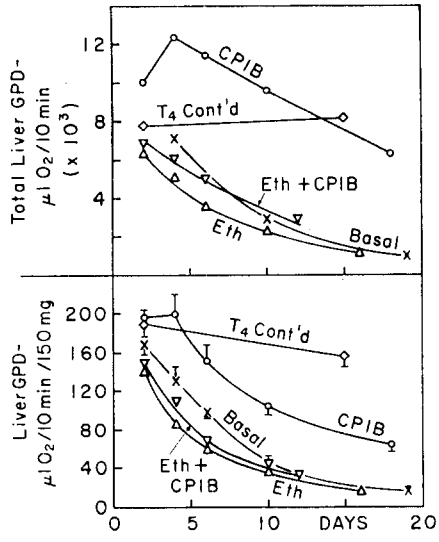

FIG. 7. The comparative loss of liver GPD (after T_4 stimulation) when rats were fed CPIB or ethionine or both; upper section: change in total liver GPD; lower section: change in unit concentration of GPD. Forty μg T_4/100 g body wt/day injected s.c. into weanling male rats for 2 weeks. After stopping the T_4 injections, the rats were fed the basal diet $\pm$ 0·3% CPIB $\pm$ 0·5% ethionine. The S.E. of the mean for rats fed ethionine $\pm$ CPIB was 7–9 per cent of the mean (5–6 rats/per group).

portion of the figure shows the changes in unit concentration. In the presence of CPIB, the liver enzyme concentration was maintained for several days, and the total liver enzyme content was increased for a few days, presumably due to an effect of the drug on the circulating T_4; thereafter, the enzyme decreased at a rate which was comparable with the rate obtained on a basal diet. The feeding of ethionine decreased the liver GPD somewhat faster than the basal diet, and ethionine blocked almost completely the surviving T_4 response to CPIB. When new protein synthesis was blocked by ethionine, CPIB was unable to stimulate the synthesis of additional liver GPD. CPIB did not inhibit the immediate loss of any existing GPD when protein synthesis was blocked by ethionine. When the T_4 stimulus was stopped, the liver weight (as per cent of body wt.) increased from 4·7 to 5·2 on the fifth day, and returned to 4·7 by the tenth day. The fifth- and tenth-day values for the other diets were: ethionine, 5·7 and 5·2; CPIB, 6·4 and 7·1; ethionine + CPIB, 6·0 and 5·6. Hence, ethionine by itself increased the liver weight somewhat, but partially blocked the much larger increase produced by CPIB. Fig. 8 shows the corresponding changes in kidney and heart GPD, and again there was no protection of any preformed enzyme by the CPIB.

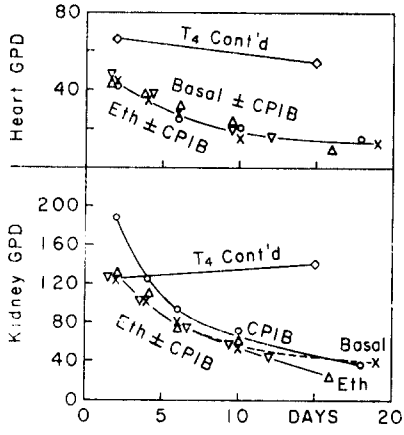

FIG. 8. Same experiment as in Fig. 7, showing the change in kidney and heart GPD (μl O_2/10 min/150 mg).

Effect of thyroid hormone on CPIB activity. The dose-response curves in Fig. 9 show the enhancement of the liver GPD response to injected T_4 when CPIB was added to the basal diet. Endogenous T_4 permitted the CPIB to increase the liver GPD from a basal level of 25 to a level of 106. Small amounts of T_4 (e.g. 1–2 μg) had little or no

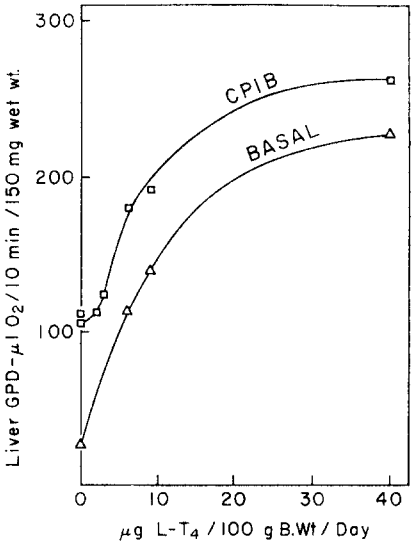

FIG. 9. Enhancement of the GPD response to injected T_4 by CPIB. Weanling male rats were fed the basal ± 0·3% CPIB (w/w) diet and were injected with the indicated dose of T_4 (μg/100 g body wt/day) for approximately 2 weeks. Each point is the mean of 6–8 rats.

additional effect on the CPIB response, but larger doses of T_4 produced a larger liver GPD response in the presence of CPIB than in its absence. The same conclusions were evident in a more dramatic way when the total liver GPD response was examined Fig. (10). The response of kidney GPD to increasing doses of exogenous T_4 was unaffected by CPIB.

Other analogs behaved similarly to T_4 in that CPIB enhanced the liver GPD response to Triac, Tetrac and isopropyl T_2 (Table 3).

Similar T_4 dose-response curves were also obtained in thyroidectomized (Tx) rats, except that CPIB without any T_4 had little effect on stimulating the liver GPD.

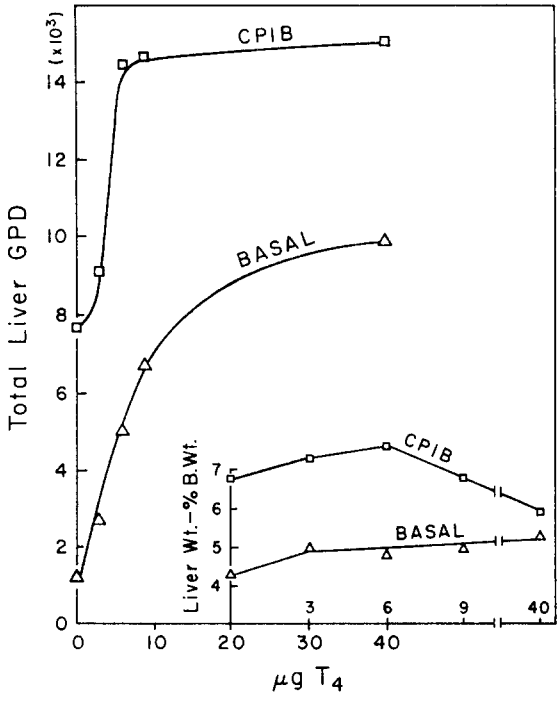

FIG. 10. Same as in Fig. 9. Total liver GPD = μl O_2/10 min total liver; liver weight = % body wt.

TABLE 3. EFFECT OF THYROXINE ANALOGS ON LIVER AND KIDNEY GPD IN THE PRESENCE OF CPIB*

Injections		Basal diet		0·3% CPIB	
Compound	Dose	GPD Liver	GPD Kidney	GPD Liver	GPD Kidney
		25 ± 4·9	31 ± 3·0	118 ± 9·5	42 ± 2·8
Triac	10	130 ± 15·6	93 ± 3·9	184 ± 2·7	100 ± 8·5
Tetrac	35	109 ± 6·2	99 ± 3·7	184 ± 6·1	122 ± 5·9
Isopropyl T_2	0·6	96 ± 8·7	79 ± 4·8	149 ± 9·1	59 ± 3·1

* Weanling male rats (6/group) from Charles River were fed the above diets and injected with 3,5,3′-triiodo-thyroacetic acid (Triac), 3,5,3′5′-tetraiodothyroacetic acid (Tetrac), 3,5-diiodo-3′iso-propylthyronine (isopropyl-T_2) at the dosage indicated (μg/100 g body wt/day) for approximately 2 weeks before determining liver and kidney GPD (μl O_2/10 min/150 mg fresh tissue). Values are expressed as the mean ± S.E.

Male rats weighing approximately 150 g were thyroidectomized (Hormone Assay Laboratories, Chicago) 8–9 days before the start of the experiment; other details were similar to those described for intact rats. In different experiments, the feeding of

CPIB to Tx rats increased the liver GPD from Tx levels of 3–5 to 5–18 units, and this small effect was attributed to small amounts of residual T_4 in the Tx rats. CPIB enhanced the liver GPD response to T_4 in the Tx rat in the manner shown for intact rats in Fig. 9. The injection of 1 μg T_4 or 0·35 μg T_3 (per 100 g body wt/day) increased liver GPD slightly above the euthyroid level in both intact and Tx rats fed the basal diet (liver GPD = 25–45), but gave values of 80–125 in the presence of CPIB in both types of rats. Hence an amount of thyroid hormone which was equivalent to the normal endogenous output was enough to allow the CPIB to reproduce the effect obtained with this drug in intact rats. The total liver GPD response to exogenous T_4 in Tx rats fed basal $\pm$ CPIB diets was also similar to the results in Fig. 10 for intact rats (except that the Tx values of 150–300 were increased to only 300–1000 by CPIB alone).

The results showed that: (1) CPIB had little or no effect on liver GPD in the absence of the thyroid hormone and is therefore not thyromimetic *per se*; and (2) the thyroid gland was not involved in the enhancement of exogenous T_4 activity by CPIB.

Liver weight was about 4·5 per cent of body wt. in these Tx rats, and this was not altered appreciably by the administration of T_4. Feeding CPIB increased the liver weight to 5·5 per cent and this was further increased to 6·5 per cent by the simultaneous injection of T_4.

Methimazole (MI) is known to block the synthesis of thyroid hormone in the thyroid gland. Dietary MI (0·1 per cent) produces thyroidectomy levels of liver GPD (GPD for control, 25 $\pm$ 4·9 for MI, 11 $\pm$ 2·1) in rats, but otherwise has no effect on the dose-response curve.[22] Like thyroidectomy, MI prevented the large increase in liver GPD produced by CPIB in intact rats (GPD for 0·3% CPIB, 118 $\pm$ 9·5; for MI $\pm$ CPIB, 16 $\pm$ 1·1)*.

A sensitive test for thyromimetic activity in mammals is a growth response in rats which have been thyroidectomized[23,24] or fed a goiterogenic diet.[25,26] When weanling male rats were fed the 0·1% MI diet, their body weights reached a plateau at 175–180 g by the sixth week. When 0·3% CPIB was then added to the MI-containing diet, there was no growth response over the next 8 weeks, and at the end of the 14 weeks, the liver GPD averaged 4 units. The lack of any response was expected, since the MI blocked formation of thyroid hormone by the thyroid gland and is further evidence that CPIB possesses no thyromimetic action *per se*.

All species do not respond to thyroid hormone with the marked elevation in liver GPD and metabolic rate which characterizes the rat,[27] and CPIB is not uniformly effective in all species.[28] If CPIB increases liver GPD through some effect of the thyroid hormone, then those species which fail to respond to T_4 should also fail to respond to CPIB. Table 4 shows the results of such studies. In the mouse, liver and kidney GPD were markedly increased by T_4, and the enzyme activities in both tissues were elevated by CPIB. The hamster also responded to T_4, but the magnitude of the effect was much less than in the rat, and the effect of CPIB was minimal. Thyroid hormone had little or no effect on guinea pigs and birds, and CPIB $\pm$ T_4 was also ineffective in increasing liver GPD in these species.

* Methimazole was found to restrict the food intake by 30 per cent and therefore the CPIB consumption was equivalent to approximately 0·2 per cent of the diet. According to the data in Table 1, the GPD could be expected to reach about two-thirds of the value produced by 0·3% CPIB. Hence, the MI prevented the stimulation of GPD by CPIB.

Thyroid function. The administration of CPIB to rats had no obvious effect on the thyroid gland. Thyroid weights of animals in the experiments shown in Fig. 1 averaged 4·5 mg/100 g body wt. for controls and 4·2 for rats fed 0·3% CPIB. The S.E.M. for groups of 16 rats was approximately 6 per cent of the mean. There was no histological evidence of thyroidal stimulation in rats receiving the CPIB. After feeding 0·3% CPIB (Na salt or ester) for 3–5 weeks, the metabolic rates[15] were unchanged: basal

TABLE 4. EFFECT OF FEEDING CPIB ± INJECTED T_4 ON THE LIVER AND KIDNEY OF DIFFERENT SPECIES*

Species		Basal diet	T_4	CPIB	T_4 + CPIB
Mice	Liver GPD	57 ± 4·5	169 ± 7·1	93 ± 9·9	180 ± 15·6
	Kidney GPD	35 ± 4·3	170 ± 6·8	60 ± 11·8	158 ± 7·2
	Liver wt.	5·8 ± 0.4	4·5 ± 0·2	6·2 ± 0·5	7·9 ± 0·3
Hamsters	Liver GPD	24 ± 1·9	66 ± 2·1	32 ± 2·2	70 ± 2·5
	Kidney GPD	27 ± 2·2	42 ± 2·1	25 ± 1·2	44 ± 3·1
	Liver wt.	4·7 ± 0·1	5·2 ± 0·1	5·9 ± 0·4	6·8 ± 0·4
Guinea pigs	Liver GPD	7 ± 0·6	11 ± 1·6	7 ± 0·9	9 ± 0·3
	Kidney GPD	18 ± 0·9	17 ± 1·8	15 ± 2·1	18 ± 1·7
	Liver wt.	3·9 ± 0·1	3·9 ± 0·2	4·3 ± 0·1	3·5 ± 0·3
Chicks	Liver GPD	2 ± 0·8	2 ± 0·6	4 ± 0·8	4 ± 1·0
	Kidney GPD	2 ± 0·4	3 ± 0·5	4 ± 0·8	4 ± 0·8
	Liver wt.	2·5 ± 0·1	3·1 ± 0·1	2·7 ± 0·1	2·8 ± 0·1
Pigeons	Liver GPD	9 ± 1·3	5 ± 1·6	6 ± 1·9	7 ± 2·9
	Kidney GPD	4 ± 1·2	7 ± 0·7	6 ± 1·9	4 ± 1·7
	Liver wt.	2·3 ± 0·2	2·2 ± 0·1	2·6 ± 0·1	2·6 ± 0·4

* Young male animals (6–8 animals/group) were fed an appropriate commercial diet ± 0·3% CPIB (either Na salt or ethyl ester) and were injected s.c. with 40 μg T_4/100 g body wt/day for approximately 2 weeks. Average body weights when sacrificed were: mice, 28; hamsters, 83; guinea pigs, 277; chicks, 86; pigeons, 484 g. GPD = μl O_2/10 min/150 mg fresh tissue; liver wt. = per cent of body wt. Values are expressed as the mean ± S.E.

= 7·91 ± 0·23; 0·3% CPIB, Na salt = 8·16 ± 0·15; 0·3% CPIB, ethyl ester = 7·87 ± 0·18 l. O_2/m^2/hr (mean ± S.E. for 30–45 individual determinations). In another experiment, weanling rats were fed the basal or 0·3% CPIB diets and injected with 40 μg T_4/100 g/day. Metabolic rate determinations were made repeatedly after day 18, and were: basal = 8·21 ± 0·21; CPIB = 8·24 ± 0·17; basal + T_4 = 13·1 ± 0·30; CPIB + T_4 = 10·2 ± 0·15 (mean ± S.E. for 23 determinations). Thorp[29] also found no effect of the drug on metabolic rate or respiratory quotient, and further reported that CPIB partially inhibited the calorigenic effect of T_4. CPIB did not increase the thyroid hormone secretion by these criteria, and the localized GPD response also argues against a general increase in endogenous thyroid hormone output. More than the normal endogenous output of T_4 was not required, since a constant dose of 1 μg T_4 allowed the CPIB to produce its typical liver GPD response in Tx rats.

Since the thyroidal effect of CPIB was confined largely to the liver, the liver might be hyperthyroid while all other tissues remained essentially euthyroid; any increased metabolism occurring in the liver might contribute too little to the overall oxygen consumption to be detected by a metabolic rate measurement. Hence, the effect

of CPIB on liver slice Q_{O_2} was determined, and the results are shown in Fig. 11. The endogenous liver slice Q_{O_2} was increased 30 per cent (from 61 ± 2·1 to 77 ± 3·9 μl O_2/100 mg wet wt/hr.) when the liver GPD was elevated to 114 by feeding CPIB, and the magnitude of this increase was comparable with the effect of T_4 alone or of T_4 plus CPIB. When α-glycerophosphate was added as substrate, the oxygen

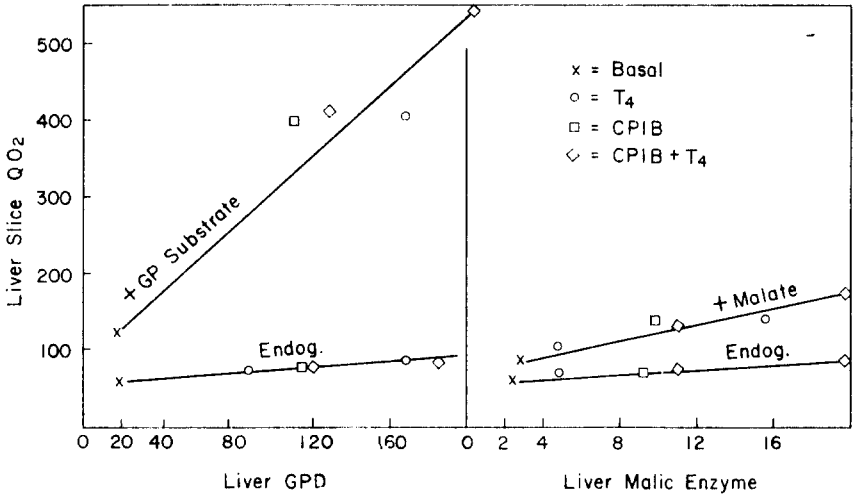

FIG. 11. Correlation of liver slice Q_{O_2} with liver GPD or malic enzyme activity. Weanling male rats were fed the basal or 0·3% CPIB diets and injected with 4 or 40 μg T_4/100 g body wt/day for 2–3 weeks. Liver slice Q_{O_2} was determined as previously described,[16] with and without glycerophosphate or malic acid substrate, and has been expressed as μl O_2/100 mg wet wt/hr. Liver GPD and malic enzyme were determined with homogenates, and have been recorded as μl O_2/10 min /150 mg and μmoles NADPH/min/g fresh tissue, respectively. Each point represents the average of 12–18 determinations for CPIB, 12–30 determinations for the basal diet and 5–12 determinations for the others.

consumption by the liver slice was also increased in proportion to the increased liver GPD whether such increases were produced by T_4, by CPIB or by both together. Similar correlations were also true of the malic enzyme (Fig. 11) except that the addition of malate substrate had much less effect on the liver slice Q_{O_2} than did the glycerophosphate substrate. Hence, CPIB produced a moderate hyperthyroidism in the liver on the basis of Q_{O_2} as well as typical enzyme changes.

DISCUSSION

CPIB had no thyromimetic activity *per se*, but was responsible for converting a small thyromimetic stimulus into a large liver GPD and malic enzyme response. When the thyroid gland was removed, the administration of an amount of T_4 which was equivalent to the normal endogenous output (1 μg/100 g/day) produced a much larger effect in the presence of CPIB. Since this effect was confined largely to the liver, the most obvious possible explanations are: (1) circulating T_4 was concentrated in the liver by displacement from the plasma proteins;[21] or (2) the drug inhibited the normal destruction or removal of T_4 by the liver. In both of these situations the T_4 stimulus to the liver could be increased without a simultaneous stimulation of other tissues. Previous studies have reported a normal thyroidal I^{131} uptake[11] and response

to TSH,[10] as well as a normal T_3-RBC-uptake and unchanged plasma protein-bound iodine concentration[30] in patients receiving the drug.

Neither of these possibilities can be evaluated critically from the available evidence, but the following four points discount both theories. (1) The total amount of T_4 in the plasma of a 100-g rat is less than 0·5 μg, while the effect of the drug is equivalent to a dose of 4–8 μg T_4/day. (2) Any T_4 immediately displaced from the plasma proteins by the drug could not sustain an elevated GPD for 6 weeks. It could be postulated that the activity of any additional endogenous T_4, which is prevented from binding to plasma proteins by the drug, acts almost exclusively on the liver and is 4–8 times as active in the liver as it would otherwise be. However, Osorio *et al.*[31] have reported that the drug does not displace T_4 from plasma proteins and does not affect its biliary excretion. (3) The total liver GPD response with T_4 plus CPIB is larger than the effect produced by large doses of T_4 alone. It is difficult to see how a simple displacement of T_4 from plasma proteins could enhance the "maximal" response to T_4. (4) The injection of small doses of T_4 into rats receiving CPIB had no more effect on liver GPD than the CPIB alone. If the drug were blocking the destruction of T_4, this increased supply should have given a larger response.

Another possible mechanism of CPIB action is suggested by the results shown in Fig. 4. CPIB alone increased the total liver GPD as rapidly as the stimulus produced by 40 μg T_4, and this increased GPD can be attributed to new protein synthesis. Hence, the small stimulus for new protein synthesis normally provided by endogenous thyroid hormone became a large stimulus for new protein synthesis in the presence of CPIB. Some thyroid hormone was required to provide the initial stimulus, but once initiated, the CPIB had the effect of keeping the synthetic machinery "open"; i.e. it prevented the normal cutoff in the synthetic process. Since the liver was synthesizing GPD as rapidly in the presence of CPIB alone as it could do with a large T_4 stimulus, small amounts of additional T_4 had no synergistic effect. The rate at which a large dose of T_4 normally increased the liver GPD was still limited by the normal cutoff mechanisms, but when the latter were blocked by CPIB, this rate was increased still further.

How much of the total effect that CPIB exerts on the liver can be attributed to an intensification of a small T_4 stimulus cannot yet be estimated. The increase in liver mitochondria after CPIB is characteristic of thyroid hormone stimulation, and can be associated with the increased mitochondrial GPD activity described in this report. Both T_4 and CPIB enhance the effectiveness of anticoagulant drugs in increasing prothrombin time.[32] Similarly, the histological appearance of liver glycogen depletion after CPIB[21] resembles the effect of T_4, and some of the increased protein synthesis[21] and protein content[9,21] can also be attributed to T_4. Thorp and Waring[28] have pointed out that a seasonal variation in CPIB activity coincides with a concomitant variation in thyroid and adrenal function. However, thyroid hormone has relatively little effect on liver weight, and it seems unlikely that an intensification of a T_4 stimulus could be solely responsible for the large increase in liver weight which results from CPIB treatment.

Whether the changes in liver GPD or malic enzyme are specifically related to the effectiveness of the drug in lowering blood lipids has yet to be determined. An increase liver GPD activity could be expected to decrease the amount of glycerophosphate substrate available for lipid synthesis, and this in turn could limit the rate at which

triglycerides are synthesized and released from the liver into plasma.[3,9,33,34] Thorp[29] found no effect of CPIB on plasma lipids in hypophysectomized rats. Best and Duncan[20] reported a small lowering of plasma cholesterol in thyroidectomized but not in thiouracil-treated rats. D-T_4 also increases liver GPD (unpublished) and decreases plasma lipids,[35] but acts primarily on the S_f 0–20 rather than the S_f 20–400 lipoproteins.[36] Irrespective of mechanism, CPIB has a limited thyroxine-like effect which alters lipid metabolism without being calorigenic.

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