Biochemical Pharmacology, Vol. 22, pp. 797–801. Pergamon Press, 1973. Printed in Great Britain.

ALLYLISOPROPYLACETAMIDE-INDUCED PORPYRIA-PROTECTIVE EFFECT OF 3,5-DIMETHYLISOOXAZOLE

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(*Received* 11 *September* 1972; *accepted* 25 *October* 1972)

Abstract—Allylisopropylacetamide (AIA) administered to rats, increases the liver δ-aminolevulinic acid (ALA) synthetase activity and porphyrin values elevate plasma triglyceride levels and decreases plasma-free fatty acids. 3,5-Dimethylisooxazole (3,5-DMI) markedly lowers the liver ALA synthetase activity, porphyrin levels and also decreases plasma triglyceride levels in AIA-treated rats. 3,5-DMI exerts its antiporphyric activity probably by increasing the protein catabolism.

SEVERAL alterations in lipid metabolism have been reported in experimentally-induced porphyria. A significant increase of plasma lipid phosphorus and total lipids have been described in allylisopropylacetamide (AIA)-treated rabbits.[1] An elevation of plasma total lipid values have also been shown in rats made porphyric with griseofulvin.[2] In rats injected with AIA liver fatty acid synthesis was increased 2-fold.[3]

Also in human patients affected by acute intermittent porphyria some alterations of lipid metabolism have previously been described, consisting of an increase in plasma β-lipoprotein levels.[4] These findings suggest that the alterations of lipid metabolism may be closely related to the derangement of porphyrin metabolism. If this hypothesis is correct, a drug affecting lipid metabolism could display some antiporphyric activity.

We therefore administered to AIA-treated rats, 3,5-dimethylisooxazole (3,5-DMI), a compound known to decrease the free fatty acids (FFA) release[5] and plasma FFA levels,[6,7] to observe whether antiporphyric activity is associated with the antilipidic effect of 3,5-dimethylisooxazole.

MATERIALS AND METHODS

Male Wistar rats, weighing 200 g, were starved for 48 hr before injecting the drugs.

A group of rats were intraperitoneally injected with AIA (400 mg/kg). AIA was dissolved in a solution of water–polyethylene glycol–ethanol (60:30:10). Animals were injected with the vehicle only.

Another group of rats received intraperitoneally 3,5-dimethylisooxazole (100mg/kg). A third group of rats was injected intraperitoneally with both drugs.

Rats were sacrificed by sectioning the jugular veins.

In the first experiment the animals were killed 4 hr after AIA administration.

In the second experiment the animals were sacrificed 4 and 8 hr after AIA injection; plasma FFA,[8] triglycerides,[9] urea,[10] blood glucose[11] were measured. The liver δ-aminolevulinic acid (ALA) synthetase activity was assayed,[12] the liver porphyrins extracted[13] and measured spectrophotofluorimetrically.[14]

TABLE 1. EFFECTS OF AIA, 3,5-DIMETHYLISOOXAZOLE AND COMBINED TREATMENT ON PLASMA TRIGLYCERIDE AND FFA VALUES

Groups	Treatment	No. animals	Triglycerides* (mg/100 ml of plasma)	Free fatty acids (μequiv./100 ml of plasma)
1	Controls	6	48·63 ± 2·32‡	63·19 ± 6·01
2	AIA	6	64·89 ± 1·85	42·25 ± 2·47
3	3,5-Dimethylisooxazole	6	26·60 ± 3·96	36·43 ± 2·25
4	3,5-Dimethylisooxazole† + AIA	6	34·04 ± 2·26	38·53 ± 2·28

Significance levels	Triglycerides	FFA
1–2	$P < 0·01$	$P < 0·05$
1–3	$P < 0·01$	$P < 0·01$
1–4	$P < 0·01$	$P < 0·01$
2–3	$P < 0·01$	N. S.§
2–4	$P < 0·01$	N. S.
3–4	N. S.	N. S.

* Triglyceride and FFA values were measured 4 hr after the AIA administration.
† 3,5-Dimethylisooxazole was intraperitoneally administered 40 min before the AIA injection.
‡ Values shown are mean ± S. E. M.
§ N. S., not significant.

TABLE 2. EFFECTS OF AIA, 3,5-DIMETHYLISOOXAZOLE AND COMBINED TREATMENT ON BLOOD GLUCOSE VALUES AND PLASMA UREA LEVELS

Groups	Treatment	No. animals	Glucose,* (mg/100 ml of blood)	Urea,* (mg/100 ml of plasma)
1	Controls	6	39·72 ± 0·63	47·50 ± 1·20
2	AIA	6	51·47 ± 2·02	52·16 ± 1·61
3	3,5-Dimethylisooxazole	6	34·68 ± 1·95	68·91 ± 2·41
4	3,5-Dimethylisooxazole† +AIA	6	57·32 ± 8·86	66·58 ± 2·33

Significance levels	Glucose	Urea
1–2	$P < 0·01$	$P < 0·05$
1–3	$P < 0·05$	$P < 0·01$
1–4	N. S.§	$P < 0·01$
2–3	$P < 0·01$	$P < 0·01$
2–4	N. S.	$P < 0·01$
3–4	$P < 0·05$	N. S.

* Glucose and urea values were measured 4 hr after the AIA administration.
† 3,5-Dimethylisooxazole was intraperitoneally administered 40 min before the AIA injection.
‡ Values shown are mean ± S. E. M.
§ N. S., not significant.

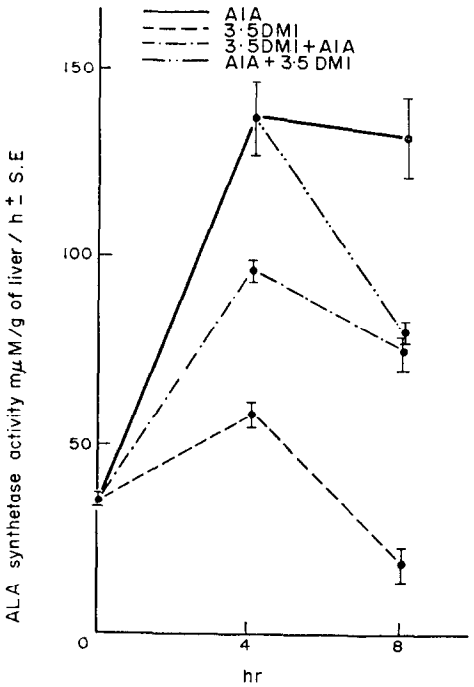

FIG. 1. Liver ALA synthetase activity in rats injected with AIA, 3,5-dimethylisooxazole and both drugs, measured 4 and 8 hr after AIA administration. 3,5-Dimethylisooxazole was administered 40 min before AIA treatment in one group of rats, and 4 hr after AIA injection in another group of animals. The values are the mean of six individual determinations ± S. E.

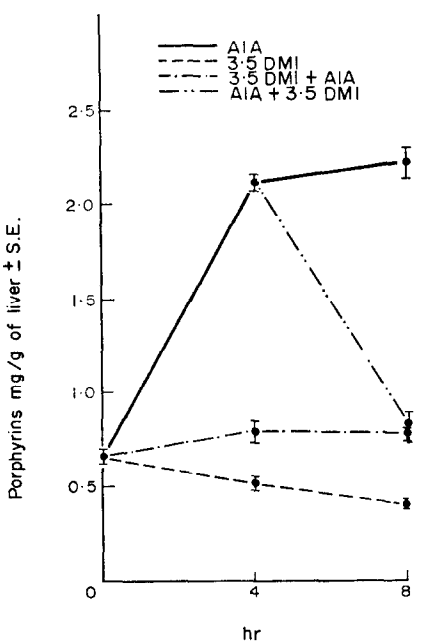

FIG. 2. Liver porphyrin levels in rats injected with AIA, 3,5-dimethylisooxazole and both drugs, measured 4 and 8 hr after AIA administration. 3,5-Dimethylisooxazole was administered 40 min before AIA treatment in one group of animals and in another group of rats it was given 4 hr after AIA injection. The values are the mean of six animals ± S. E.

RESULTS

The results reported in Table 1 show that 3,5-dimethylisooxazole decreased triglyceride and FFA levels in normal and in AIA-injected rats; it was particularly capable of depressing the high triglyceride values induced by AIA administration.

As is shown in Table 2, 3,5-dimethylisooxazole administration does not depress the hyperglycemia observed in AIA-treated rats, while it elevates the plasma urea values in rats even if injected with AIA.

Figure 1 shows a marked elevation of ALA synthetase activity after AIA administration, and a significant lowering of this enzyme in AIA-injected rats whether 3,5-dimethylisooxazole was given before or after AIA administration.

The liver porphyrin values are highly increased in AIA-treated rats and markedly depressed in AIA-injected animals, if treated with 3,5-dimethylisooxazole, whether administered before or after AIA injection, as it is shown in Fig. 2.

DISCUSSION

Hypertriglyceridemia induced by AIA administration had been suppressed by treatment with 3,5-dimethylisooxazole, a well-known antilipemic drug.[5–7] 3,5-Dimethylisooxazole reduces triglyceride levels by depressing FFA release from adipose tissue,[2,5,6] but probably also affects liver fatty acid synthesis, which is increased inAIA-treated rats.[3]

However, it is important to observe that 3,5-dimethylisooxazole exerts not only an antilipemic, but also an antiporphyric activity in the AIA-treated rats.

The diminished ALA synthetase activity and porphyrin amount, in animals receiving 3,5-dimethylisooxazole and AIA is not due to better glucose utilization, which is known to exhibit antiporphyric activity,[15] because glycemia is increased in animals receiving the combined treatment.

The antiporphyric activity of 3,5-dimethylisooxazole could be due to an action on protein breakdown.

Our results show that 3,5-dimethylisooxazole increases plasma ureogenesis in AIA-treated rats. It is known that ureogenesis is linked to increased protein breakdown and amino acid oxidation.[16] Therefore, 3,5-dimethylisooxazole may also stimulate in AIA-injected rats, the oxidation of glycine and δ-aminolevulinic acid and consequently may decrease their incorporation into porphyrins.

By activating protein catabolism, 3,5-dimethylisooxazole may repress the increased RNA and protein synthesis observed in AIA-treated animals;[17] particularly it may lower the messenger RNA synthesis for ALA synthetase[18] and thus decrease ALA synthetase activity and porphyrin levels in animals, even if treated with a porphyrogenic drug.

REFERENCES

1. L. TADDEINI, K. L. NORDSTROM and C. J. WATSON, *Metabolism* **13,** 691 (1964).
2. F. DE MATTEIS, *Biochem. J.* **98,** 23c (1966).
3. R. F. LABBÉ, Y. HANAWA and F. I. LOTTSFELD, *Archs Biochem. Biophys.* **92,** 393 (1961).
4. R. S. LEES, C. S. SONG, R. D. LEVERE and A. KAPPAS, *New Eng. J. Med.* **282,** 432 (1970).
5. G. C. GERRITSEN, W. E. DULIN and F. P. KUPIECKI, in *Drugs Affecting Lipid Metabolism* (Eds. W. L. HOLMES, L. A. CARLSON and R. PAOLETTI), p. 93. Plenum Press, New York (1969).
6. W. E. DULIN, G. H. LUND and G. C. GERRITSEN, *Proc. Soc. exp. Biol. Med.* **118,** 499 (1965).
7. U. SCHWABE and A. HASSELBLATT, *Klin. Wschr.* **44,** 707 (1966).
8. V. P. DOLE and H. MEINERTZ, *J. biol. Chem.* **235,** 2595 (1960).

9. L. A. CARLSON, *J. Atheroscler. Res.* **3,** 334 (1963).
10. W. H. MARSH, B. FINGERHUT and H. MILLER, *Clin. Chem.* **11,** 524 (1965).
11. A. HYVARINEN and E. A. NIKKILA, *Clin. Chim. Acta* **7,** 140 (1962).
12. H. S. MARVER, D. P. TSCHUDY, M. G. PEARLROTH and A. COLLINS, *J. biol. Chem.* **241,** 2803 (1966).
13. S. SCHWARTZ, M. H. BERG, I. BOSSENMAYER and E. DINSMORE, in *Methods of Biochemical Analysis* (Ed. D. GLICK) Vol. 8, p. 221 (1960).
14. A. PINELLI and R. GASPARI, *Clin. Chim. Acta* **39,** 135 (1972).
15. D. P. TSCHUDY, F. M. WELLAND, A. COLLINS and G. P. HUNTER, *Metabolism* **13,** 396 (1964).
16. A. HASSELBLATT, U. PANTEN and W. POSER, in *Metabolic Effects of Nicotinic Acid and its Derivatives* (Eds. K. F. GEY and L. A. CARLSON), p. 1023. H. Huber, Bern (1971).
17. F. DE MATTEIS, *S. Afr. J. Lab. Clin. Med.* (Special Issue) **17,** 126 (1971).
18. H. S. MARVER, A. COLLINS, D. P. TSCHUDY and M. RECHGIGL, *J. biol. Chem.* **241,** 4323 (1966).