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Stimulation of Ketogenesis After Glycogen Depletion by Nicotinic Acid in Perfused Rat Liver*

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Treatment with nicotinic acid produced an enhancement of ketone bodies production from endogenous substrates, either oleate or octanotate. The enhancement was accounted for by an increase of acetoacetate synthesis. These results suggest that the increase of acetoacetate production may be due to the enhancement of extramitochondrial ketogenesis as a consequence of the inhibition of lipogenesis.

Nicotinic acid administration produces a mobilization of liver glycogen (1) which leads to its depletion within 5 h (14). This effect is probably brought about by the enhancement of glucose utilization due to the antilipolytic effect of nicotinic acid (3). MCGARRY *et al.* (13) have recently suggested that the onset of ketogenesis may be related with glycogen mobilization. In this paper, we have investigated the effect of glycogen depletion by treatment with nicotinic acid on the ketogenic capacity of the perfused rat liver.

Materials and Methods

Treatment of animals. Female albino Wistar rats, fed on a stock laboratory diet, were used for experiments between 9 and 10 a.m. Nicotinic acid was injected intraperitoneally in neutral solution (500 mg/kg body wt.) 6 or 8 h before perfusion, the controls being treated with 0.9% NaCl solution. After injection, the animals were deprived of food but allowed to take water *ad libitum*.

Perfusion technique. The perfusion method was as described by HEMS *et al.*

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(5). The perfusion medium (150 ml) consisted of Krebs-Henseleit physiological saline (6), dialysed bovine serum albumin (fraction V) and washed human erythrocytes. Substrates were added to the perfusate disolved in 5% dialysed albumin after 38 min of perfusion. The rates of ketogenesis were calculated from the ketone bodies produced by the liver in the 40 to 85 min perfusion period.

Analytical methods and reagents. Acetoacetate and 3-hydroxybutyrate were assayed as described by WILLIAMSON *et al.* (18) 3-hydroxybutyrate-dehydrogenase and coenzymes were purchased from Boehringer (Mannheim, Germany). Bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. (Chicago, Ill. USA). Nicotinic acid, oleate and octanoate were obtained from Sigma Chemical Co. (St. Louis, Mo. USA). Sodium pentobarbital was a gift from Abbot Laboratories (Madrid, Spain).

Results

Tables I-III show the ketone bodies production from endogenous substrates, oleate or octanoate in liver pretreated with nicotinic acid. The ketone bodies production from endogenous substrates, oleate or octanoate was increased 8 h after administration of nicotinic acid. In these conditions, rates of ketone bodies production rose near to the values reported for 48 h starved rats (7, 8). The increase of ketone bodies production was accounted for by acetoacetate whith minimal changes in the rates of 3-hydroxybutyrate production. The effect of the pretreatment with nicotinic acid on the

Table I. *Ketone bodies production from endogenous sources in perfused liver from rats previously treated with nicotinic acid.*
Well-fed rats treated by intraperitoneal injection of NaCl (0.9 %) or nicotinic acid (500 mg/kg body wt.) 6 or 8 h before perfusion were perfused in the absence of added substrate. Results are expressed in μmoles/h per g liver w.wt. and are means ± S.E.M. (n = 4 — 7).

Treatment of animals	Elapsed time from treatment h	Acetoacetate	3-hydroxybutyrate	Ketone bodies	3-HB/AcAc
NaCl	6	7±2	2±1	9±2	0.29
Nicotinic acid	6	20±1**	2±1	22±1**	0.10
NaCl	8	8±2	2±1	10±2	0.25
Nicotinic acid	8	20±3*	2±1	22±2**	0.11

* $p < 0.025$; ** $p < 0.005$.

Table II. *Ketone bodies production from oleate in perfused liver from rats previously treated with nicotinic acid.*
Well-fed rats treated by intraperitoneal injection of NaCl (0.9 %) or nicotinic acid (500 mg/kg body wt.) 6 or 8 h before perfusion were perfused in presence of 2 mM oleate. Results are expressed in μmoles/h per g liver w.wt. and are means ± S.E.M. (n = 4 — 7).

Treatment of animals	Elapsed time from treatment h	Acetoacetate	3-hydroxybutyrate	Ketone bodies	3-HB/AcAc
NaCl	6	37±2	26±4	63±4	0.70
Nicotinic acid	6	33±4	26±2	59±5	0.78
NaCl	8	34±6	35±5	69±8	1.02
Nicotinic acid	8	56±7*	44±5	100±9*	0.78

* $p < 0.025$.

Table III. *Ketone bodies production from octanoate in perfused liver from rats previously treated with nicotinic acid.*
Well-fed rats treated by intraperitoneal injection of NaCl (0.9 %) or nicotinic acid (500 mg/kg body wt.) 6 or 8 h before perfusión were perfused in presence of 5 mM octanoate. Results are expressed in μmoles/h per g liver w.wt. and are means ± S.E.M. (n = 4 — 6).

Treatment of animals	Elapsed time from treatment h	Acetoacetate	3-hydroxybutyrate	Ketone bodies	3-HB/AcAc
NaCl	6	38±6	51±6	89±4	1.34
Nicotinic acid	6	60±2*	40±1	100±1*	0.66
NaCl	8	51±7	64±6	115±4	1.25
Nicotinic acid	8	87±3**	59±1	146±3**	0.68

* $p < 0.025$; ** $p < 0.005$.

ketone bodies production from endogenous substrates or octanoate was detectable 6 h after treatment while a delayed onset was found when oleate was used. These results suggest that several factors should be involved in the effect produced on ketogenesis by treatment with nicotinic acid.

Discussion

The increase of the ketogenic capacity of the liver by pretreatment with nicotinic acid might be mainly due to 1) decrease of free fatty acids esterification, 2) enhancement of the fatty acids transport accross the mitochondrial membrane and 3) decrease of the acetyl-CoA oxidation through the tricarboxylic acid cycle. Althoug any of these factors may not be absolutely precluded, it seems unlikely that the increase of ketogenic capacity brought about by pretreatment with nicotinic acid may be solely accounted for the decrease of free fatty acids esterification. Actually, the increase of ketone bodies production was also observed from fatty acids which do not undergo esterification i.e. octanoate (11). In addition, the increase of ketogenesis from oleate might be achieved by the increase of the palmitoyl-CoA-carnitine transferase activity which is the main responsable of the long-chain fatty acid transport through the inner mitochondrial membrane (4). However, the increase of ketone bodies production was also observed from octanoate which does not require the activity of palmitoyl-CoA-carnitine transferase but a carnitine-independent transport system which seems not to be adaptable to nutritional or hormonal changes (12). Consequentely, our results suggest that some other factors, besides the decrease of free fatty acids esterification and/or the enhancement of the fatty acids transport into mitochondria may play an important rôle on the increase of ketogenic capacity of the liver pretreated with nicotinic acid.

It is noteworthy that the increase of ketone bodies production observed in the treated liver is accounted for by acetoacetate formation. This disbalance might be accomplished by a decrease of the NADH/NAD^+ ratio. As nicotinic acid is a precursor of NAD^+, its conversion in the nucleotide would change the NADH/NAD^+ ratio (20). However, a decrease of the NADH/NAD^+ ratio may increase the rate of acetyl-CoA oxidation through the tricarboxilic acid cycle decreasing ketogenesis (17). Likewise, a direct effect of nicotinic acid may be reasonably precluded in our experimental conditions taking into account that the livers were perfused when the hepatic concentrations of nicotinic acid are expected to be very low (3). On the other hand, the overproduction of acetoacetate by the treated liver might be accounted for by the stimu-

lation of extramitochondrial ketogenesis. Actually, the activities of the enzymes involved in acetoacetate synthesis have been detected in the mitochondria-free supernatants (16) and the inhibition of ketogenesis by hidroxycitrate in livers of fed rats has been recently reported (2). Consequentely, it is reasonable to assume that the acetoacetate is produced outside the mitochondria in the liver pretreated with nicotinic acid. If so, the increase of acetoacetate production may be explained by the inhibition of fatty acids and/or cholesterol synthesis in the treated liver (19). This inhibition might be accomplished by the decrease of plasma immunoreactive insulin observed between 4 and 8 h after administration of nicotinic acid (results not shown) through the decrease of the activities of acetyl-CoA carboxylase (10) and/or hydroxymetyl glutaryl-CoA reductase (9, 15).

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Resumen

El tratamiento con ácido nicotínico aumenta la producción de cuerpos cetónicos a partir de sustratos endógenos, oleato y octanato. El aumento observado tiene lugar como consecuencia del incremento de la síntesis de acetoacetato. Los resultados obtenidos sugieren que el incremento en la producción de acetoacetato puede ser debido a un aumento de la cetogénesis extramitocondrial, probablemente como consecuencia de la inhibición de la lipogénesis.

References

1. AMMON, H. P. T. and ESTLER, C. J.: *Life Sci.*, **6**, 641-647, 1967.
2. BRUNENGRABER, H. and LOWENSTEIN, J. M.: *FEBS Lett.*, **65**, 251-253, 1976.
3. CARLSON, L. A. and NYE, E. R.: *Acta Med. Scand.*, **179**, 453-461, 1966.
4. FRITZ, I. B. and YUE, T. N.: *J. Lipid Res.*, **4**, 279-288, 1963.
5. HEMS, R., ROSS, B. D., BERRY, M. N. and KREBS, H. A.: *Biochem. J.*, **101**, 284-292, 1966.
6. KREBS, H. A. and HENSELEIT, K.: *Hoppe-Seyler's Z. T. Physiol. Chem.*, **210**, 33-36, 1932.
7. KREBS, H. A., WALLACE, P. G., HEMS, R. and FREEDLAND, R. A.: *Biochem, J.*, **112**, 595-600, 1969.
8. KREBS, H. A. and HEMS, R.: *Biochem, J.*, **119**, 525-533, 1970.
9. LAKSHMANAN, M. R., NEPOKROEFF, C. M., NESS, G. C., DUGAN, E. and PORTER, W.: *Biochem. Biophys. Res. Comm.*, **50**, 704-710, 1973.
10. LEE, K. H., THRALL, T. and KIM, K. H.: *Biochem. Biophys. Res. Comm.*, **54**, 1133-1140, 1973.
11. MCGARRY, J. D. and FOSTER, D. W.: *J. Biol. Chem.*, **246**, 1149-1159, 1971.
12. MCGARRY, J. D. and FOSTER, D. W.: *J. Biol. Chem.*, **249**, 7984-7990, 1974.
13. MCGARRY, J. D., WRIGHT, P. H. and FOSTER, D. W.: *J. Clin. Invest.*, **55**, 1202-1209, 1975.
14. MORENO, F. J., SÁNCHEZ-URRUTIA, L., MEDINA, J. M., SÁNCHEZ-MEDINA, F. and MAYOR, F.: *Biochem. J.*, **150**, 51-58, 1975.
15. NEPOKROEFF, C. M., LAKSHMANAN, M. R., NESS, G. C., DUGAN, E. and PORTER, W.: *Arch. Biochem. Biophys.*, **160**, 387-393, 1974.
16. SAUER, F. and ERFLE, J. D.: *J. Biol. Chem.*, **241**, 30-37, 1966.
17. WIELAND, O.: In «Advances in Metabolic Disorders» (LEVINE, R. and LUFT, R., eds.), vol. 3, Academic Press, New York, 1968, pp. 1-47.
18. WILLIAMSON, D. H., MELLANBY, J. and KREBS, H. A.: *Biochem. J.*, **82**, 90-96, 1962.
19. WILLIAMSON, D. H., BATES, M. W. and KREBS, H. A.: *Biochem. J.*, **108**, 353-361, 1968.
20. WILLIAMSON, D. H., MAYOR, F., VELOSO, D. and PAGE, M. A.: In «Metabolic effects of Nicotinic Acid and its Derivatives» (GEY, K. F. and CARLSON, L. A., eds.), Hans Huber Publishers, Bern, 1971, pp. 227-236.