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EFFECT *IN VITRO* OF NICOTINIC ACID 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 nicotinic acid increases the glucose incorporation into lipids only when great amounts of tissue FFA are available, as after fasting or norepinephrine treatment. The data indicate that nicotinic acid has no effect on glucose uptake and on lipid synthesis after phosphatidic acid formation. It is suggested that under the influence of nicotinic acid *in vitro* an increased re-esterification from α-GP and tissue FFA takes place.

In RECENT years it has been demonstrated several times that nicotinic acid lowers the level of the free fatty acids (FFA) of plasma in human beings and experimental animals and inhibits both *in vitro* and *in vivo* the lipolytic effect of catecholamines or ACTH.[1, 2, 3, 4] In addition, it also proved to be able to antagonize the increased FFA release during fasting, i.e. when the lipid mobilization from the adipose tissue is independent of the catecholamine-sensitive lipolytic system.[5] The suggestion that nicotinic acid acts directly in the adipose tissue against FFA mobilization, was supported recently by the macroautoradiographic demonstration of a rapid accumulation of nicotinic acid in adipose tissue,[6] as a characteristic phenomenon for its initial distribution in the organism.

The mode of action of this drug, however, has not yet been elucidated. Referring to the dynamic state of the stored triglycerides (TG), i.e. to their simultaneous breakdown and resynthesis, there are two main possibilities for its effect:

(1) the inhibition of the different lipolytic enzyme-systems responsible for FFA mobilization[7, 8] and/or

(2) an increased resynthesis, which can also be influenced on different steps.[9]

In the present investigations we wanted to study the latter possibility, i.e. the influence of nicotinic acid on the resynthesis of the adipose tissue lipids. Recently Östman[10] observed in alloxan-diabetic rats that nicotinic acid increased the incorporation of palmitate-1-^{14}C into the neutral lipids *in vitro* which may be the consequence of an enhanced esterification. The close relationship between glucose utilization and FFA metabolism in the tissues—meaning that a decreased glucose metabolism (diabetes, fasting) is generally accompanied by increased FFA release and, conversely, an increased glucose metabolism leads to an enhanced esterification and consequently reduces the release of FFA[11, 12]—prompted us to investigate the *de novo* TG synthesis by the incorporation of glucose-U-^{14}C into the adipose tissue lipids. On the other hand,

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because of the lack of glycerokinase activity, the adipose tissue is unable to re-incorporate free glycerol which is produced in TG hydrolysis due to the lipolytic enzymes and thus α-glycerophosphate (α-GP)—the major source of which is certainly glucose—has a particular importance in the synthesis of new TG in adipose tissue[13, 14]

To check the possible effect of nicotinic acid on the lipid synthesis from glucose, investigations *in vitro* were made at different fatty acid pools and metabolic states using adipose tissue fragments from fed and fasted rats, in the absence and presence of norepinephrine in the medium.

MATERIALS AND METHODS

The experiments were carried out *in vitro* on epididymal fat pads from adult, male Sprague-Dawley rats, weighing 250 ± 20 g, maintained on standard diet. Normal fed rats were deprived of food for 3 hr, fasted ones for 48 hr, but with free access to tap water. They were killed by a blow on the head, the distal part of both epididymal fat pads immediately excised, weighed and incubated. After 30 min of preincubation in 5 ml Krebs-Ringer phosphate buffer (pH 7·4) containing 1 % bovine serum albumin, the tissues were transferred to the incubation medium, consisting of the same buffer solution with 2·5 % albumin content and actually of substances in investigation. The incubations were carried out for 60 min in final volume of 5 ml with continuous shaking and using air as the gas phase. For an analysis of the drug's effect the controlateral epididymal fat pad of the same animal was used as control.

Na-nicotinate.$2H_2O$ (Bracco, Milano, Italy) was added to medium in 0·1 ml volume to have a final concentration of 2×10^{-3}, 2×10^{-4} or 2×10^{-5} M. These doses of nicotinic acid were selected in order to obtain an important reduction in output of FFA from adipose tissue to the medium (F. BERTI and M. M. USARDI: Personal communications). Norepinephrine bitartrate.H_2O (Recordati, Milano, Italy) was used in 0·4 γ/ml or 1 γ/ml concentration in the medium. D-glucose-U-^{14}C (specific activity 48·3 mc/mM, Department Biologie, CEA, France) was dissolved in buffer and the activity in the medium was 0·4 μc/ml. Insulin (Boots Pure Drug. Co. Ltd , Nottingham, England) was added in 0·1 U/ml concentration. Special conditions of the incubation are mentioned in the tables.

After the incubation the tissue fragments were washed in buffer and saline solutions and total lipids were extracted according to Folch *et al.*[15] From the chloroform phase aliquots were taken for total radioactivity measurement and thin-layer chromatography for separation of the lipid components. The latter was carried out on silica gel G layers 250μ thick and the developing solvent was petroleum ether:diethyl ether: acetic acid (70:30:1) as described by Privett *et al.*[16] In some experiments monoglycerides (MG) were analysed on another plate with a solvent system consisting of petroleum ether:diethyl ether: acetic acid in 10:90:1 proportion, to separate them from the more polar lipids (phospholipids, etc., PL). After the chromatograms were removed from the chamber and dried, the spots were visualized by spraying the plates first with the toluene liquid scintillation solution (containing PPO and POPOP) and then with distilled water. The wet chromatograms were observed under u.v. light and the spots outlined.[17] For the better identification of the components a standard mixture of mono-, di-, tripalmitin and palmitic acid was also used on every plate. After the chromatograms have been dried on air, each spot corresponding to a component was scraped out and transferred directly into the scintillation vial. One millilitre of petroleum ether: diethyl

ether: acetic acid (10:90:1) solvent—to facilitate the separation of lipids from silica gel—and 5 ml toluene liquid scintillator were then added to the vial. The activities recovered from the chromatograms were compared tot hose of a similar aliquot of the lipid extraction placed directly in the vial and after the evaporation of the solvent treated in the same way. The samples were counted in a Packard Tricarb scintillation spectrometer. An internal standard of ^{14}C-toluene was used to evaluate the quenching characteristics of the samples. Our results obtained with this direct combination of thin-layer chromatography and liquid scintillation counting, agree with those of Snyder and Stephens[18] or Brown and Johnston[19] concerning the high percentage recovery from the chromatoplates.

The radioactivity of the medium was measured according to the method of Herberg.[20]

RESULTS

In the first series of experiments epididymal fat pads of fed animals were used and the results are in Table 1. Nicotinic acid has no effect on the glucose incorporation into lipids at 10^{-4} M concentration in normal condition, but causes a significant, if moderate, elevation of total lipid radioactivity when norepinephrine is also present in the medium. Norepinephrine itself does not influence significantly the total activity of lipids in such conditions, it changes, however, the distribution of radioactivity in the diglycerides (DG) and TG. It induces a marked decrease in DG activity and a respective increase in that of the TG. Nicotinic acid has no effect on the distribution of radioactivity in the different lipid components, either in the absence or presence of norepinephrine in the medium. It is to be mentioned, referring also to the results below, that the radioactivity of FFA and MG spots or regions were also measured, but it was always very low (under 1% of the total) and therefore it is not shown in the tables. Though there is some possibility that the glucose used as precursor was providing not only the glycerol moiety but also fatty acids in the newly formed neutral lipids, in this case it does not seem to have any importance from the point of view of esterification processes.

The total lipid radioactivity is much lower in adipose tissue from fasted rats than in that of fed ones (Table 1 and Table 2). In fasted state, nicotinic acid shows a moderate effect also at 10^{-5}M concentration. More evident and significant increase is observed, however, in the lipid radioactivity at 10^{-3} M concentration. At the same time there is no important change in the radioactivity of the medium (glucose uptake). The relative radioactivity of the lipid components does not vary either. On the contrary, at the dose used in these experiments, insulin causes an extreme increase in total lipid activity associated with a significantly lower medium radioactivity (increased glucose uptake) and with a decrease in PL and some increase in TG radioactivity (Table 2).

When the adipose tissue fragments from fasted animals were incubated in the presence of norepinephrine, the total lipid radioactivity was even lower. The change in the medium radioactivity also indicates lesser glucose uptake than was observed in the absence of norepinephrine (compare the control groups in Tables 2 and 3). The above mentioned effect of norepinephrine on the distribution of radioactivity in the lipid components is also very apparent in fasted state. The decrease of DG and increase of TG is particularly significant when norepinephrine and labelled glucose are added at the same time to the medium similar to the experimental conditions used at fat pads

TABLE 1. EFFECT OF NICOTINIC ACID ON GLUCOSE-U-^{14}C INCORPORATION IN THE LIPID FRACTIONS OF ADIPOSE TISSUE FROM FED RATS

Group	Treatment	No. of Exper.	Tissue weight (mg ± S.E.)	Total DPM × 10^3/g	% increase	Radioactivity of lipids (mean value ±S.E.) % of total in lipid components PL	DG	TG	Recovery (%)
I	None	7	243 ± 10·2	249·9 ± 26·3	—	5·9 ± 0·4	20·7 ± 1·5	65·9 ± 2·3	92·6 ± 1·9
II	NA	7	242 ± 18·7	248·9 ± 19·3	0·0	6·2 ± 0·2	21·8 ± 1·7	64·7 ± 1·5	92·6 ± 1·9
III	NE	7	238 ± 9·5	271·1 ± 21·9	—	5·5 ± 1·3	11·0 ± 2·4	77·8 ± 1·3	94·3 ± 1·1
IV	NE + NA	7	238 ± 9·4	343·5 ± 43·4	26·6	5·2 ± 0·2	11·6 ± 0·4	78·7 ± 0·8	95·7 ± 1·0

NA = nicotinic acid, in 2×10^{-4} M final concentration.
NE = norepinephrine, in 1 μg/ml concentration.
PL = polar lipids (phospholipids).
DG = diglycerides.
TG = triglycerides.
DPM × 10^3/g = disintegrations per minute/g tissue wet wt.

TABLE 2. EFFECT OF NICOTINIC ACID ON GLUCOSE-U-^{14}C INCORPORATION IN THE LIPID FRACTIONS OF ADIPOSE TISSUE FROM FASTED RATS

Groups (No. of Exper.)	Treatment	Tissue weight (mg ± S.E.)	Medium % decrease	Radioactivity (mean values ±S.E.) Tissue lipids DPM × 10^3/g	% increase	% of total in lipid components PL	DG	TG	Recovery (%)
I (8)	None	267 ± 6·0	8·3 ± 1·0	94·3 ± 12·1	—	12·5 ± 0·7	27·1 ± 2·9	58·9 ± 3·1	98·4 ± 0·9
II (8)	NA ^{0}	272 ± 3·6	8·4 ± 0·8	149·6 ± 24·3	58·6	8·1 ± 0·7	30·4 ± 0·9	56·4 ± 1·1	95·0 ± 1·1
III (9)	None	267 ± 12·2	7·0 ± 0·5	110·5 ± 34·6	—	10·4 ± 1·4	27·2 ± 1·8	60·0 ± 5·3	97·7 ± 1·6
IV (9)	NA ^{00}	272 ± 1·5	8·1 ± 0·7	139·5 ± 29·2	26·1	9·2 ± 0·9	28·1 ± 1·7	60·0 ± 2·0	97·4 ± 0·7
V (5)	None	264 ± 10·0	8·2 ± 1·1	109·7 ± 10·9	—	13·6 ± 0·7	27·1 ± 4·5	58·6 ± 5·9	99·1 ± 1·3
VI (4)	Ins.	266 ± 1·6	13·9 ± 1·7	613·9 ± 119·0	459·3	6·0 ± 0·7	24·6 ± 3·2	66·4 ± 6·7	97·2 ± 1·8

NA ^{0} = nicotinic acid, in 2×10^{-3} M final concentration.
NA ^{00} = nicotinic acid, in 2×10^{-5} M final concentration.
Ins. = insulin, in 0·1 U/ml concentration.

TABLE 3. EFFECT OF NICOTINIC ACID AND NOREPINEPHRINE ON GLUCOSE-U-^{14}C INCORPORATION IN THE LIPID FRACTIONS OF ADIPOSE TISSUE FROM FASTED RATS

Groups (No. of Exper.)	Treatment	Tissue weight (mg ±S.E.)	Medium % decrease	Radioactivity (mean values ±S.E.) Tissue lipids Total DPM × 10^3/g	% increase	% of total in lipid components PL	DG	TG	Recovery (%)
I (6)	NE	265 ± 4·2	4·4 ± 0·2	34·6 ± 4·8	—	13·4 ± 4·0	7·3 ± 1·4	76·6 ± 4·5	97·5 ± 2·1
II (6)	NE + NA	262 ± 13 1	4·6 ± 1·2	52·8 ± 12·3	52·6	12·1 ± 3·6	6·7 ± 0·7	78·6 ± 2·8	97·4 ± 2·4
III (8)	NE	248 ± 6·1	5·9 ± 0·8	51·8 ± 7·0	—	18·4 ± 1·1	15·6 ± 4·0	63·8 ± 4·1	97·7 ± 1·7
IV (8)	NE + NA	244 ± 15·2	5·8 ± 0·8	66·7 ± 9·3	28·7	19·9 ± 2·2	14·3 ± 4·1	63·8 ± 3·7	98·1 ± 0·6

NE = norepinephrine, in 0·4 μg/ml concentration.
NA = nicotinic acid, in 2 × 10^{-4} M final concentration.
Group III–IV: the incubation medium did not contain albumin; the tissue fragments were incubated first with NE for 60 min, then transferred to the second medium containing glucose-U-^{14}C and NA without NE and also incubated for 60 min.

from fed animals. In another experiment the tissues have been incubated first with norepinephrine and then in another medium with labelled glucose but without albumin in the medium, in order to have a greater amount of FFA in the tissue.[21] In this case the decreased activity in DG is accompanied by a significant increase in the PL activity and only a moderate increase in TG activity can be observed (Tables 2 and 3). Nicotinic acid at 10^{-4} M concentration increases the total radioactivity without any effect on the norepinephrine induced changes in the radioactivity of lipid components or on that of the medium.

DISCUSSION

The significant differences between the radioactivity of the neutral lipids of the control fat pads under different experimental conditions are in good agreement with the actual dynamic state of the lipid metabolism in the adipose tissue. In fed rats the principal metabolic process is the synthesis and therefore only a small amount of FFA is mobilized or found in the adipose tissue. The incorporation of glucose into lipids in in the adipose tissue from fed rats is greater than in those from fasted ones, which agrees with the data of Shapiro *et al.*[22] obtained with labelled stearate. It is possible that the TG synthesis has optimal conditions in the fed state and therefore nicotinic acid cannot have any positive effect on it. If, however, under the influence of norepinephrine a greater amount of FFA is available as a "substrate" for re-esterification, nicotinic acid can enhance the glucose utilisation for lipid synthesis.

A similar condition—increased lipolysis—may be the explanation of the effect of nicotinic acid in fasted state. The fact that nicotinic acid, also in such circumstances has a relatively slight effect on glucose incorporation to lipids in comparison to insulin, may be explained by the lack of its influence on glucose uptake from the medium. The effects of insulin on the metabolism of adipose tissue are mainly the consequences of a facilitation of glucose transport across the cell membrane[13, 23] and our data agree with this. According to the unchanged radioactivity of the different lipid components under the influence of nicotinic acid, in comparison to the respective controls, there is no effect on the steps of TG biosynthesis after phosphatidic acid formation.[9] Thus as an explanation of the observed effect two main possibilities are given: (1) facilitation of α-GP formation or (2) the conjugation of it with FFA for phosphatidic acid formation. In fact, the carbohydrate metabolism in fasted state is altered[24, 25] and the α-GP content is markedly reduced.[26] It is possible that in this case the α-GP content is the rate-limiting factor because FFA is available in a relatively great amount. This is also suggested by the fact that the effect of nicotinic acid is not greater in the presence of norepinephrine, which results in an even greater amount of FFA in the tissue. According to recent investigations epinephrine treatment causes an acute decrease of the α-GP content of rat liver.[26] If this is also true in the case of the adipose tissue, the results obtained may indicate an effect of nicotinic acid somehow on α-GP level. We did not observe any significant increase in the lipid radioactivity in normal fed state under the influence of norepinephrine as it has been described by Cahill *et al.*[28] In fasted state the total lipid radioactivity was even lower in the presence of norepinephrine and no increase in glucose uptake was shown either. It must be mentioned, however, that in the experiments of Cahill *et al.* the incubation time was three hours, while in that of us it was only one hour. These authors concluded that there is an

accelerated lipolysis and re-esterification at the same time under the effect of norepinephrine, but the changes of glucose metabolism are secondary to the increased FFA levels caused by the enhanced lipolysis. The striking changes in the radioactivty of the lipid components observed by us, may also be the consequence of an acceleration in some steps (e.g. DG→TG) of lipid synthesis. Whether it would be a direct or indirect effect of norepinephrine, needs further investigations.

These *in vitro* data do not permit any final conclusion referring to the mode of action of nicotinic acid on FFA mobilization. It is clearly suggested, however, that an enhanced re-esterification from α-GP and tissue FFA and an influence on tissue carbohydrate metabolism[27] may have a role in it. The fact that nicotinic acid *in vivo* decreases plasma FFA levels even in fed state, while in such conditions it does not show any effect on TG synthesis from glucose, suggests that this is not the only or principal site of its action.

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