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INHIBITION OF *p*-CHLOROPHENOXYISOBUTYRATE BY 1-METHYL-2-MERCAPTOIMIDAZOLE AND RELATED COMPOUNDS*

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Abstract—The induction of both rat liver mitochondrial α-glycerophosphate dehydrogenase (GPD) and the soluble malic enzyme by thyroid hormone is enhanced by *p*-chlorophenoxyisobutyrate (CPIB). CPIB also affects lipid metabolism by a mechanism which is unrelated to the thyroid hormone.

The intensification of the T_4 response by CPIB was blocked by the simultaneous administration of the goitrogen, 1-methyl-2-mercaptoimidazole (methimazole, MI), and this particular inhibition of CPIB by MI was shared by a number of other compounds containing the ureido or substituted ureido grouping: 1-methylimidazole, imidazole, thiourea, 2-thiouracil, 2-mercaptobenzimidazole, 2-hydroxybenzimidazole and 2-mercaptopyrimidine. However, the effect of CPIB in (a) preventing an orotic acid fatty liver, or (b) intensifying the β-lipoprotein band in serum gel electrophoresis was not inhibited by imidazole.

Rat liver malic enzyme activity was increased somewhat by MI and other ureido compounds *per se*.

THE ADMINISTRATION of the hypolipidemic drug CPIB† increases the activity of two rat liver enzymes: (1) mitochondrial α-glycerophosphate dehydrogenase (1-glycerol-3-phosphate; (acceptor) oxidoreductase, EC 1.1.99.5, GPD) and (2) soluble malic enzyme (L-malate: NADP oxidoreductase (decarboxylating), EC 1.1.1.40).[1–3] Thyroidectomy abolishes the influence of CPIB on these liver enzymes, but replacement of the hormone from exogenous sources restores this effect of CPIB.[4] When MI was given along with CPIB and T_4, the enhancement effect of CPIB was abolished, and a number of other related ureido compounds were found to have the same effect as MI in this test. These substances probably do not interfere with the hypolipidemic effect of CPIB;[5] at least imidazole did not block the removal of liver fat or the intensification of serum β-lipoproteins by CPIB.

The activity of rat liver malic enzyme is influenced by a number of factors, including dietary alterations, which influence lipogenesis. In the absence of dietary carbohydrate, malic enzyme activity is very low;[6] conversely, it is increased when the diet is rich in glucose or fructose.[7] Fasting and then refeeding a normal diet increases the activity of this enzyme markedly, but fasting and refeeding a diet devoid of carbohydrate results in no elevation of malic enzyme.[6] Meal-fed rats show the same sensitivity to dietary carbohydrate and fat as do fasted-refed rats.[8] In addition to dietary factors,

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† Abbreviations used: CPIB, *p*-chlorophenoxyisobutyrate; GPD, α-glycerophosphate dehydrogenase; MI, methimazole or 1-methyl-2-mercaptoimidazole; T_3, L-3,5,3′ triiodothyronine; T_4, thyroxine.

malic enzyme is increased 8- to 10-fold by the administration of thyroid hormone,[2] and is increased somewhat by the administration of phenobarbital.[9] The present studies showed that MI also increased the activity of the malic enzyme, and that this effect is additive with the response to T_4 or dietary manipulation.

METHODS

Unless specified otherwise, all experiments were conducted with male Sprague–Dawley rats (Holtzman or Blue Spruce) weighing 120–150 g. The compounds were added to a purified diet[10,11] at the expense of carbohydrate and fed *ad lib.* for 14 days. T_3 or T_4 was injected subcutaneously in alkaline (pH 8–9) saline daily on a body weight basis. Liver CPD[11,12] and malic enzyme[2,3] activities were determined as previously described. The GPD activity has been expressed as microliters of O_2/10 min/150 mg of fresh liver and the malic enzyme activity as micromoles of NADPH/min/g of fresh liver. When these enzyme activities were calculated on the basis of protein concentration or total liver activity, the results were either the same as those reported or the reported differences were exaggerated. The results are given as the mean ± S.E. of the mean for groups of eight to twelve rats.

Serum triglyceride concentrations were determined by the method of Fletcher[13] except that the reagent concentrations and the use of arsenite to halt the periodate oxidation were according to Kessler and Lederer.[14] The distribution of alimentary lipid was determined by oral administration (gavage) of a dose of corn oil in which tritiated palmitic acid was dissolved, and subsequent determination of the tissue distribution of the tritium followed the procedure of Bragdon and Gordon.[15]

RESULTS

Methimazole vs GPD response to CPIB. Figure 1 shows the liver GPD response to feeding a diet containing 0·3% CPIB ± 0·1% MI (w/w) at varying doses of T_3.*

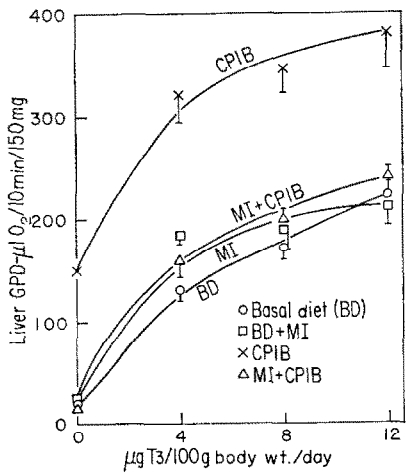

FIG. 1. Effect of MI on the response of liver GPD to CPIB in T_3-treated rats. CPIB (0·3%) and 0·1% MI were included in the diet while T_3 was injected subcutaneously for 14 days. BW = body wt.

* Methimazole and other ureido compounds were obtained from Aldrich Chemical Company, Inc. and *p*-chlorophenoxyisobutyrate (ethyl ester or sodium salt) was provided by Ayerst Laboratories, Inc.

Endogenous thyroid hormone in the euthyroid rats allowed the CPIB to increase the GPD from a basal level of 20 to a level of 150 units. Exogenous T_3 alone increased the liver GPD with increasing doses and all of these responses to T_3 were also enhanced by CPIB. However, no enhancement by CPIB was obtained in the presence of MI. MI alone slightly enhanced the GPD response to T_3 (but not to T_4) and the major effect of MI was the abolition of the CPIB effect. Similar results were obtained when T_4 was used instead of T_3 or when the CPIB was gavaged instead of being added to the diet. MI in the diet reduced food consumption and the CPIB intake to 85–90 per cent of the controls, and the gavage experiments showed that this had no effect on the results.

TABLE 1. ENHANCEMENT OF THE LIVER GPD RESPONSE TO THYROID HORMONE BY CPIB, AND THE INHIBITION OF THIS CPIB EFFECT BY METHIMAZOLE AND RELATED COMPOUNDS*

	Liver GPD (μl O_2/10 min/150 mg)	
	(1 μg T_3)	(1·5–2 μg T_4)
Control	66 ± 6	36–38 ± 2–4
CPIB (0·3% in diet)	184 ± 14	122–140 ± 10–15
CPIB + 0·1% methimazole	76 ± 15	23 ± 2
CPIB + 0·2% 1-methylimidazole	75 ± 10	64 ± 5
CPIB + 0·2% imidazole	82 ± 5	83 ± 5
CPIB + 0·2% thiourea	65 ± 7	40 ± 3
CPIB + 0·2% 2-thiouracil	95 ± 5	
CPIB + 0·1% 2-mercaptopyrimidine	92 ± 8	35 ± 4
CPIB + 0·06% 2-mercaptobenzimidazole		29 ± 2
CPIB + 0·06% 2-hydroxybenzimidazole		39 ± 3

* All rats received the indicated amounts of T_3 or T_4 per 100 g body wt/day for 14 days subcutaneously. CPIB and the other compounds listed were incorporated in the diet at the indicated concentrations. In the absence of exogenous thyroid hormone, the control liver GPD was 20 ± 2 Units. Values are recorded as mean ± S.E.

Table 1 shows that several other compounds containing the ureido or substituted ureido grouping also inhibited this CPIB effect. The liver GPD response to a small dose of either T_3 or T_4 was greatly enhanced by dietary CPIB. MI and the other compounds listed in Table 1 prevented much of this CPIB enhancement. The following compounds had little or no such inhibitory effect on CPIB when the initial stimulus was either T_3 or T_4: benzimidazole (0·2 per cent), 8-mercaptopurine (0·1 per cent), and 2-methylimidazole (0·2 per cent). In the absence of CPIB none of these compounds inhibited the induction of liver GPD by exogenous T_3.

Both CPIB and MI individually increased the liver weight (controls, *ad lib.* = 4·7 ± 0·1; controls, pair-fed = 4·4 ± 0·3; MI = 5·5 ± 0·1; CPIB = 7·1 ± 0·1. When CPIB and MI were fed together, the livers were 6·2 ± 0·1 per cent of the body wt. Other compounds also inhibited somewhat the hepatomegaly produced by CPIB. The liver weights obtained with CPIB plus the test substance were: thiourea = 5·3 ± 0·2; 1-methylimidazole = 5·9 ± 0·2; 2-mercaptoimidazole = 5·4 ± 0·1, 2-hydroxybenzimidazole = 5·2 ± 0·1. The effect of the latter compounds in the absence of CPIB was not determined.

Methimazole vs liver malic enzyme. GPD and malic enzyme are induced concurrently by thyroid hormone.[3] However, the data in Table 2 show that, when T_4 or its analogs were fed in a diet containing MI, the increase in malic enzyme activity was greater than that obtained with the hormone alone. (The apparent inhibition of the GPD response by MI in these experiments can be attributed to a 10–15 per cent reduction in the hormone intake when the diet contained MI, since the hormone was incorporated in the diet in these experiments instead of being injected.) Since the malic enzyme activity varies with diet, the above MI effect might also have been due to diet rather than the drug. Pair-feeding experiments were unsatisfactory for testing this possibility

TABLE 2. INFLUENCE OF METHIMAZOLE (MI) ON THE LIVER MALIC ENZYME RESPONSE TO DIETARY THYROID HORMONE ANALOGUES*

Analog/kg of diet	MI	Malic enzyme (μmoles NADPH/min/g)	GPD (μl O_2/10 min/150 mg)
None	—	2·1 ± 0·2	23 ± 1·3
None	+	2·1 ± 0·3	6 ± 1·3
1·5 mg T_4	—	10·8 ± 0·5	103 ± 16·9
1·5 mg T_4	+	16·5 ± 1·7	81 ± 7·0
0·3 mg Triac	—	6·8 ± 0·9	80 ± 14·0
0·3 mg Triac	+	12·6 ± 1·5	57 ± 4·4
8·0 mg Tetrac	—	16·5 ± 1·5	176 ± 8·8
8·0 mg Tetrac	+	26·0 ± 1·8	140 ± 7·5
15 mg Tetraprop	—	7·1 ± 0·9	88 ± 5·5
15 mg Tetraprop	+	13·8 ± 1·1	64 ± 6·2
0·15 mg Isopropyl-T_2	—	12·9 ± 0·9	165 ± 12·6
0·15 mg Isopropyl-T_2	+	18·0 ± 2·3	108 ± 6·0

* The thyromimetic compounds were mixed in the basal or 0·1% MI diet and fed *ad libitum* to weanling male rats for 3 weeks. T_4, thyroxine; triac, 3,5,3′-triiodothyroacetic acid; tetrac, 3,5,3′,5′-tetraiodothyroacetic acid; tetraprop, 3,5,3′,5′-tetraiodothyropropionic acid; isopropyl-T_2, 3,5-diiodo-3′-isopropyl thyronine.

because the restricted rats became meal-eaters while the rats fed *ad lib.* were nibblers. The effect of diet was ruled out by feeding the MI in a high fat diet which eliminates the fasting–refeeding or meal-eating, nibbling effect. One group of rats was fed a high fat diet[3] containing 0·1% MI *ad lib.* and the other group was pair-fed the same diet without MI for 15 days.* All were injected with 15 μg of T_4/100 g body wt/day. Malic enzyme values for the two groups were 8·4 ± 0·3 and 6·0 ± 0·2 units (μmoles/min/g of fresh liver), respectively; the increased malic enzyme activity produced by MI was significant at $P = 0{\cdot}01$. In another experiment the effect of diet was also ruled out by showing that the stimulation of liver malic enzyme by MI could be superimposed upon the stimulation caused by food restriction alone (Fig. 2).

The same ureido compounds that inhibited the CPIB effect on GPD were tested for malic enzyme stimulation in rats receiving 1 μg of T_3/100 g body wt/day for 14 days. Rats receiving T_3 alone had a liver malic enzyme activity of 6·6 ± 0·33 units. The corresponding activities in the presence of the various test substances were: 0·1% MI, 9·0 ± 0·83; 0·2% imidazole, 12·9 ± 0·83; 0·2% thiourea, 10·1 ± 0·66; 0·2% 2-thiour-

* Protein calories (30%) were the same in the regular basal diet and in this high fat diet: 44% lard, 42% casein, 5% salt mixture, 8% non-nutritive fiber and vitamins.

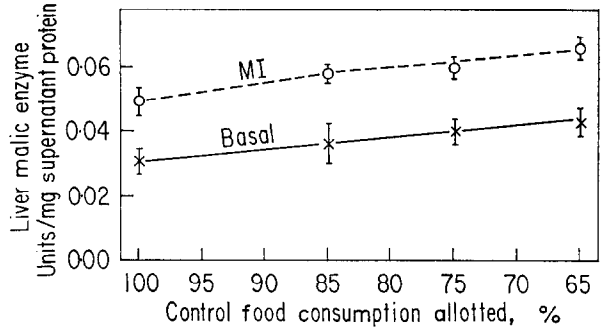

FIG. 2. Effect of food restriction and the additive effect of MI on rat liver malic enzyme activity. Groups of rats were fed the basal diet or 0·1% MI *ad lib.* (100 per cent) or some percentage (85, 75, 65 per cent) of the *ad lib.* consumption. At all levels the MI-fed rats received about 85 per cent of the food given the basal rats. All rats were also injected with 3 μg of T_3/100 g body wt/day for 14 days. The malic enzyme activities have been expressed as micromoles of NADPH per min per milligram of supernatant protein; the results were similar when the enzyme activity was expressed as units per g of fresh liver.

acil, 11·8 ± 0·66; 0·1% 2-mercaptopyrimidine, 10·8 ± 1·10; 0·06% 2-hydroxybenzimidazole, 9·10 ± 0·66; and 0·06% 2-mercaptobenzimidazole, 11·3 ± 0·66; all were significantly increased at $P = 0·01$. 1-Methylimidazole had no effect (7·1 ± 0·55 at 0·2% of the diet). One of the most effective analogs (imidazole) did not decrease food consumption or inhibit growth.

Even though MI increased the malic enzyme activity *per se*, it inhibited the much larger effect produced by CPIB. When all rats were injected with 1·5 to 2·0 μg of T_4/100 g body wt/day to prevent the hypothyroid effects of MI, the following liver malic enzyme activities were obtained: basal diet = 2·6 ± 0·4; 0·3% CPIB = 10·6 ± 0·3; 0·1% MI = 4·5 ± 0·5; CPIB + MI = 4·2 ± 0·4. Thiourea and 2-mercaptoimidazole also inhibited this CPIB effect (5·9 ± 0·4 and 4·2 ± 0·4 respectively). The

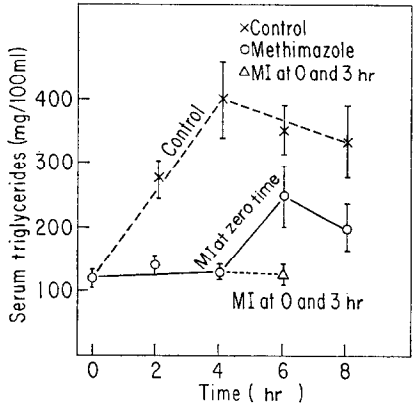

FIG. 3. Effect of MI on serum triglycerides after an oral dose of corn oil. The rats were fasted overnight and given 1·5 ml of corn oil by stomach tube at zero time. The methimazole (7·5 mg/100 g body wt) was injected intraperitoneally at zero time to one group (solid line) and again after 3 hr to another group (dotted line).

effect of the other ureido compounds was ambiguous because any inhibition of the CPIB effect was counterbalanced by a direct stimulation of the malic enzyme activity by the test compound.

In other experiments, 2-mercaptoimidazole or MI (plus 1·5 μg of T_4/100 g body wt/day) gave the same malic enzyme activity in adipose tissue as T_4 alone. Similarly the liver demethylase activity was unchanged or slightly decreased by MI or 2-mercaptoimidazole in these experiments.

Effect on lipids. Serum triglycerides following an oral fat load were comparatively low when the rats were given MI simultaneously (Fig. 3). The normal increase in serum triglycerides to a peak in about 4 hr was decreased and delayed by MI to 6 hr, and the latter peak was further decreased by a second dose of MI 3 hr after the first.

The following experiments showed that MI delayed the emptying of the stomach and thereby delayed the absorption of the lipid into the serum. Control rats receiving tritiated palmitic acid dissolved in corn oil had a higher percentage of the radioactivity in serum, liver, adipose and small intestine 3 hr after oral administration than did the MI-treated rats (Table 3, A). Conversely, the stomachs of MI-treated rats retained

TABLE 3. EFFECT OF METHIMAZOLE (MI) ON THE DISTRIBUTION OF PALMITIC ACID-^{3}H PLACED IN THE STOMACH OR SMALL INTESTINE*

	Per cent of dose of palmitic acid-^{3}H			
	A (oral)		B (injected into sm. intest.)	
	Control	MI	Control	MI
Serum	9·1 ± 2·2	2·2 ± 0·9	1·7 ± 0·2	2·1 ± 0·3
Liver	5·2 ± 0·9	1·5 ± 0·3	4·8 ± 0·5	3·9 ± 0·8
Adipose (epididymal)	7·1 ± 0·9	2·5 ± 0·2	4·7 ± 1·7	7·4 ± 1·3
Stomach plus contents	20·0 ± 4·8	56·2 ± 4·1		
Small intestine plus contents	30·0 ± 1·2	9·0 ± 2·3	50·6 ± 8·2	48·4 ± 5·0
Large intestine plus contents	0·3 ± 0·1	0·2 ± 0·1	0·1 ± 0·01	0·1 ± 0·02

* All rats were offered a 15% sucrose solution in place of solid food for 24 hr prior to administration of the oil. Rats in group A were given by gavage the labeled palmitic acid dissolved in corn oil (5 μCi/0·1 ml; 0·25 ml/100 g body wt). Either methimazole (7·5 mg/100 g body wt) or saline was also injected intraperitoneally at zero time, and the animals were sacrificed after 3 hr. In the rats of group B, a ligature was placed around the region of the pyloric valve and the tritiated palmitic acid–corn oil mixture (5 μCi/0·1 ml) was injected directly into the small intestine (0·1 ml/100 g body wt).

more than twice the radioactivity of the controls. Absorption from the small intestine did not appear to be impaired by MI since the radioactivity in the serum, liver and adipose was comparable in the two groups when the oil was injected directly into the small intestine (Table 3, B).

The effect of MI on fat absorption was unrelated to its stimulation of the malic enzyme since three of the analogs which were most effective in elevating the liver malic enzyme activity (imidazole, 1-methylimidazole, 2-mercaptobenzimidazole) had no effect on the serum triglyceride curve following an oral fat load. A fourth analog (2-hydroxybenzimidazole) actually increased the serum triglycerides at the 2-hr point (438 ± 41 vs controls of 231 ± 13).

Tests on the inhibition of CPIB by MI with respect to the prevention of an orotic acid fatty liver were unsatisfactory because the combination of MI plus orotic acid was toxic and the rats refused to eat. However, the various combinations of 1% orotic acid, 0·3% CPIB and 0·2% imidazole were tested by the procedures previously described[16,17] and gave the following results: (1) imidazole did not alter the liver triglyceride or cholesterol concentrations *per se* (controls = 11 and 5 mg/100 g, respectively) and did not prevent the marked increase in these constituents produced by orotic acid (96 and 35, respectively); (2) imidazole also did not block the prevention of an orotic acid fatty liver by CPIB (10 and 4, respectively); (3) imidazole had no effect on the serum pre-β or β-lipoprotein bands *per se*, and did not prevent the elimination of these bands by orotic acid; and (4) imidazole also did not prevent the intensification of the β-band by CPIB in the presence or absence of orotic acid.

DISCUSSION

CPIB exaggerated the liver GPD and malic enzyme responses to T_4 but had no effect on these enzyme activities in the absence of T_4. CPIB exerted this effect at all levels of T_4 administration but had the most effect relatively at or near physiological levels of thyroid hormone (1–3 μg of T_4/100 g body wt/day). MI is a potent goitrogen which does not inhibit the peripheral effects of T_4 or T_3.[18–21] However, it interfered directly with some of the effects of CPIB since T_4 was administered routinely in all these experiments to prevent any hypothyroid effects from the MI. This property of MI was also shared by some other ureido compounds which have relatively little effect on the synthesis of T_4. The mechanism by which CPIB enhances this effect of T_4 is unknown, but in some way it must be related to an increased synthesis of this enzyme beyond the levels achieved by hormone induction alone. MI did not interfere with the induction process *per se*, but blocked out the added response created by CPIB. Imidazole blocked the enhancement of the T_4 effects by CPIB, but did not block the effects of CPIB on lipid metabolism; this is further evidence that the latter effects are not mediated via an enhancement of the thyroid hormone.[16,17]

Both the liver GPD and malic enzyme activities respond simultaneously and comparably to the administration of thyroid hormone. The GPD response appears to be relatively specific for thyroid hormone, but diet and other factors can alter the malic enzyme. MI and some other ureido compounds increased the malic enzyme activity *per se*, and this effect was additive with the response to food restriction (Fig. 2) or T_4 administration.[16] The exaggerated response obtained with both enzymes in the presence of CPIB was blocked by MI and related compounds, but this effect was more difficult to demonstrate with the malic enzyme because these ureido compounds also had an independent effect on the malic enzyme.

An increased malic enzyme activity after the administration of a drug could theoretically be required to supply an increased demand for NADPH by the microsomal drug-metabolizing enzymes.[9] This does not appear to be the case with these ureido compounds since two that were tested (MI and 2-mercaptoimidazole) did not increase liver demethylase activity.

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