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M. EFFECT OF DRUGS ON MOBILIZATION OF FREE FATTY ACID

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The mobilization of free fatty acid (FFA) from adipose tissue is subjected to a multiple control responsible for the enhancement or the reduction of lipolysis and of release of FFA into the circulation (18). Insulin at low doses (24) and glucose (7) are typical inhibitors of lipolysis while catecholamines (18), as well as ACTH and other polypeptides (10), are known to activate FFA mobilization. Because of the suggested importance of FFA as a source of energy in situations of emergency and as a substrate for the synthesis of other lipids, it was logical to look for inhibitors of FFA mobilization.

Nicotinic acid has been reported to lower plasma FFA in a variety of experimental situations (6, 9) and to decrease FFA and glycerol release from triglycerides in the adipose tissue *in vitro* (6, 8). β-Adrenergic blockers can also counteract the mobilization of FFA stimulated by noradrenaline (NE) *in vitro* (16), whereas *in vivo* scanty and contradictory data have been obtained (6). More recently salicylic acid (1, 3, 6) and 3,5-dimethylpyrazole (3,5-DMP) (2, 11) have raised considerable interest because of their activity in lowering plasma FFA. Finally butoxamine (see chemical structures in fig. 1), a derivative of methoxamine, interferes with the elevation of plasma glucose and FFA without showing any effect on the conventional tests suitable to detect β-adrenergic blocking activity (5).

This report is intended to summarize some observations made with 3,5-DMP and to propose a tentative classification of the differences observed among the various drugs known to inhibit FFA mobilization *in vivo*.

On injecting 3,5-DMP intraperitoneally, a fall of plasma FFA occurs rapidly and attains its maximum after about 30 min. The proportionality between dose and effect is quite consistent particularly considering the duration of action. Figure 2 shows the effect of 3 doses of 3,5-DMP on plasma FFA in rats fasted 18 hr. The larger dose (10 mg/kg) is still active 4 hr after the administration (the LD50 of 3,5-DMP in rats by intraperitoneal injection is >350 mg/kg). The effect on FFA is not accompanied by an effect on blood glucose (fig. 2). There may be a late hypoglycemic action, which is probably related to an increasing utilization of glucose resulting from the long-lasting decrease of circulating FFA (14). These results are confirmed by those reported in figure 3, where the effect of 3,5-DMP on blood FFA, glycerol and glucose was measured over a large range of doses at a short time (30 min) after the administration of the drug. Even 100 μg per kg of 3,5-DMP can lower FFA and glycerol in fasted animals, while 3.5 mg per kg of the same drug do not affect blood glucose.

A first attempt to demonstrate that the effect on FFA was the result of a reduced lipid mobilization from adipose tissue was to study at the same time the changes of FFA and glycerol in plasma after administration of 3,5-DMP in

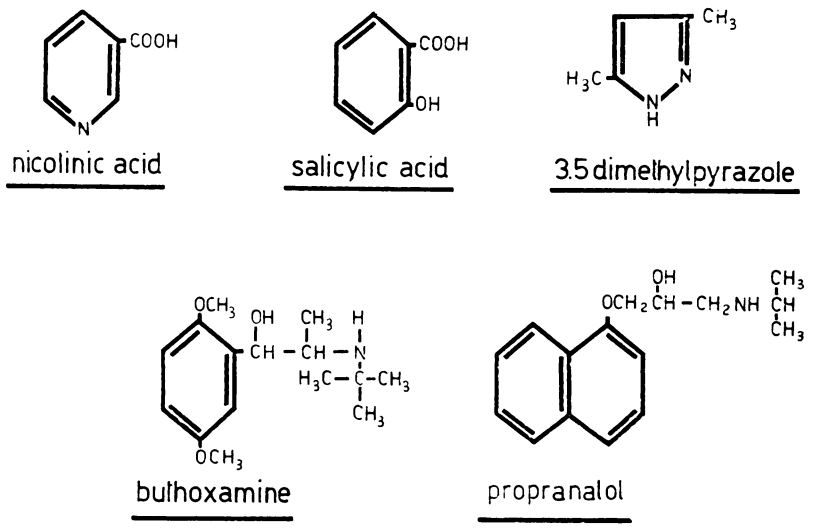

FIG. 1. Chemical structures of drugs that lower plasma FFA

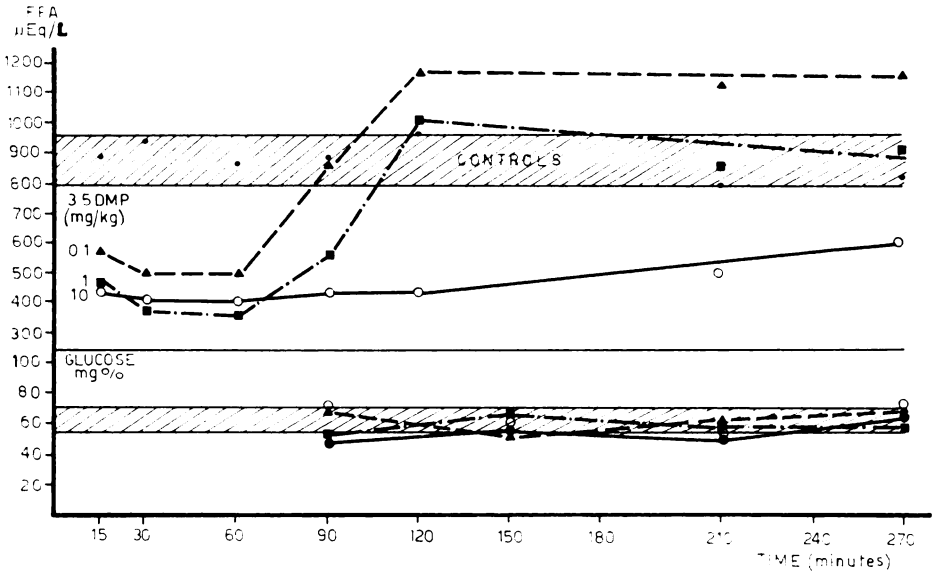

FIG. 2. Effect of 3,5-DMP at different doses (0.1, 1 and 10 mg/kg intraperitoneally) on plasma FFA of 18-hr-fasted rats. Animals were killed by decapitation at the times indicated on the abscissae. Plasma FFA were determined according to Dole (7), blood glucose with the glucose oxidase method according to Hugget (12). Each point represents a minimum of 5 determinations.

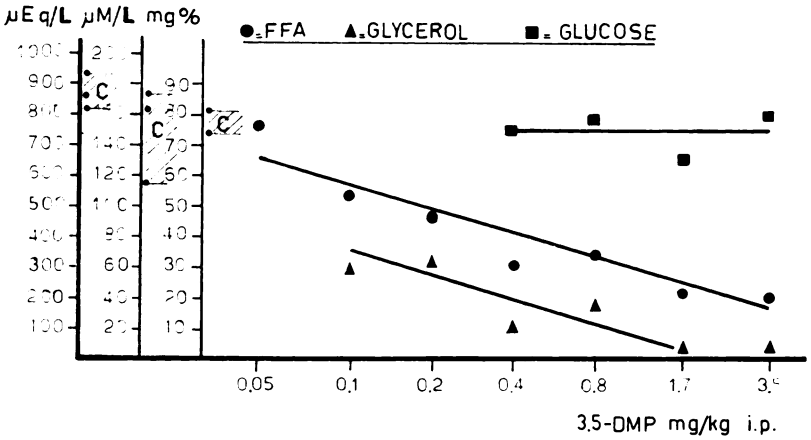

FIG. 3. Effect of 3,5-DMP at different doses (0.05 to 3.5 mg/kg intraperitoneally) on plasma FFA, glycerol and glucose of 18-hr-fasted rats. Animals were killed by decapitation 30 min after the administration of 3,5-DMP. Plasma glycerol was determined with an enzymatic method according to Vaughan (21). C indicates the range of values obtained on untreated animals.

TABLE 1

Effect of 3,5-DMP (7 mg/kg intraperitoneally) on plasma FFA and glycerol 30 min after administration in various experimental conditions

Experimental Condition or Drug	Plasma FFA (μEq/l ± S.E.)		Plasma Glycerol (μM/l ± S.E.)	
	Controls	3,5 DMP	Controls	3,5 DMP
Fed	370 ± 33	253 ± 12 (68)	76 ± 8	52 ± 9 (68)
Fasted 18 hr	600 ± 36	162 ± 10 (26)	112 ± 8	50 ± 4 (43)
Fasted 48 hr	761 ± 25	171 ± 25 (22)	100 ± 6	20 ± 2 (20)
ACTH	620 ± 66	235 ± 12 (38)	239 ± 20	54 ± 10 (22)
NE	928 ± 53	399 ± 17 (43)	258 ± 16	165 ± 9 (64)
Theophylline	656 ± 43	176 ± 15 (27)	250 ± 24	72 ± 5 (29)

ACTH (200 I.U./kg s.c.) was given 2 hr before sacrifice; NE bitartrate (1 mg/kg s.c. as a base in oil suspension) and theophylline (150 mg/kg i.p.) were given at the same time as 3,5-DMP. Numbers in parenthesis represent the percent of the value found in untreated animals.

normal as well as in elevated conditions of lipid mobilization. Table 1 shows that there is indeed a parallel between the decrease of FFA and that of glycerol. The possibility that the decrease of both could have been related to an increased utilization of FFA and glycerol for triglyceride synthesis was excluded because the effect of 3,5-DMP, if any, is to decrease liver and plasma triglycerides in fed resting animals. Furthermore the increase of liver and plasma triglycerides induced by ACTH was prevented by 3,5-DMP; this fact suggests that there was really a lack of circulating FFA, a necessary substrate for triglyceride accumulation in liver.

When 3,5-DMP was given *in vivo* it was possible to demonstrate that the amount of FFA in the rat epididymal adipose tissue was considerably reduced as soon as 5 min after an intraperitoneal injection. Direct experiments aiming to show the effect of 3,5-DMP on fat pad *in vitro* were completely negative in a range of doses from 0.1 to 60 μg per ml of medium. Similar results were obtained on using large or small pieces of adipose tissue and a medium with or without albumin or glucose, or both (unpublished results). Salicylic acid and butoxamine are also inactive *in vitro*, while propranalol shows a clear inhibition on the release of FFA and glycerol only after stimulation with NE. More consistent inhibition was observed with nicotinic acid particularly after stimulation with NE and theophylline, an inhibitor of phosphodiesterase which can increase the level of cyclic 3′,5′-AMP (19).

The lack of effect of 3,5-DMP *in vitro* may be related to the necessity for formation of an active metabolite *in vivo*. This hypothesis is supported by a recent paper (17) suggesting that 3,5-DMP acts on carbohydrate metabolism through the formation of 3-carboxyl-5-methylpyrazole. A demonstration of the effect of 3,5-DMP on lipolysis in adipose tissue was performed by injecting 3,5-DMP into rats in various experimental conditions and examining the release of FFA and glycerol by the adipose tissue excised and incubated *in vitro*. For the *in vitro* part of the experiment the method described by Vaughan (22) was

TABLE 2

Effect of 3,5-DMP (15 mg/kg intraperitoneally) injected 30 min before sacrifice in the experimental conditions described under table 1

Experimental Condition and Drugs	FFA μEq/g Adipose Tissue To	FFA μEq/g/hr Balance a	Glycerol μM/g/hr Balance b	Total Lipolysis μM/g/hr (b x 3)	Re-esterification μEq/g/hr (b x 3) − a
mg/kg					
Fed-resting	3.39	2.68	1.58	4.74	2.06
Fed-resting + 3,5-DMP 15 i.p.	2.73	1.59	0.85	2.55	0.97
18-hr fasting	7.39	2.05	1.89	5.68	3.62
18-hr fasting + 3,5-DMP 15 i.p.	3.12	1.63	1.04	3.15	1.50
48-hr fasting	7.61	4.97	3.10	9.30	4.33
48-hr fasting + 3,5-DMP 15 i.p.	3.03	2.11	2.16	6.48	4.37
ACTH 200 I.U. s.c.	4.78	3.61	3.04	9.12	5.48
ACTH 200 I.U. + 3,5-DMP 15 i.p.	1.91	1.52	1.36	4.08	2.56
NE 1 s.c.	7.97	5.11	4.32	12.96	7.85
NE + 3,5-DMP 15 i.p.	2.38	1.27	2.29	6.87	5.60
Theophylline 150 i.p.	10.59	9.41	6.65	19.95	10.54
Theophylline + 3,5-DMP 15 i.p.	4.03	2.23	1.46	4.38	2.15

Adipose tissue was incubated for 1 hr at 37°C in Krebs-Ringer bicarbonate without albumin or glucose. For details see the footnote in the text. a = [FFA adipose tissue (T 60) + FFA medium (T 60)] − [FFA adipose tissue (To)]. b = [glycerol adipose tissue (T 60) + glycerol medium (T 60)] − [glycerol adipose tissue (To)].

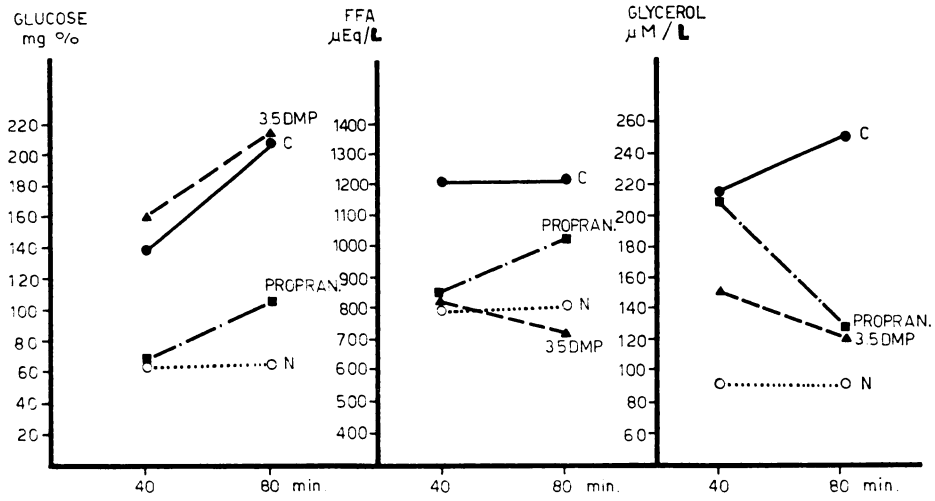

FIG. 4. Effect of 3,5-DMP (7 mg/kg intraperitoneally) and propranalol (10 mg/kg intraperitoneally) on the increase of blood glucose, plasma FFA and glycerol induced by E (1 mg/kg s.c. in oil suspension). Drugs were given together with E and animals were sacrificed 40 and 80 min after E. C, untreated rats (only E); N, control rats (only saline).

substantially followed.[1] Table 2 shows that 3,5-DMP not only decreases the concentration of FFA in the adipose tissue, but it also lowers the formation of FFA and glycerol during incubation. As a result of this effect 3,5-DMP decreases lipolysis in the adipose tissue and, to a lesser extent, inhibits re-esterification. This observation made *in vitro* is sufficient to account for the lowering of FFA and of glycerol observed in plasma after administration of 3,5-DMP. Since the effect of 3,5-DMP on re-esterification is calculated by difference, further studies are needed.

In relation to the strong inhibitory effect exerted by 3,5-DMP on the lipolysis stimulated by NE, it was interesting to investigate possible differences between β-adrenergic blockers and 3,5-DMP. A direct comparison was made by evaluating the effect of propranalol and 3,5-DMP on the increase of blood glucose, FFA and glycerol induced by adrenaline (E) (fig. 4). Propranalol inhibits all the metabolic effects induced by E, while 3,5-DMP is active only in lowering FFA and glycerol, but not glucose. That 3,5-DMP does not act on the hyperglycemia elicited by E was further established by using a wide range of doses (from 1.7 to 30 mg/kg intraperitoneally).

Another difference between β-adrenergic blockers and 3,5-DMP was ob-

[1] The amount of FFA was calculated as the concentration (in μEq/g per hr) present in the medium (without albumin and glucose) after 60 min of incubation (T 60) plus the concentration in the tissue at T 60, minus the concentration present in the tissue at T 0. The glycerol produced was calculated (μM/g per hr) in the same way. The lipolysis was assumed to be 3 times the glycerol produced, as all the glycerol would come from the hydrolysis of triglycerides. The value obtained by subtracting the FFA found from the lipolysis was assumed to be the amount of FFA re-esterified.

TABLE 3

Effect of various drugs on the level of FFA in fed and in 48-hr-fasted rats

Drug	Fed	48-hr Fasting	Remarks
Propranalol	>40	>40	
Pronethalol	>40	>20	Stimulates
MJ 1999	>40	>40	
Butoxamine	>100	>50	Hypoglycemic
3,5-DMP	2.35	0.3	
Nicotinic acid	80	6.2	
Salicylic acid	56	>100	

The ED50, calculated by a graphic method, refers to the dose in milligrams per kilograms able to lower the level of plasma FFA of 50%.

served on determining their effect on the level of FFA of fed and 48-hr fasting rats. As reported in table 3, the β-adrenergic blockers propranalol (4), pronethalol (13), and MJ 1999 (15) do not affect FFA in fed and fasted animals even at very high doses. In many instances there was actually a stimulation, particularly in the case of pronethalol. When the effect observed in fed animals was plotted against the level of FFA in untreated animals it was evident that propranalol increased plasma FFA only if the levels of FFA were low. With higher levels of FFA, propranalol induced a significant decrease (fig. 5). These results can be interpreted to suggest that when for some reasons (noise, stress, *etc.*) there is a relatively high level of FFA, this is due to more catecholamines acting on the adipose tissue and therefore propranalol can exert its adrenolytic effect. When

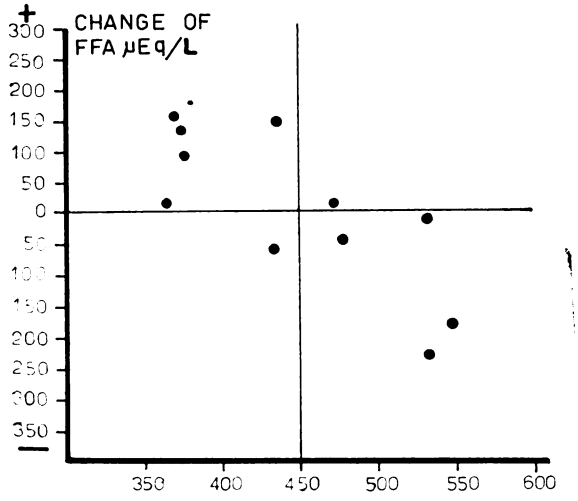

FIG. 5. Changes of plasma FFA after propranalol (5 to 20 mg/kg intraperitoneally). On the ordinates the change observed; on the abscissae the level of plasma FFA of the control group. Animals were sacrificed 30 min after administration of propranalol. Each point represents the average of at least 5 determinations.

TABLE 4

Effect of propranalol, 3,5-DMP and nicotinic acid on various parameters

Drug	FFA 48-hr Fasted	FFA NE	Glycerol NE	Glucose E	Heart Rate IPNE	Blood Pressure IPNE	FFA *in Vitro* NE
Propranalol	0[a]	+	+	+	+	+	+
3,5-DMP	+	+	+	0	0	0	0
Nicotinic acid	+	+	+	0	0	0	+

Heart rate and blood pressure were determined in anesthetized rats (ethylurethane, 2.5 g/kg s.c.) after administration of isoprenaline (IPNE).

[a] 0 = no effect; + = inhibition.

instead the animals are fed and resting, the sympathomimetic activity of propranalol predominates. Table 4 summarizes differences and similarities among propranalol, 3,5-DMP, and nicotinic acid on a number of parameters. 3,5-DMP and nicotinic acid cannot be classified as β-adrenergic blockers. The only difference between 3,5-DMP and nicotinic acid is the failure of the first drug to inhibit the stimulation exerted by NE on adipose tissue *in vitro*. A more detailed analysis of the similarities between 3,5-DMP and nicotinic acid is presented in table 5. On considering doses equipotent in lowering plasma FFA in fasted rats, although 3,5-DMP is considerably more potent than nicotinic acid, it is evident that also their effects on fed animals, whether stimulated or not with NE, ACTH, or theophylline, are very similar, with the possible exception of a greater effect of nicotinic acid in ACTH-treated animals. Table 6 presents a summary of the action of various compounds in conditions of normal or elevated plasma FFA. 3,5-DMP and nicotinic acid are more or less active in all the experimental conditions considered. Salicylic acid differs from the latter drugs because it has no effect on fasted animals. Propranalol inhibits only the increase of plasma FFA induced by NE or ACTH. Butoxamine is weakly active only at high doses and it shows hypoglycemic activity; therefore a conclusion about its effect cannot be drawn. It is possible that in rats butoxamine undergoes rapid metabolism, unlike what has been observed in dogs (J. J. Burns, personal communication).

Taking into account all the results obtained up to now, it is possible to draw a tentative classification of the drugs that lower FFA in plasma. This classification is based upon conditions only in fed-resting, fasted and NE-treated rats. It should be noted also that so far drugs have shown parallel effects on NE and ACTH. From this classification we have omitted drugs which alter FFA as a result of or concomitantly with major alterations of blood glucose. Furthermore, this should be considered a descriptive classification preliminary to a more precise understanding of the biochemical effects of the drugs here considered.

Within these limitations it is possible to distinguish the following groups of drugs lowering FFA. Type 1: active in fed, fasted and NE-treated rats (examples: 3,5-dimethylpyrazole and nicotinic acid). Type 2: active in NE-treated rats but not in fed or fasted animals (examples: propranalol, pronethalol, MJ 1999, and possibly other β-adrenergic-blocking agents). Type 3: active in fed and in

TABLE 5

Comparison of the effect on plasma FFA exerted by nicotinic acid and 3,5-DMP

Drug	Dose	Fasted	Fed	NE (Fed)	ACTH (Fed)	Theophylline (Fed)
	mg/kg i.p.					
Nicotinic acid	6.2	466	75	39	539	494
	12.5	386	158	250	643	521
	25.0		130	493		
3,5-DMP	0.4	426	133	117	322	466
	0.8	407	179	178	364	514
	1.6		130	485		

Animals were sacrificed 30 min after drug administration. Experimental conditions as in table 1.

TABLE 6

Effect of various drugs on plasma FFA in different experimental conditions (see table 1)

Drug	Range of Doses	Fed	18-hr Fasted	48-hr Fasted	NE	ACTH	Theophylline
	mg/kg i.p.						
Propranalol	5–40	↗[a]	±	0	+	+	±
Butoxamine	25–100	±	±	0	0	+	0
3,5-DMP	0.1–15	+	+	+	+	+	+
Nicotinic acid	3.1–50	+	+	+	+	+	+
Salicylic acid	25–100	+	0	0	+	+	+

[a] ↗, stimulation; 0, no effect; ±, occasional effect never exceeding 50% inhibition; +, inhibition proportional to the dose exceeding 50%. The effect was calculated after having subtracted the inhibition exerted by each drug in fed animals.

NE-treated rats but not in fasted animals (examples: salicylic acid). This classification may reflect differences in the biochemical site at which these drugs act. These drugs may be useful tools to investigate the importance of FFA mobilization in a number of physiological and pathological conditions.

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