J. Pharm. Pharmac. 1966, **18**, 611–614 Received April 6, 1966

Lowering of plasma triglycerides as a result of decreased free fatty acid mobilization

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3,5-Dimethylpyrazole and its metabolite 3-carboxy-5-methylpyrazole lower plasma FFA and decrease the level of plasma triglycerides. The metabolite given intravenously is more rapidly effective than 3,5-dimethylpyrazole. Plasma cholesterol and phospholipids as well as blood glucose were not affected by these acute treatments.

A RELATION between changes of lipid mobilization and alterations of plasma lipids in several animal species has been suggested by several authors. For instance, adrenaline, a potent releaser of free fatty acids (FFA), increases plasma triglycerides in dogs and man (Dury & Treadwell, 1955; Shafrir, Sussman & Steinberg, 1959; Carlson, Boberg & Högstedt, 1965). Adrenocorticotrophic hormone increases plasma triglycerides in rabbits (Woods, Freedman & Kellner, 1962). On the other hand, many drugs known to depress lipid mobilization have been described as hypolipaemic agents. Nicotinic acid decreases plasma triglycerides in rats (Jacobs, Grebner & Cook, 1965), and like salicylates (Alexander, MacDougall, Oliver & Boyd, 1959; Reid, 1961), ganglionic and adrenergic blocking agents (Hollister, Kanter, Powell & Henrich, 1957; Deming, Hodes, Baltazar, Edreira & Torosday, 1958) it reduces plasma cholesterol in man (Altschul, Hoffer & Stepnen, 1955). Recently, 3,5-dimethylpyrazole (DMP) was reported to decrease plasma FFA in a variety of experimental conditions (Gerritsen & Dulin, 1965a; Bizzi, Jori, Veneroni & Garattini, 1964).

This drug has since been shown to decrease lipolysis in adipose tissue (Gerritsen & Dulin, 1965b; Garattini & Bizzi, 1966; Bizzi & Garattini, 1966a) and the formation of triglycerides in the liver (Bizzi & Garattini, 1966b; Bizzi, Tacconi, Veneroni & Garattini, 1966).

These effects of DMP are probably mediated by an acid metabolite, 3-carboxyl-5-methylpyrazole (CMP) (Gerritsen & Dulin, 1965b) which has been isolated from the urine of animals treated with DMP (Smith, Forist & Dulin, 1965).

We report the effect of DMP and of its metabolite on the level of the plasma lipids (triglycerides, cholesterol and phospholipids) in rats.

Experimental

MATERIALS AND METHODS

Male Sprague-Dawley rats, from ALAL breeding, fasted overnight were used. DMP and its metabolite were given orally or intravenously, at doses and times indicated in the Tables. Plasma FFA levels were determined by Dole's method (1956) with minor modifications. A washing with 0·05% sulphuric acid according to Trout, Estes & Friedberg (1960)

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Supported by a grant made from Public Health Service National Heart Institute, National Institutes of Health, Bethesda, Md. 20014 U.S.A. (1-RO1 HE09971-01 SRC).

was adopted to avoid the interference of CMP in the titration. Lipids were extracted with chloroform-methanol 2:1, and washed with saline. The phosphorus of phospholipids was determined in th chloroform extracts according to Lowry, Roberts, Leiner & Farr (1954). The residual chloroform extracts were shaken with silicic acid and centrifuged. Cholesterol and triglycerides were determined in the supernatant using the Lieberman & Burchard reaction, and Van Handel & Zilversmit's (1957) method respectively. Blood glucose was determined according to Hugget & Nixon (1957).

Compounds. DMP was obtained via Fluka AG, Buchs, Switzerland; CMP was kindly synthesised by Dr. F. Rubessa, Department of Pharmaceutical Chemistry, University of Trieste. Other samples of these compounds were subsequently kindly supplied by Dr. G. C. Gerritsen, Upjohn Co., Kalamazoo, Michigan, U.S.A.

Results

The effect on plasma triglycerides of DMP and its metabolite CMP, given orally to fasted rats is shown in Table 1. A lowering of plasma triglycerides occurred with a dose of 1·5 mg/kg for both compounds. The effect increases with increase in dose up to about 50% inhibition; further increasing the dose after this point has been reached does not cause a significantly greater lowering of plasma triglycerides. Previous studies with DMP indicated that increasing the dose resulted in a prolongation of the lowering of plasma FFA rather than in an absolute increase in the fall of the plasma FFA (Bizzi & Garattini, 1966a).

Table 2 shows the time course of the activity of a single dose, 7·5 mg/kg orally, of DMP or its metabolite on FFA, triglycerides, cholesterol and phospholipids and also the level of blood glucose.

The most significant changes were at the expense of FFA and triglycerides, while phospholipids were slightly but not significantly lowered. The decrease in the concentration of plasma FFA always preceded the lowering of plasma triglycerides. The values of FFA were higher 6–8 hr after the treatment than after the controls. Plasma cholesterol and blood glucose were practically unchanged in these experimental conditions. The rate of decrease of plasma FFA and triglycerides when DMP and its metabolite were given intravenously was also investigated. Table 3 shows that the effect of the metabolite was rapid, a statistically significant decrease of plasma FFA occurring after 1 min. At 5 min there was a fall of about 50% while DMP decreased plasma FFA more slowly, showing a 50% decrease only after about 10 min. Plasma triglycerides changed only slightly during this experiment.

Discussion

The present results show that in fasted animals DMP and its metabolite, decrease not only FFA mobilization, but also the level of plasma triglycerides. The two drugs are similar in action orally, but show some differences when given intravenously. By the latter route the metabolite reduced plasma FFA in a shorter time than DMP. The fast rate at which

TABLE 1. EFFECT OF VARIOUS DOSES OF 3,5-DIMETHYLPYRAZOLE (DMP) OR 3-CARBOXY-5-METHYLPYRAZOLE (CMP) ON THE LEVELS OF PLASMA TRIGLYCERIDES

Treatment mg/kg oral	Triglycerides mg/100 ml ± s.e.	
	DMP	CMP
0	86 ± 5	86 ± 5
0·75	86 ± 4	80 ± 8
1·5	48 ± 1*	64 ± 4*
3·7	56 ± 1†	63 ± 4†
7·5	48 ± 3*	43 ± 3*
15	40 ± 2*	46 ± 4*
30	41 ± 2*	57 ± 4*

Rats fasted overnight; determination were made 4 hr after the treatment. Each figure represents the average of at least 5 determinations.
* $P < 0.01$; † $P < 0.05$ relative to controls.

TABLE 2. EFFECT OF 3,5-DIMETHYLPYRAZOLE (DMP) OR 3-CARBOXY-5-METHYLPYRAZOLE (CMP) ON PLASMA LIPIDS AND BLOOD GLUCOSE

Time** min	FFA		Triglycerides		Phospholipids (phosphorus)		Cholesterol		Glucose	
	DMP	CMP	DMP	CMP	DMP	CMP	DMP	CMP	DMP	CMP
30	58*	58*	86	75	102	122	103	106	107	103
60	36*	29*	62*	76	85	83	86	88	103	94
120	42*	37*	76†	70†	93	80	90	—	103	120
240	69*	71*	53*	54*	88	85	96	91	86	106
360	143*	129	97	89	98	88	99	94	82	105
480	146	139*	124	87	97	88	108	100	68	98

** Time between dose and death; drugs were given orally in a single dose of 7·5 mg/kg; each figure represents the average of at least 5 determinations. Controls = 100. The absolute values were for FFA 633 ± 51 μ-equiv/litre; for triglycerides 74 ± 6 mg/100 ml; phosphorus in phospholipids 4·7 ± 0·3 mg/100 ml; cholesterol 71 ± 8 mg/100 ml; glucose 62 ± 7 mg%.
* $P < 0.01$; † $P < 0.05$ in respect to controls.

TABLE 3. LEVEL OF PLASMA FFA, TRIGLYCERIDES AND OF BLOOD GLUCOSE AFTER THE INTRAVENOUS ADMINISTRATION OF 3,5-DIMETHYLPYRAZOLE (DMP) OR 3-CARBOXY-5-METHYLPYRAZOLE (CMP)

Time after treatment (min)	FFA μ-equiv/litre ± s.e.			Triglycerides mg/100 ml ± s.e.		Glucose mg/100 ml ± s.e.	
	Control	CMP	DMP	Control	CMP	Controls	CMP
1	663 ± 22	509 ± 24*	715 ± 10	130 ± 8	130 ± 7	61 ± 5·7	70 ± 2·6
3	668 ± 35	507 ± 48*	732 ± 79	81 ± 4	108 ± 8	57 ± 4·1	67 ± 6·6
5	691 ± 49	299 ± 13*	554 ± 81*	87 ± 7	77 ± 1	66 ± 3·8	63 ± 3·2
10	594 ± 44	207 ± 21*	325 ± 7*	81 ± 2	68 ± 6	72 ± 6·6	72 ± 6·1
20	524 ± 41	146 ± 26*		91 ± 2	78 ± 5	70 ± 6·8	70 ± 4·6
30	529 ± 47	116 ± 21*		90 ± 2	69 ± 6†	70 ± 3·3	75 ± 4·6

Drugs were given i.v. at a dose of 3·7 mg/kg. Each figure is the average of at least 5 determinations.
* $P < 0.01$; † $P < 0.05$ in respect to controls.

plasma FFA decrease after the metabolite was expected because of the known rapid turnover of FFA (Carlson & others, 1965).

The results obtained are also consistent with previous data showing that CMP but not DMP was effective in reducing lipolysis *in vitro* (Smith & others, 1965; Bizzi & Garattini, unpublished), and the findings agree with the hypothesis that the metabolite is the active compound responsible for the effect of DMP. It is interesting to note that the depression of plasma FFA was followed by a period of time (6–8 hr after treatment) in which a large increase of plasma FFA occurred. After the depression of plasma FFA there was always a decrease of plasma triglycerides probably related to a decrease of the liver triglyceride synthesis (Bizzi & Garattini, 1966b; Bizzi & others, 1966).

These results indicate that an important factor controlling the level of plasma and liver triglycerides in fasted rats may be the availability of plasma FFA. The level of plasma cholesterol and phospholipids was unaffected during the period in which FFA were depressed. Blood glucose was only slightly affected and the hypoglycaemia appeared to follow the decrease of FFA. These results are consistent with Randle's hypothesis that a depression of FFA should stimulate glucose utilization (Randle, Garland, Hales & Newsholme, 1963).

Since a high level of plasma triglycerides has been considered a possible negative factor in the development of atherosclerosis, thrombosis and coronary diseases (Albrink, 1960; Schrade & Boehle, 1960; Bizzi, Howard & Gresham, 1963) it is suggested that DMP and its metabolite, by lowering the levels of plasma FFA and triglycerides, might have some therapeutic value in the treatment of such diseases.

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