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LEVELS OF PLASMA-FREE FATTY ACIDS AND LIVER TRIGLYCERIDES IN RATS TREATED WITH ADRENALINE OR THEOPHYLLINE: EFFECTS EXERTED BY ADMINISTRATION OF URIDINE DIPHOSPHATE GLUCOSE

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Abstract—Adrenaline or theophylline administration to rats causes increased levels of plasma-free fatty acids and liver triglycerides; however, plasma FFA and liver triglyceride values are reduced in animals treated either with adrenaline or theophylline if they receive UDPG pretreatment. The protective effect exerted by UDPG in animals receiving the combined treatment is attributable to a decrease of cAMP-dependent lipolytic activity. Furthermore, the antilipemic lowering lipid effect exhibited by UDPG is linked to the intact nucleotide molecule, because compounds structurally associated to UDPG, like uridine, UMP, UDP or glucose-1-phosphate, are incapable of affecting the increased lipolytic activity observed in adrenaline injected rats.

Many compounds affecting lipid metabolism have been reported to cause an increase in liver triglyceride levels by raising the free fatty acid (FFA) release from adipose tissue [1–4]. Liver triglyceride storage had been prevented by drugs capable of blocking FFA release from adipose tissue [1–4].

Among compounds affecting lipid metabolism, some phosphorylated nucleotides like ATP [5], and UDPG [6, 7] have been reported as protecting the liver from fatty infiltration induced by several agents. UDPG administration in particular decreased total liver lipid levels in carbon tetrachloride or ethanol treated rats [6, 7]. However, the authors have not elucidated whether the protective mechanism exerted by UDPG in carbon tetrachloride- or ethanol-injected animals was linked to a blockage of FFA release from adipose tissue. It seemed to us of great interest to study some aspects of the protective action exerted by UDPG in animals treated with drugs capable of increasing liver triglyceride levels.

We have therefore treated rats with adrenaline and theophylline, compounds known to stimulate FFA release from adipose tissue [8, 9] and increase liver triglyceride values; we have observed whether UDPG administration might affect FFA and triglyceride concentration in rats injected with adrenaline or theophylline. We have also administered to rats other substances structurally related to UDPG, like uridine, UMP, UDP or glucose-1-phosphate, and have observed whether the effect on FFA plasma levels is linked only to the intact UDPG molecule, or to its constituents as well.

MATERIALS AND METHODS

Uridine diphosphate glucose (UDPG), uridine, UMP (uridine monophosphate), uridine diphosphate (UDP) and glucose-1-phosphate (G-1-P) were obtained from Biochemia (Milan). The reagents used in these experiments were from BDH (Milan).

Male Sprague–Dawley rats weighing 200 ± 10 g were starved for 48 hr before the drugs were injected, and were kept without food for the duration of the experiments.

Three experiments were performed. In the first experiment one group of animals, kept as a control, was injected with saline solution; a second group was treated intraperitoneally with UDPG (400 mg/kg) divided into three doses administered 7.5, 5 and 2.5 hr before death. Animals in the third group received adrenaline (500 μg/kg) intraperitoneally either 20, 30 or 40 min before death. Rats of the fourth group were treated with both UDPG and adrenaline, administered as mentioned above.

In the second experiment, a control group received a saline solution and another group was treated with UDPG as previously described; rats in the third group were injected intraperitoneally with theophylline (60 mg/kg) 10 or 60 min before death; animals in the fourth group received UDPG and theophylline combined treatment, again following the aforementioned protocol.

In the third experiment several trials were performed. In each trial a group of rats acted as control and was treated with saline solution; another group received adrenaline (500 μg/kg) intraperitoneally 20 min before death. Rats of the third group were treated intraperitoneally with one of the following compounds given at the dose of 0.66 mM/kg divided into three administrations, injected 7.5, 5 and 2.5 hr before death. The drugs tested were UDPG (400 mg/kg), uridine (160 mg/kg), UMP (210 mg/kg), UDP (260 mg/kg) and glucose-1-phosphate (170 mg/kg). Animals of the fourth group were treated with adrenaline combined either with UDPG or with each of the previously mentioned compounds structurally related to UDPG, injected as described above.

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Table 1. Levels of plasma FFA in rats injected with adrenaline, UDPG and their combination

Treatment	Plasma FFA (μmoles/ml ± S.E.)*		
Time after adrenaline administration	20 min	30 min	40 min
Controls	0.642 ± 0.082	0.778 ± 0.047	0.763 ± 0.069
Adrenaline†	1.625 ± 0.143§	0.874 ± 0.052	0.655 ± 0.079
Adrenaline† + UDPG‡	1.013 ± 0.066§	0.676 ± 0.092	0.632 ± 0.061
UDPG‡	0.615 ± 0.071	0.604 ± 0.033	0.577 ± 0.049

* Average of six animals + S.E.
† Adrenaline (500 μg/kg) was given, respectively 20, 30 and 40 min before death.
‡ UDPG (400 mg/kg) was divided in three doses, given 7.5, 5 and 2.5 hr before death.
§ Significance level 2–3 ($P < 0.01$).

The rats, after a brief ether anaesthesia, were killed by dissection of jugular vessels, 7.5 hr after the first UDPG injection. The blood was collected and the livers were immediately removed. Plasma FFA levels were evaluated according to Pinelli [10]; liver triglyceride values were analysed according to Carlson [11].

RESULTS

The data related to the first experiment are shown in Tables 1 and 2. Adrenaline administration to rats increased plasma FFA and liver triglyceride levels. Higher values of plasma FFA were observed 20 min after adrenaline injection, while increased levels of liver triglycerides were seen 40 min after epinephrine treatment.

It is important to note that UDPG pretreatment counteracts the effects of adrenaline injection. In animals receiving the combined treatment, UDPG is capable of significantly lowering either plasma FFA values or liver triglyceride levels even when one would have expected them to be increased by adrenaline administration (see Tables 1 and 2).

The results achieved in the second experiment are

Table 2. Levels of liver triglycerides in rats injected with adrenaline, UDPG and their combination

Treatment	Liver triglycerides (μM/g of wet tissue		
Time after adrenaline administration	20 min	30 min	40 min
Controls	3.80 ± 0.34	4.36 ± 0.25	3.95 ± 0.36
Adrenaline†	5.23 ± 0.27	6.24 ± 0.82	7.84 ± 0.84§
Adrenaline† + UDPG‡	5.37 ± 0.44	5.31 ± 0.49	4.38 ± 0.43§
UDPG‡	4.65 ± 0.19	3.75 ± 0.55	4.20 ± 0.76

* Average of six animals ± S.E.
† Adrenaline (500 μg/kg) was given, respectively 20, 30 and 40 min before death.
‡ UDPG (400 mg/kg) was divided in three doses given 7.5, 5 and 2.5 hr before death.
§ Significance level 2–3 ($P < 0.01$).

Table 3. Levels of plasma FFA in rats treated with theophylline, UDPG and their combination

Treatment	Plasma FFA (μmoles/ml ± S.E.)*	
Time after theophylline administration	10 min	60 min
Controls	0.633 ± 0.078	0.633 ± 0.078
Theophylline†	1.396 ± 0.106§	0.678 ± 0.052
Theophylline† + UDPG‡	1.026 ± 0.078§	0.739 ± 0.092
UDPG‡	0.514 ± 0.049	0.514 ± 0.049

* Average of six animals ± S.E.
† Theophylline (60 mg/kg) was given, respectively 10 and 60 min before death.
‡ UDPG (400 mg/kg) was divided in three doses and given 7.5, 5 and 2.5 hr before death.
§ Significance level 2–3 ($P < 0.05$).

Table 4. Levels of liver triglycerides in rats injected with theophylline, UDPG and their combination

Treatment	Liver triglycerides (μM/g of wet tissue ± S.E.)*	
Time after the theophylline administration	10 min	60 min
Controls	3.98 ± 0.45	3.98 ± 0.45
Theophylline+	5.31 ± 0.38	6.99 ± 0.64§
Theophylline+ + UDPG‡	4.98 ± 0.28	5.35 ± 0.19§
UDPG‡	4.02 ± 0.44	4.02 ± 0.44

* Average of six animals ± S.E.

+ Theophylline (60 mg/kg) was given, respectively 10 and 60 min before death.

‡ UDPG (400 mg/kg) was divided in three doses and given 7.5, 5 and 2.5 hr before death.

§ Significance level 2–3 ($P < 0.05$).

shown in Tables 3 and 4. Theophylline injection caused a marked increase of plasma FFA and liver triglyceride levels. The effect of theophylline on FFA values was quite evident 10 min after its administration, while the action on liver triglyceride concentration appeared 60 min after theophylline treatment. It is important to observe that in rats treated with UDPG and theophylline, UDPG significantly lowers FFA and triglyceride levels, even though they might be expected to be increased by theophylline injection.

Data related to the third experiment are reported in Fig. 1. Adrenaline markedly increased plasma FFA levels 20 min after injection. It is of interest to observe that only UDPG lowered plasma FFA values, while compounds structurally related to UDPG, like uridine, UMP, UDP and glucose-1-phosphate, were incapable of lowering plasma FFA levels in adrenaline injected rats.

DISCUSSION

Adrenaline or theophylline administration increases plasma FFA levels and liver triglyceride values. These results are attributable to a higher activity of adipose

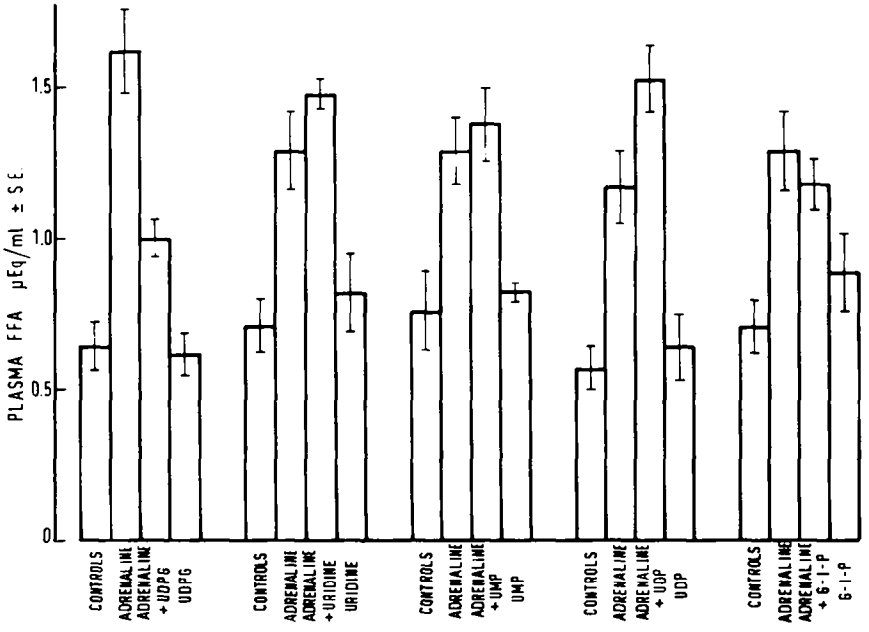

Fig. 1. Plasma levels observed 20 min after adrenaline injection in controls and in rats treated with adrenaline, UDPG, uridine, UMP, UDP, G-1-P and the association of adrenaline combined with UDPG or a compound structurally related to UDPG. Five different experiments are shown. Each column represents the mean ± S.E. for six animals. Uridine, G-1-P, UMP and UDP did not lower plasma FFA levels stimulated by adrenaline injection. Only UDPG markedly decreased FFA levels in adrenaline injected rats.

tissue cAMP-dependent lipase [12]. In fact, the lipolytic activity of this enzyme is increased by adrenaline adenylcyclase stimulation or by theophylline phosphodiesterase inhibition [12, 13]. It is of interest to observe that UDPG pretreatment is capable of significantly lowering plasma FFA and liver triglyceride levels in animals receiving either adrenaline or theophylline. A total lipid lowering effect, exerted by UDPG, has also been observed by other authors in rats injected with carbon tetrachloride [6] or ethanol [7].

It is also important to observe that the antilipolytic effect exerted by UDPG administration seems to be linked to its intact molecule. In fact, no chemical compound related to UDPG, like uridine, UMP, UDP or glucose-1-phosphate, is capable of lowering plasma FFA levels increased by adrenaline injection.

The results observed in our experiments agree with the effects exerted on lipolysis by ATP [5]. This phosphorylated nucleotide had been shown to inhibit lipolysis. The authors have suggested that the decreased lipolytic activity might be linked to higher glucose availability [5].

At the present time the mechanism of the decreased lipolytic activity exhibited by UDPG in our experiments remains speculative. UDPG could lower plasma FFA and liver triglyceride concentration, by inhibiting hormone-stimulated lipolysis or by increasing reesterification or peripheral utilization of fatty acids. Further experiments are needed to elucidate the lipid-lowering effect exhibited by UDPG in epinephrine or theophylline injected rats.

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