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EFFECT *IN VITRO* OF THEOPHYLLINE AND PROSTAGLANDIN E_1 ON FREE FATTY ACID RELEASE AND ON TRIGLYCERIDE SYNTHESIS IN RAT ADIPOSE TISSUE

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Abstract—Experiments *in vitro*, carried out on paired rat epididymal fat pads, show that theophylline increases free fatty acid release and decreases triglyceride synthesis from glucose and palmitic acid. Prostaglandin E_1 is able to antagonize the former without influencing the latter effect of theophylline. Prostaglandin E_1 alone has no effect on basal free fatty acids release and on palmitic acid incorporation, slightly increases, however, glucose incorporation into lipids. It may be concluded that both theophylline and prostaglandin E_1 are affecting free fatty acid release by interfering rather with the breakdown than with the resynthesis of adipose tissue triglycerides.

THERE is a good evidence that triglycerides (TG) in adipose tissue are in a dynamic state, being continuously broken down (lipolysis) and resynthesized, and that the net release of free fatty acids (FFA) depends on the rate of lipolysis and re-esterification. The availability and metabolism of glucose is particularly important in the re-esterification processes, providing, through α-glycerophosphate formation, the glycerol moiety of TG. Thus, the close relationship between glucose utilization and FFA metabolism[1,2] is to be taken into consideration especially when the mode of action of drugs affecting FFA release from adipose tissue is investigated.

It has been found by Vaughan[3] that caffeine inhibits the *in vitro* incorporation of glucose-U-^{14}C into glycerides in adipose tissue and increases at the same time the release of FFA. The inhibition of glucose metabolism (production of $^{14}CO_2$ and incorporation into lipids) has been recently confirmed and caffeine proved to be able to antagonize the effect of insulin on glucose metabolism in adipose tissue.[4] Since insulin is known to reduce FFA release[1] the above mentioned effect of caffeine on glucose utilization might well contribute to its effect on FFA mobilization. However, it has been generally accepted that caffeine increases lipolysis[5] by the inhibition of the phosphodiesterase activity[6] which results in the accumulation of adenosine-3′,5′-phosphate (3′,5′-AMP) and finally in activation of hormone-sensitive lipolytic enzymes.[7] Among the methyl xanthines, theophylline is a more potent inhibitor of phosphodiesterase[8] and also has a true lipolytic activity causing significant increase of glycerol release *in vitro* from adipose tissue.[9] Thus catecholamines and methyl xanthines seem to have cumulative effects on lipolysis, increasing the synthesis and

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inhibiting the break-down of 3′,5′-AMP, respectively, while the effect of caffeine on glucose metabolism in adipose tissue is entirely different from that of catecholamines.[3,10]

On the other hand, prostaglandin E_1 (PGE_1) has been found to be a potent inhibitor of FFA mobilization caused by catecholamines and other lipolytic hormones *in vitro* and *in vivo*.[11–14] Although some of these data indicate an effect of PGE_1 on lipolysis, the mode of its action on adipose tissue metabolism is still under investigation.

The present experiments have been designed in order to establish a possible interrelation between FFA release and TG synthesis in adipose tissue after treatment with theophylline and PGE_1.

MATERIALS AND METHODS

The experiments were carried out *in vitro* on epididymal fat pads from adult, Sprague–Dawley rats, weighing 220 ± 20 g, maintained on standard diet. They were decapitated, the distal part of both epididymal fat pads excised immediately, weighed and incubated. The incubation medium consisted of Krebs–Ringer phosphate buffer (pH 7·4) containing 2·5% bovine serum albumin (Armour Pharmaceutical Company, U.S.A.). After 30 min of preincubation the drugs and labelled precursors for lipid synthesis were added to the medium and the incubation was carried out for a further 60 min, at 37°, in a final volume of 5 ml with continuous shaking and using air as the gas phase. In each experiment the controlateral fat pad of the same rat was used as the control. The final concentration in the medium was 5×10^{-4} M for theophylline monohydrate (Recordati, Milano) and 1×10^{-6} M for PGE_1 (11-α-15-dihydroxy-9-keto-prost-13-enoic acid, supplied by Prof. S. Bergström, Stockholm, Sweden). The dose of PGE_1 was repeated after 30 min incubation, since it is known to be rapidly metabolized. Glucose-U-^{14}C (sp. act. 123 mc/mM) and albumin-bound palmitate-9,10-^{3}H (sp. act. 350 mc/mM, the Radiochemical Centre, Amersham, U.K.) were added to the medium and the total radioactivity was 2 μc of each labelled precursor in the incubation system.

At the end of the incubation time, the tissue and medium were extracted, and FFA concentrations determined, according to the Dole-procedure, as modified by Trout.[15] Aliquots of tissue extract were used for radioactivity measurement of total lipids and TG. TG were separated by thin-layer chromatography[16] using silica-gel G plates impregnated with toluene liquid scintillation solution in alcohol, in order to obtain a direct visualization of the spots under u.v. light.[17] The spots corresponding to TG were scraped out and transferred directly into the scintillation vials. The samples were counted in a Packard Tricarb scintillation spectrometer and internal standards of ^{3}H-toluene and ^{14}C-toluene were used to evaluate the counting efficiency.

The FFA concentrations were expressed as μequiv., the radioactivity values as dpm/g tissue wet weight. The drug effects are shown by expressing the values of tissue incubated with drugs as a percentage of the values obtained for the controlateral, untreated fat pad of the same rat.

RESULTS

The *in vitro* addition of theophylline highly increases the FFA concentration in the incubation system (Table 1, group II). This effect on FFA release is accompanied by a significantly reduced incorporation of both labelled palmitate and glucose into

total lipids and TG (Table 2, group II). In the control fat pads, in the case of palmitate incorporation, 75·3 ± 1·6 per cent of total lipid radioactivity was found in TG, while in the tissues treated with theophylline, only 60·3 ± 2·7 per cent ($P < 0.02$). This may be the reason why the decrease of palmitate incorporation into TG is even greater

TABLE 1. EFFECT OF THEOPHYLLINE AND PGE_1 ON FFA MOBILIZATION IN RAT EPIDIDYMAL ADIPOSE TISSUE INCUBATED *in vitro*

Group	Drug	No. of expts.	Changes in percentage* of FFA concentrations (mean value ± S.E.)	
			Tissue	Tissue + medium
I	PGE_1	5	90 ± 7	99 ± 12
II	Theophylline	6	182 ± 24	493 ± 60
III	Theophylline + PGE_1	6	129 ± 28	221 ± 43
Significance levels:		*C*— I	n.s.	n.s.
		C— II	$P < 0.01$	$P < 0.001$
		C—III	n.s.	$P < 0.02$
		II—III	n.s.	$P < 0.01$

C = controls = 100 ± 0.
* All values expressed as percent of the value obtained for the controlateral, untreated, control fat pad of the same rat.

TABLE 2. EFFECT OF THEOPHYLLINE AND PGE_1 ON PALMITATE-9,10-^{3}H AND GLUCOSE-U-^{14}C INCORPORATION INTO LIPIDS OF ADIPOSE TISSUE *in vitro*

Group	Drug	No. of expts.	Changes in percentage* of the incorporation of:			
			Palmitate-9,10-^{3}H		Glucose-U-^{14}C	
			Total lipids	Triglycerides	Total lipids	Triglycerides
I	PGE_1	5	114 ± 9	111 ± 9	125 ± 8	123 ± 6
II	Theophylline	6	48 ± 4	37 ± 4	62 ± 8	70 ± 9
III	Theophylline + PGE_1	6	62 ± 5	59 ± 6	67 ± 5	68 ± 4
Significance levels:		*C*— I	n.s.	n.s.	$P < 0.02$	$P < 0.01$
		C— II	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.02$
		C—III	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
		II—III	$P < 0.05$	$P < 0.02$	n.s.	n.s.

C = controls = 100 ± 0.
* All values expressed as percent of the value obtained for the controlateral, untreated, control fat pad of the same rat.

than into total lipids. The ratio between palmitate and glucose incorporation (^{3}H:^{14}C) is also lower, because of the greater decrease in palmitate than in glucose incorporation (Table 3, group II). The difference is especially significant in TG in agreement with the above mentioned findings.

PGE_1 alone, at the dose used (10^{-6} M), has no effect on FFA concentrations or palmitate incorporation. However, glucose incorporation into lipids is slightly increased (Tables 1 and 2, group I). On the other hand, PGE_1 is able to reduce significantly the effect of theophylline on FFA release. It failed, however, to influence its effect on glucose incorporation into lipids (Tables 1 and 2, group III).

The decrease in the incorporation of palmitic acid is less, but still significant, in presence of PGE_1 than with theophylline alone (Table 2, groups II and III). In the case of interaction between theophylline and PGE_1 the relative TG radioactivity also increases, with reference to treatment with theophylline alone, and becomes similar to that of controls (in untreated fat pads 77·4 ± 1·7, in theophylline + PGE_1 treated

TABLE 3. CHANGES* IN THE RATIO ^3H:^{14}C IN LIPIDS OF ADIPOSE TISSUE INCUBATED WITH PALMITATE-9,10-^3H AND GLUCOSE-U-^{14}C *in vitro*

Group	Drug	No. of expts.	^3H:^{14}C Radioactivity ratio	
			Total lipids	Triglycerides
I	PGE_1	5	0·92 ± 0·06	0·91 ± 0·06
II	Theophylline	6	0·78 ± 0·07	0·54 ± 0·04
III	Theophylline + PGE_1	6	0·93 ± 0·07	0·87 ± 0·09
Significance levels:		*C*— I	n.s.	n.s.
		C— II	$P < 0·01$	$P < 0·001$
		C—III	n.s.	n.s.
		II—III	n.s.	$P < 0·01$

C = controls = 1·00 ± 0.

* Taking as unity the values obtained for the controlateral, untreated, control fat pad of the same rat.

ones 72·6 ± 5·3 per cent of total lipid radioactivity is found in TG). In agreement with these data the ^3H:^{14}C radioactivity ratio becomes, especially in TG, greater than that obtained after treatment with theophylline alone (Table 3, groups II and III), and does not differ significantly from that of controls.

DISCUSSION

The results obtained with theophylline are in good agreement with previous observations concerning the effects of another methyl xanthine, caffeine, on FFA release[3,18] and glucose incorporation into lipids.[3,4] It seems unlikely, however, that reduced TG synthesis from glucose caused by theophylline would play an important role in its acute action increasing FFA release. The facts that PGE_1 is able to antagonize the effect of theophylline on FFA output without influencing the reduced glucose incorporation into lipids, and that theophylline increases both FFA and glycerol release,[9,19,20] indicate that theophylline is causing FFA mobilization rather through increased breakdown than decreased resynthesis of TG.

The reduced incorporation of palmitic acid into tissue lipids and especially into TG, under the effect of theophylline, is very similar to that observed when palmitic acid incorporation has been investigated in the presence of ACTH, epinephrine[21] or norepinephrine[22] in the medium. In these cases a marked decrease was found in palmitate incorporation into neutral lipids, accompanied with higher radioactivity values in FFA. Detailed studies in this field indicated that such results alone should not be interpreted as a suppression of TG synthesis.[23] Our results, concerning especially the differences in palmitate incorporation into TG and ^3H:^{14}C ratios in TG of tissues treated with theophylline and theophylline + PGE_1, also suggest that the

reduced palmitate incorporation is, in part, an effect secondary to the increased FFA concentrations in the medium and to changes in FFA pools in the tissue. However, taking into consideration that in experiments where FFA release is reduced by PGE_1 the incorporation of palmitate is still decreased significantly and ^3H:^{14}C ratios do not increase above the normal values, these data together with those of glucose incorporation support the conclusion that theophylline decreases TG synthesis, from both glucose and palmitic acid, in adipose tissue. Further investigations are required to establish if this is a consequence of an effect of theophylline on the uptake of glucose and FFA.

As far as the effect of PGE_1 is concerned, it was found in this laboratory that PGE_1 was able to antagonize the effect of theophylline not only on FFA release, as presented here, but to similar extent also on glycerol release.[19,20] It fails, however, to influence the depression of glucose incorporation into lipids caused by theophylline. Furthermore, it seems to have no direct effect on palmitate incorporation and the slight increase in glucose incorporation into lipids does not seem to be of importance in its effect on FFA release. These facts together indicate that PGE_1 is primarily acting on lipolysis and not on the re-esterification against FFA mobilization.

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