and rupture of lysosomal membranes by other fat soluble compounds including vitamins and steroids are well documented.[7]

Δ^1-THC and cannabidiol interact with the erythrocyte membrane in a way leading to protection against hypotonic hemolysis at a concentration range[3] at which they cause total rupture of lysosomal integrity. The marked difference in the effect of the two components on the two membrane limited structures might be explained by a higher expending capacity of the erythrocyte membrane (inferred from measurements of increase in volume as a result of the interaction with hashish components)[3] as compared to that of the lysosomal membrane.

Though the action of Δ^1-THC and cannabidiol does not seem to be specific, the irreversible damage and release of lysosomal enzymes may be of biological significance. A high concentration of these components in the liver or the nervous system may lead, aside from the psychomimetic effect, to permanent damage on the cellular level. When testing Δ^1-THC on cells and subcellular organelles and inferring from its action on the psychomimic phenomenon it seems worthwhile to test also non psychoactive components as cannabidiol as it gives an indication to the degree of specificity of the biological effect.

*Department of Biophysics,
The Weizmann Institute of Science
Rehovot, Israel
and
The Negev Institute for Arid Zone Research,
Beer Sheba, Israel

AVRAHAM RAZ*
AVITAL SCHURR
AVINOAM LIVNE
RACHEL GOLDMAN*

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Effect of an amphetamine derivative on rat adipose tissue lipolysis and glycerol utilization *in vitro*

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NUMEROUS synthetic analogues of the naturally occurring sympathomimetic amines have been shown to possess varying capacities for mobilizing fat.[1] One of these analogues, amphetamine, may exert its fat-mobilizing action by releasing endogenous catecholamines or through an effect on serotonin receptors.[2–4] We have studied the effect of an amphetamine derivative, the chlorhydrate of [(methyl-1 phenyl-2) ethylamine]-3 propionitrile (CMPEP) on adipose tissue metabolism *in vitro*. As we have observed previously that glycerol utilization by adipose tissue is considerably higher than previously thought,[5,6] in this study we have determined the effect of CMPEP on the *in vitro* production and utilization of glycerol by rat epididymal fat-pads.

Pieces of epididymal fat pads from fed rats were incubated at 37° for 120 min in Krebs–Ringer bicarbonate buffer, pH 7·4 in the presence of 10 mg/ml of bovine albumin purified according to Chen,[7] 0·5 μCi/ml of (1-^{14}C) glycerol (15·3 mCi/mmole) and different concentrations of CMPEP (gift of Roussel-Amor Gil laboratorios). Details of the incubation and the processing of the samples have already been described.[5,6] As it is shown in Table 1, the amount of glycerol formed by the

tissues during the incubation was not affected by the presence of 0·01 and 0·05 mg/ml of CMPEP in the medium. However, higher doses of the drug (0·5 mg/ml) produced a significant increase in the production of glycerol by the tissue (Table 1), which was specially apparent when each value was expressed as a percentage of its respective basal control, i.e. the same tissue, incubated without the drug ($P < 0·01$). The effect of 5 mg/ml of CMPEP was exactly the opposite: it produced a dramatic inhibition in the production or release of glycerol by the tissue (Table 1). The amount of glycerol found in the medium after the incubation in the presence of these doses of the drug was lower than that found in its absence.

TABLE 1. EFFECT OF THE CHLORHYDRATE OF [(METHYL-1 PHENYL-2) ETHYLAMINE]-3 PROPIONITRILLE (CMPEP) ON GLYCEROL FORMATION AND UTILIZATION BY RAT EPIDIDYMAL FAT-PAD INCUBATED *in vitro**

Additions to the medium	Formation of glycerol† (μmoles/100 mg)	Utilization of (1-^{14}C) glycerol (% of initial radioactivity/100 mg)	
		(^{14}C)CO_2	[^{14}C]labelled total lipid
None	0·102 ± 0·017	2·18 ± 0·42	22·7 ± 2·7
CMPEP, 0·01 mg	0·113 ± 0·015	2·69 ± 0·47	18·0 ± 2·4
P‡	N.S.	N.S.	N.S.
CMPEP, 0·05 mg	0·104 ± 0·017	3·05 ± 0·46	17·7 ± 1·8
P	N.S.	N.S.	N.S.
CMPEP, 0·5 mg	0·159 ± 0·017	1·73 ± 0·31	13·5 ± 1·7
P	< 0·05	N.S.	< 0·02
CMPEP, 5 mg	0·054 ± 0·010	2·68 ± 0·57	14·3 ± 2·2
P	< 0·05	N.S.	< 0·05

* The drug was added at zero time, and the incubation lasted 120 min. Values are means ± S.E.M. of six rats.

† Glycerol in tissue + medium.

‡ P corresponds to the differences between each group and its basal control (incubated in the absence of the drug). N.S. = not significant, i.e. $P > 0·05$.

As we have shown previously, adipose tissue incubated *in vitro* utilizes glycerol, mainly for the synthesis of lipids and for its complete oxidation to CO_2[5,6]: an interpretation of these results in terms of the effect of the drug on the rate of lipolysis and esterification demanded some index of the amount of glycerol that was taken up by the tissue under these experimental conditions. Thus the incubation was carried out in the presence of trace concentrations of (1-^{14}C) glycerol. The percentage of labelled glycerol converted to (^{14}C) CO_2 was affected only by 0·5 mg/ml of CMPEP which produced a decrease in this parameter (Table 1) ($P < 0·001$ vs the basal controls, when calculated as percentage of each respective basal value). The amount of cold glycerol in these vials was higher than that in the controls which reduced the number of counts converted to (^{14}C) CO_2 due to dilution of the tracer, although the actual synthesis of the compound was unaltered. The results indicate that these doses of CMPEP did not affect the net oxidation of glycerol to CO_2. Similarly, although higher doses of CMPEP reduced the amount of glycerol in the system, the specific activity of the tracer was higher. The unaffected percentage of radioactive glycerol converted to CO_2 might indicate therefore that the net synthesis of CO_2 is actually inhibited. Similar results were observed when the formation of [^{14}C]labelled total lipids from (1-^{14}C) glycerol was considered. The percentage of radioactivity converted to total lipids decreased as the concentration of the drug in the incubation medium increased, the difference from the basal controls being statistically significant at concentrations of 0·5 and 5 mg of CMPEP (Table 1). Here again, the effect produced by 0·5 mg of the drug may just be due to the decreased specific activity of the tracer in these vials and not to an effect on the net synthesis of lipids. The reduction of the radioactive glycerol converted to lipids, produced by 5 mg of CMPEP, would be even greater when the small amount of glycerol in the system (higher specific activity) of these vials is taken into account.

In all the tissues, incubated with or without the drug, more than 99·1 per cent of the radioactivity incorporated into total lipids was in the glyceride glycerol fraction, as we found previously under other conditions.[5,6]

DISCUSSION

We have demonstrated that the amphetamine derivative, CMPEP, has an *in vitro* effect on adipose tissue metabolism, which is dose dependent. A dose of 0·5 mg/ml produced an increase in the rate of lipolysis, affecting very slightly the reutilization of the glycerol by the tissue, since the reduction of (^{14}C) CO_2 and ^{14}C-total lipids formation from (1-^{14}C) glycerol could simply be explained by a decrease in the specific activity of the tracer and not by an action on the net synthesis of these compounds. The effect is biphasic as higher doses of the drug produced an intense inhibition of the lipolysis and of the reutilization of glycerol by the tissue, specially for the synthesis of glyceride glycerol. This biphasic effect of CMPEP on adipose tissue lipolysis could be related to the biphasic effect of this drug on the release of catecholamines by the perfused adrenals.[8] It is possible that the drug acts on adipose tissue metabolism by affecting the release of catecholamines from the sympathetic terminals, still present in the fat pads incubated *in vitro*, and not by a direct action. Further investigation is required to support or refute this possibility.

A second explanation of the biphasic effect of the drug could be that, at high concentrations, it inhibits lipolysis by an enhanced accumulation of free fatty acids into the adipocyte as a result of a previous activation of lipolysis.[9] This possibility seems unlikely as data not shown demonstrate that these doses of CMPEP produce an inhibition in the release and reutilization of glycerol by adipose tissue after shorter periods of incubation (30 and 60 min) than those used here.

In the present study we have demonstrated once more that adipose tissue reutilizes glycerol for the synthesis of glyceride glycerol and for its complete oxidation to CO_2 and it seems therefore that glycerokinase activity might be considerably higher than previously thought. The rate of reutilization of glycerol by adipose tissue does not necessarily parallel the rate of lipolysis, it must therefore be taken into account when the true rates of lipolysis and esterification are calculated.

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Cátedra de Fisiología General
Facultad de Ciencias
Universidad de Barcelona
Barcelona-7, Spain

EMILIO HERRERA
ANGEL PASCUAL

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Inhibition of brain adenylate cyclase by ethacrynic acid and dithiobisnitrobenzoic acid

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THE ESSENTIAL role of cyclic AMP as a second messenger in biological systems is well established.[1] The biochemical and physiological effects of cyclic AMP are frequently studied by utilizing inhibitors of phosphodiesterase which delay degradation of cyclic AMP. A more direct evaluation of the role of cyclic AMP in biological responses might be possible if potent inhibitors of adenylate cyclase, the