ELSEVIER

Environmental Toxicology and Pharmacology 6 (1998) 59–66

ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP

Pantethine stimulates lipolysis in adipose tissue and inhibits cholesterol and fatty acid synthesis in liver and intestinal mucosa in the normolipidemic rat

Carlos Bocos *, Emilio Herrera

Facultad de Ciencias Experimentales y Técnicas, Universidad San Pablo-CEU, P.O. Box 67, 28660 Boadilla del Monte, Madrid, Spain

Received 8 October 1997; received in revised form 1 April 1998; accepted 8 April 1998

Abstract

In vitro effects of pantethine on adipose tissue lipolysis and on both hepatic and intestinal cholesterol and fatty acid synthesis in normolipidemic rats are determined and related to their respective in vivo hypolipidemic effects after acute oral administration. At 3, 5, 7 and 24 h after a single high dose of pantethine to rats, free fatty acids (FFA), cholesterol and triglycerides levels decreased whereas plasma glycerol increased, the effect becoming significant at 7 h. The release of glycerol and FFA by epididymal fat pad pieces from rats was measured in Krebs Ringer bicarbonate-albumin buffer supplemented or not with epinephrine and several concentrations of pantethine (0, 10^{-5}, 10^{-4}, or 10^{-3} M), and it turned out to be enhanced as pantethine concentration increased. Besides, when glucose was present in the medium, this drug lowered fatty acid re-esterification in a dose-dependent manner, the effect being specially evident in the presence of epinephrine. In vitro synthesis of both cholesterol and fatty acids by slices of liver or intestinal epithelial cells was depressed as the concentration of pantethine increased in the medium. Thus, an inhibition of both cholesterolgenesis and lipogenesis seems to contribute to the hypocholesterolemic and hypotriglyceridemic effects of pantethine. On the other hand, the stimulation of lipolysis and the inhibition of fatty acid re-esterification on adipose tissue caused by pantethine must be counteracted by a high fatty acid oxidation in the liver which would explain the decrease in FFA and the increase in glycerol levels detected in the plasma of the pantethine-treated animals.

Keywords: Pantethine; Lipolysis; Cholesterolgenesis; Lipogenesis; Adipose tissue; Intestinal mucosa; Hypolipidemic drug; Rat

1. Introduction

Pantethine is a natural compound which is the stable disulfide form of pantetheine, the major component and precursor of coenzyme A and the prosthetic group of acyl carrier protein in fatty acid syntethase (Maggi et al., 1982).

Pantethine has shown to be an efficient lipid-lowering agent both in experimental animal models and clinical studies. Thus, pantethine reduces total cholesterol, low-density-lipoprotein cholesterol and triglycerides, and increases high-density-lipoprotein cholesterol in patients with dyslipidemia of types II A, II B, and IV (Maggi et al., 1982; Avogaro et al., 1983; Arsenio et al., 1986). The hypolipidemic effects of pantethine in animal models have been associated with a stimulation of fatty acid oxidation, since under increased fatty acid mobilization conditions, the administration of pantethine caused a significant reduction of triglycerides, free fatty acids (FFA) and ketones (Kameda and Abiko, 1980; Morisaki et al., 1983). However, this agent was unable to reduce the FFA levels in ACTH-induced lipolysis animals (Farina et al., 1982). In vitro pantethine has proved to be efficient at inhibiting cholesterol synthesis, due to a direct influence on the

* Corresponding author. Tel.: +34 1 3520144; fax: +34 1 3510496; e-mail: carbocos@ceu.es

1382-6689/98/$19.00
PII S1382-6689(98)00020-9

3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) reductase activity (Cighetti et al., 1988), to an effect on the conversion of lanosterol to cholesterol (Ranganathan et al., 1982), or to a preferential drive of acetyl-CoA towards fatty acid synthesis rather than to cholesterol production (Maggi et al., 1982; Sirtori and Donati, 1986). Nevertheless, pantethine has been shown to be efficient in reducing the fatty acid synthesis from acetate in isolated hepatocytes (Cighetti et al., 1987; Hsu et al., 1992) and skin fibroblasts (Ranganathan et al., 1982).

Although few reports describe the metabolic effects of pantethine in animals and in humans, the mechanism of action has been poorly described and is yet to be established. Therefore, the present study was undertaken to examine the effect of a high dose of this drug on plasma lipids, and relate it to the effects of different concentrations of this agent on in vitro lipolysis, cholesterolgenesis and lipogenesis. The findings obtained indicate that hypocholesterolemic and hypotriglyceridemic effects of pantethine may be related to its capability to reduce cholesterol and fatty acid synthesis both in liver and intestinal epithelial cells. On the other hand, the strong prolipolytic effect of pantethine shown here seems to be efficiently counteracted by an active free fatty acid oxidation in liver, accounting for the reduction of plasma FFA detected after pantethine administration.

2. Materials and methods

2.1. In vivo studies

Male Sprague–Dawley rats weighing 210–240 g were fed ad libitum purina chow diet (Panlab, Barcelona, Spain) and subjected to a 12-h on-off light cycle and kept at 22–24°C. At 10:00 h a single dose (1.2 mmol/kg body weight) of pantethine [D-bis-(*N*-pantothenyl-β-aminoethyl) disulphide] (Sigma) freshly suspended in 2% Tween-80 or the medium (controls) was administered to rats by stomach tube without anesthesia. At different times thereafter (3, 5, 7 and 24 h), blood was collected from the tip of the tail into ice-chilled heparinized receptacles. After centrifugation at $3000 \times g$ at 4°C for 15 min, plasma was stored at −30°C until processed. Aliquots of total plasma were used for FFA, triglycerides and cholesterol analysis with commercial kits (Wako, Germany; Boehringer Mannheim, Germany; and Menarini, Italy, respectively). Other plasma aliquots were deproteinized (Somogyi, 1945) for glycerol measurement (Garland and Randle, 1962).

2.2. In vitro experiments

2.2.1. Lipolytic activity in adipose tissue

Male Sprague–Dawley rats weighing 180 220 g, maintained under the same conditions as above, were decapitated and their epididymal fat pad pieces used to determine lipolytic activity following the method previously described (Domínguez and Herrera, 1976), with a few modifications. After weighing, fresh epididymal fat pads were cut into small pieces and 18–20 mg were placed in vials containing 2 ml of Krebs Ringer bicarbonate (KRB) buffer (pH 7.4), with 20 mg/ml of fatty acid-free bovine albumin, and supplemented or not with 5 mM D-glucose as final concentration. Half of the vials were also supplemented with 0.1 μg/ml of epinephrine bitartrate (Sigma). At zero time, 20 μl of dimethyl sulfoxide (Merck, Darmstadt, Germany) containing D-pantethine to give a final concentration of 0, 10^{-5}, 10^{-4}, or 10^{-3} M was added to the corresponding vial. Vials were capped, gassed for 5 min with O_2/CO_2 and incubated for 120 min as previously described (Domínguez and Herrera, 1976). Incubations were stopped by placing the vials in an ice-bath. In each experiment a set of vials corresponding to each of the conditions used was placed directly in an ice-bath without incubation, being considered zero time values. An aliquot of 750 μl of each medium was treated with 10% $HClO_4$ for protein precipitation and the neutralized supernatants were used for glycerol determination (Garland and Randle, 1962). Another aliquot of each medium was used for FFA determination with a commercial kit (Wako, Germany).

2.2.2. Lipogenic and cholesterolgenic activities in liver slices and isolated enterocytes

Male Sprague–Dawley rats weighing 200–225 g, kept under the same conditions as above were decapitated and intestine and liver were removed and placed in ice-chilled saline solution. Slices of 0.5 mm from liver were obtained with a tissue chopper (Mc Ilwain microtome, UK) and used to determine lipogenic and cholesterolgenic activities from radioactive acetate as described by Castro et al. (1972). Briefly, around 100 mg of liver slices were placed into vials containing 1 ml KRB buffer (pH 7.4), 5 mM D-glucose, and supplemented with D-pantethine at final concentrations of 0, 10^{-5}, 10^{-4}, or 10^{-3} M. At zero time, 1 μCi of 1-[^{14}C]acetate (Amersham; specific activity, 53 mCi/mmol) in 0.5 ml of medium was added. Vials were capped, gassed for 5 min with O_2/CO_2, and incubated for 90 min at 37°C; incubation was stopped by placing the vials in an ice-bath. In each experiment a set of vials corresponding to each of the conditions used was placed directly in an ice-bath to have zero time values. Incubated liver slices were placed in chloro-

Table 1
Time-response of plasma lipid concentrations in rats after the administration of a single oral dose (1.2 mmol/kg body weight) of pantethine

Parameter	Drug	Time (h)				
		0	3	5	7	24
Cholesterol (mg/dl)						
	Pantethine	–	49.3 ± 4.0*	50.3 ± 2.6*	49.3 ± 2.8*	43.8 ± 1.7**
	Controls	61.9 ± 4.3	63.3 ± 3.7	62.8 ± 3.3	61.6 ± 4.1	60.1 ± 2.6
Triglycerides (mg/dl)						
	Pantethine	–	29.5 ± 1.9**	32.3 ± 3.4**	31.3 ± 4.1**	35.5 ± 7.1*
	Controls	58.0 ± 4.8	60.0 ± 5.1	64.0 ± 5.1	61.5 ± 5.4	65.6 ± 7.2
Free fatty acids (μM)						
	Pantethine	–	309.3 ± 32.8**	376.2 ± 31.1*	385.3 ± 51.6*	356.9 ± 38.5*
	Controls	543.9 ± 58.2	541.1 ± 34.7	545.5 ± 54.3	562.9 ± 50.3	549.8 ± 46.4
Glycerol (μM)						
	Pantethine	–	163.4 ± 10.9	176.4 ± 10.6	186.5 ± 14.9*	175.4 ± 12.1
	Controls	173.9 ± 7.5	141.0 ± 11.3	147.8 ± 10.1	150.6 ± 6.6	146.5 ± 9.3

Values are means ± S.E. of five to six rats/group. Asterisks correspond to the statistical comparisons versus the respective control values (rats receiving placebo) (* $p<0.05$; ** $p<0.01$).

form:methanol (2:1, v/v) for total lipids extraction (Folch et al., 1957).

At the sample time, another operator divided the intestines into three segments that were designated as duodenum (about 10 cm from stomach), ileum (a 20–25-cm region proximal to the ileo-caecal junction), and jejunum (a 20–25-cm region between duodenum and ileum). Isolation of epithelial cells from these intestinal portions was carried out as described by Prieto et al. (Prieto et al., 1979, 1981), consisting of the eversion of the intestinal fragments, refilling with 2% NaCl, and immersion in a 2.5-mM EDTA, 1.4% NaCl medium. After the tubes had been carefully rotated end over end for 20–30 min, the intestinal portions were removed and the cell fractions centrifuged at 200 × g for 5 min, washed once in KRB buffer and, after weighing, resuspended in an appropriate volume of KRB buffer, supplemented with 5 mM glucose. Aliquots were used to determine cell viability (approx. 80–90%) with trypan blue. Then 20 mg of cell suspension was placed into vials, and supplemented with 10 μl of dimethyl sulfoxide containing D-pantethine to give a final concentration of 0, 10^{-5}, 10^{-4}, or 10^{-3} M. Other cell suspension aliquots were digested with 0.2 N NaOH to determine protein amount (Lowry et al., 1951). At zero time, 1.7 μCi of 1-[^{14}C]acetate in 0.25 ml of medium was added. Vials were capped, gassed for 5 min with O_2/CO_2, and incubated for 90 min at 37°C; incubation was stopped by placing the vials in an ice-bath. Incubation media were placed in chloroform:methanol (2:1, v/v) for extracting total lipids (Folch et al., 1957).

Fractionation of total lipids was carried out following a previously reported method (Carmaniu and Herrera, 1980; Soley et al., 1983). Briefly, extracted total lipids were saponified with 1 M KOH in 95% ethanol in a boiling-water bath. The sterol fraction was then isolated with heptane and radioactivity determined as a measurement of the cholesterolgenic activity. After acidification with H_2SO_4, the free fatty acid fraction was extracted with heptane, and radioactivity determined as a measurement of the lipogenic activity.

2.3. Expression of the results

Values correspond to means ± S.E., and the statistical comparison between the groups was performed by Student's t-test.

3. Results

To determine the in vivo hypolipidemic effect of pantethine in the rat, a single high dose (1.2 mmol/kg of body weight) was given by stomach tube and blood was collected at different times thereafter. As shown in Table 1, pantethine significantly decreased plasma cholesterol levels at 3, 5, 7 and 24 h. The acute responsiveness of plasma triglycerides levels to oral pantethine was even greater than that of cholesterol (Table 1), the reduction being also significant at the four time points studied. As also shown in Table 1, pantethine significantly decreased plasma FFA levels at the four time points studied, whereas it produced an increment in plasma glycerol levels which became significant at 7 h but not earlier.

To determine whether the hypolipidemic effect of pantethine could be related to an inhibitory effect on either cholesterolgenesis or lipogenesis (fatty acids synthesis), these pathways were studied in vitro in isolated intestinal epithelial cells (enterocytes) and liver slices.

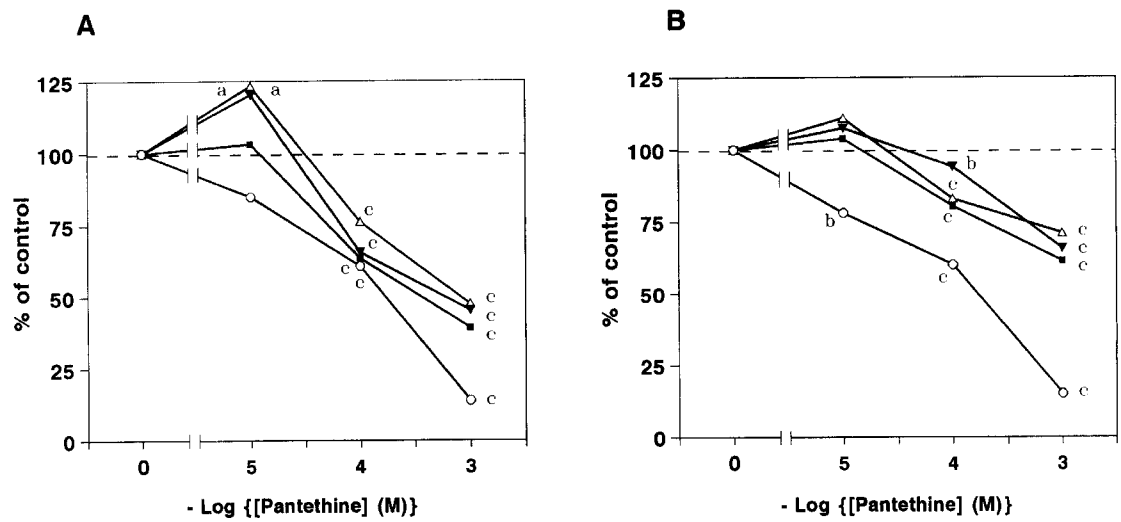

Fig. 1. Effect of pantethine on in vitro synthesis of both cholesterol and fatty acids in different tissues. Relative incorporation of [1-^{14}C]acetate into (A) cholesterol and (B) fatty acids in both isolated enterocytes from duodenum (■—■), jejunum (▼—▼) and ileum (△—△), and slices of hepatic tissue (○—○), at various concentrations of pantethine in the incubation medium. Values are means. Mean of four different experiments performed in duplicate; the percentage change from controls (incubations without the drug) is indicated. The variation between the duplicates was less than 15% in all cases. Statistical comparisons versus control values are indicated by letters (a $p < 0.05$; b $p < 0.01$; c $p < 0.001$).

Taking the incorporation of [1-^{14}C]acetate into either cholesterol or fatty acids by liver slices or isolated enterocytes from either duodenum, jejunum or ileum as 100% when incubated in the absence of the drug, the effect of different concentrations of pantethine in the incubation medium was studied. As shown in Fig. 1A, cholesterolgenesis in liver decreased progressively as pantethine concentration in the medium increased. With regard to the synthesis of cholesterol in isolated enterocytes, whereas the two highest concentrations used caused a progressive and significant reduction, the lowest concentration of pantethine (10^{-5} M) produced a significant increase in jejunum or ileum enterocytes (Fig. 1A). As shown in Fig. 1B, the lipogenic activity in the liver slices decreased in a dose-response manner with the presence of pantethine in the medium. With regard to fatty acids synthesis from acetate in the different intestinal portions, there was no effect when using the concentration of 10^{-5} M, but there was a trend to a progressive decrease as the amount of pantethine in the medium increased, although the effect was always less pronounced than in liver slices.

In order to understand the opposite effects of pantethine on plasma FFA and glycerol levels after acute administration, adipose tissue lipolytic activity was measured by incubating epididymal fat pad pieces from rats in the presence of different concentrations of pantethine in media, supplemented or not with glucose and/or epinephrine. As shown in Fig. 2A, the two lowest concentrations of pantethine used did not modify the production of glycerol, with the exception of a significant increase when adipose tissue pieces were incubated with pantethine at the concentration of 10^{-4} M, in the presence of epinephrine and in the absence of glucose. However the highest concentration (10^{-3} M) of pantethine enhanced glycerol production of fat pad pieces in all cases. Fig. 2B shows that FFA behaviour was quite similar to glycerol in epididymal fat pad pieces; thus, the lowest concentration of pantethine tested did not affect FFA production, while higher concentrations of this drug in the presence of epinephrine and the highest concentration of pantethine in the absence of hormone increased this parameter. Although a significant decrease in FFA production was found when tissue was incubated in the presence of 10^{-4} M pantethine under basal conditions and without glucose (Fig. 2B), this effect was not significant when values were expressed as percentage of respective control values (data not shown). Therefore, pantethine increased the release of both glycerol and FFA to the incubation medium in a dose-dependent manner, the effect being specially marked when tissues were incubated in the presence of a lipolytic hormone, epinephrine.

FFA released to an incubation medium may be taken up by the tissue for re-esterification (Grahn and Davies, 1980; Edens et al., 1990), whereas the glycerol is hardly reutilized (Palacín et al., 1988), and therefore the [FFA]/[glycerol] ratio can be used as an inverse index of re-esterification (Hammond and Johnston, 1985, 1987). As shown in Table 2, pantethine did not modify the low re-esterification rate determined in the absence of glucose. However, when glucose was present in the medium, FA esterification decreased with increasing concentrations of the drug; esterification became significant when incubations were done in the presence of

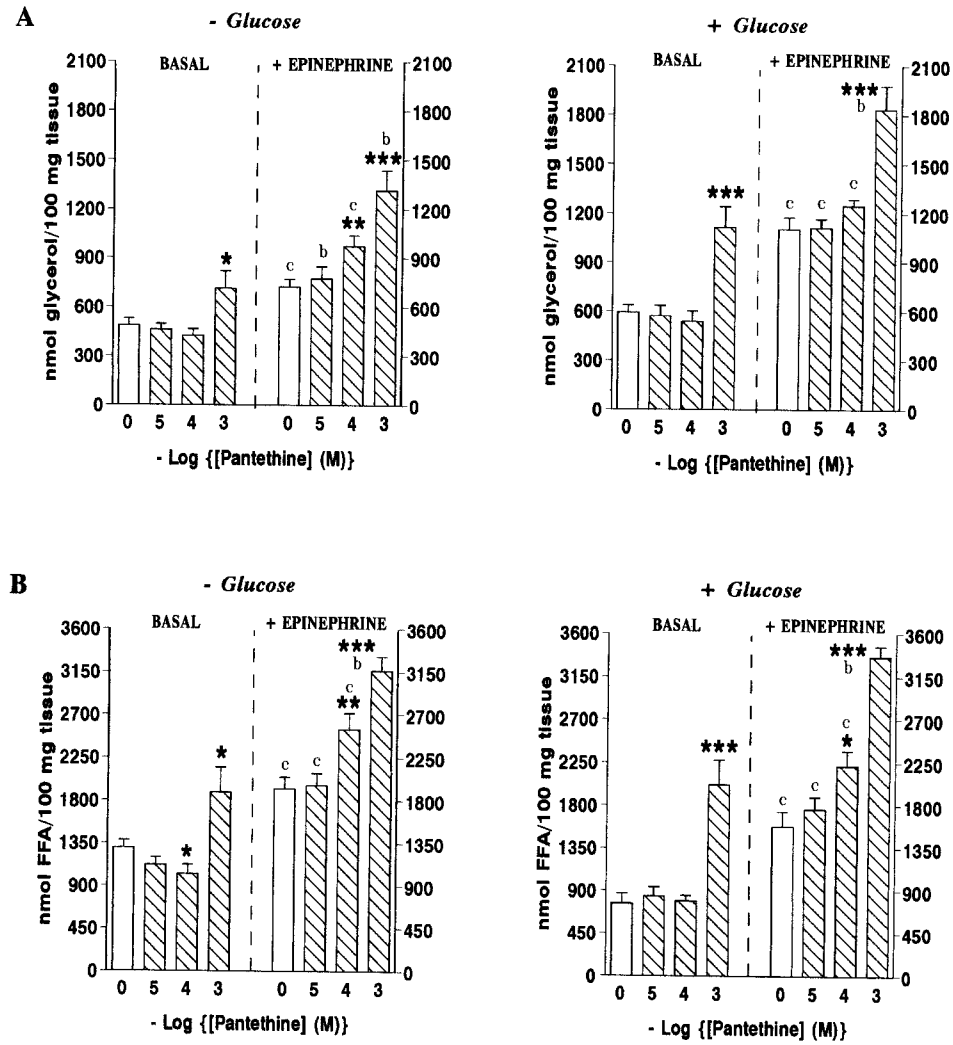

Fig. 2. In vitro lipolytic effect of pantethine. (A) Glycerol and (B) free fatty acid release by epididymal fat pieces from untreated rats incubated in vitro for 120 min with or without three different concentrations of pantethine. The presence or absence of both 0.1 μg/ml epinephrine and 5 mM glucose in the medium of incubation are also indicated. Values are means ± S.E. Mean of four experiments performed in duplicate. Asterisks correspond to the statistical comparison versus the respective control value (incubations without the drug) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), whereas the letters correspond to the comparison versus the respective value in the absence of epinephrine (a $p < 0.05$; b $p < 0.01$; c $p < 0.001$).

epinephrine and the two higher concentrations of pantethine (10^{-4} and 10^{-3} M) (Table 2).

4. Discussion

The present study shows pantethine to be an efficient hypolipidemic agent in the normolipidemic rat, reducing cholesterol, triglycerides and free fatty acids levels in plasma after acute oral treatment. The dosage used (1.2 mmol of pantethine) corresponds to 666 mg of drug/kg of body weight, which is similar or even lower than that used by others in animal experiments (Watanabe et al., 1981; Farina et al., 1982; Halvorsen, 1983). The hypocholesterolemic effect of this drug was detected 3 h after the treatment and was maintained until 24 h, proving that pantethine is effective in reducing cholesterol levels not only in hyperlipidemic conditions, as previously reported (Carrara et al., 1984; Wittwer et al., 1987), but also in normolipidemic rats. Although, the hypocholesterolemic effect of pantethine could be a secondary consequence of its intense hypotriglyceridemic effect, the inhibition that pantethine exerts on the synthesis of cholesterol from acetate, both in liver slices and in intestinal mucosa cells found here, would indicate that an effect decreasing cholesterolgenesis could contribute to its hypocholesterolemic effect, since these two organs are well known to be the main sources of plasma cholesterol in vivo (Robins et al., 1985; Field et al., 1990). The reduction that pantethine exerts on liver cholesterolgenesis has been previously reported, but not at the intestinal level, and it has been proposed to be due to an inhibitory action on 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity (Cighetti

Table 2
Effect of pantethine on free fatty acids re-esterification index (FFA/glycerol) by rat epididymal fat pad pieces in vitro

Medium of incubation	Treatment				
		Controls	Pantethine		
			10^{-5} M	10^{-4} M	10^{-3} M
Without glucose					
	Basal	2.49 ± 0.14	2.55 ± 0.11	2.45 ± 0.19	2.61 ± 0.09
	+ Epinephrine	2.67 ± 0.09	2.40 ± 0.04	2.49 ± 0.11	2.60 ± 0.12
		n.s.	n.s.	n.s.	n.s.
With glucose					
	Basal	1.30 ± 0.13	1.38 ± 0.05	1.45 ± 0.04	1.66 ± 0.05
	+ Epinephrine	1.48 ± 0.04	1.53 ± 0.03	1.72 ± 0.07**	1.99 ± 0.06***
		n.s.	<0.05	<0.01	<0.001

Values are means ± S.E. Mean of four experiments performed in duplicate. The free fatty acid re-esterification index was calculated using the (nmol FFA/100 mg tissue):(nmol glycerol/100 mg tissue) ratio. Therefore, a value near 3 means low re-esterification of free fatty acids and a value near 0 means high re-esterification. Asterisks correspond to the statistical comparisons versus the respective control value (incubations without the drug) (** $p<0.01$; *** $p<0.001$). Values corresponding to the comparisons between incubations done in the presence and the absence of epinephrine are also indicated. n.s., not significant.

et al., 1988). Despite the inhibiting effects on cholesterol synthesis both in liver or intestinal preparations of high and medium concentrations of pantethine, our results show that low concentrations (10^{-5} M) had the opposite effect, that is, it increased cholesterol synthesis. Although direct experiments on the matter would be required, we think that this stimulating effect could be related to the role this drug plays as a coenzyme A precursor under conditions in which its inhibitory effect on 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity is still not evident. In this sense, pantethine would be favouring acetyl-CoA formation and subsequently cholesterol synthesis.

Other authors explain the inhibitory effect of pantethine on cholesterolgenesis as a consequence of enhancing the preferential utilization of acetyl-CoA towards fatty acid synthesis, rather than to cholesterol synthesis (Maggi et al., 1982; Sirtori and Donati, 1986). Nevertheless, this hypothesis would be rejected in view of our findings that show that the same concentrations of pantethine reduce similarly fatty acid and cholesterol synthesis both in liver and intestinal mucosa cells. An inhibitory effect of pantethine on FAS activity similar to that reported on the HMG-CoA reductase activity has been proposed before (Cighetti et al., 1988), and the prosthetic group of acyl carrier protein (ACP) has been considered as the site of the inhibitory action on fatty acids synthesis (Cighetti et al., 1987). The inhibition of hepatic lipogenesis would correlate with the activation of β-oxidation of free fatty acids in liver by pantethine (Kameda and Abiko, 1980; Sirtori and Donati, 1986 and references therein), since it is well known that the activity of these two processes normally vary in opposite directions. A high liver oxidation of free fatty acids after pantethine administration might explain its effects in decreasing plasma FFA levels, which would counteract the pro-lipolytic action that this drug seems to have based on both its effect enhancing plasma glycerol levels in vivo and its stimulating effect on in vitro lipolytic activity found here. Present findings would be in accordance with experiments carried out in conditions of increased fatty acid mobilization, where the administration of pantethine was able to reduce plasma FFA levels (Kameda and Abiko, 1980).

Thus, pantethine stimulates in vitro adipose tissue lipolysis in a dose-dependent manner, both under basal conditions and in the presence of glucose and/or epinephrine. This effect might be related to the reported stimulating effect that pantethine has on neutral cholesteryl ester hydrolase (NCEH) activity in arterial wall homogenates (Shirai et al., 1979; Shinomiya et al., 1980), since it has been suggested that the hormone-sensitive lipase (HSL), the enzyme that controls the triglyceride breakdown in adipose tissue (Fredikson et al., 1986), is also responsible for the neutral cholesteryl ester hydrolytic activity in aortic smooth muscle cells (Hajjar et al., 1983) and in murine macrophages (Khoo et al., 1981). Besides, pantethine reduces the re-esterification of fatty acids released by adipocytes when this pathway is enhanced by the presence of glucose in the medium, and this effect decreasing re-esterification was specially marked under the highest lipolytic-stimulating conditions, that is in the presence of both glucose and epinephrine. As a matter of fact, although both agents, pantethine and epinephrine, may display differences in their respective mechanism of action, our results show that they have a similar and synergistic effect on adipose tissue lipolysis and re-esterification. These effects of pantethine on adipose tissue metabolism may explain the hyperglycerolemic effect detected after oral

administration. An additional mechanism of reducing glycerol utilization by liver, as the one found to be caused by other hypolipidemic drugs (Bocos and Herrera, 1996), that would contribute to the effect of pantethine enhancing plasma glycerol levels, could not be discarded, although more direct studies would be needed to test this hypothesis.

Interestingly, pantethine is an efficient lipid-lowering agent even in normolipidemic conditions despite its action enhancing adipose tissue triglycerides mobilization. This effect contrasts with most hypolipidemic agents, which do not affect (statins) or clearly inhibit adipose tissue lipolytic activity (fibrates, nicotinic acid and derivatives). Therefore, the present findings support that pantethine mainly exerts its hypolipidemic action by enhancing triglyceride depots breakdown and fatty acids catabolism in liver, reducing their availability for liver triglycerides synthesis and release. In this manner, the hypolipidemic action of pantethine clearly differs from that exerted by other hypolipidemic drugs, and this difference should be considered when deciding the best therapy to apply to specific dyslipidemic patients.

Acknowledgements

The authors thank Milagros Morante for her excellent technical assistance and Beatriz Ramos for her editorial help. This study was supported by a grant from Comisión Interministerial de Ciencia y Tecnología (PM96-0022) and a research grant from the Commission of the European Communities (EUROLIP, of BIOMED 1).

References

Arsenio, L., Bodria, P., Magnati, G., Strata, A., Trovato, R., 1986. Effectiveness of long-term treatment with pantethine in patients with dyslipidemia. Clin. Ther. 8, 537–545.

Avogaro, P., Bittolo Bon, G., Fusello, M., 1983. Effect of pantethine on lipids, lipoproteins and apolipoproteins in man. Curr. Ther. Res. 33, 488–493.

Bocos, C., Herrera, E., 1996. Comparative study on the in vivo and in vitro antilipolytic effects of etofibrate, nicotinic acid and clofibrate in the rat. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. 2, 351–357.

Carmaniu, S., Herrera, E., 1980. Comparative utilization in vivo of [U-^{14}C]glycerol, [2-^{3}H]glycerol, [U-^{14}C]glucose and [1-^{14}C]palmitate in the rat. Arch. Int. Physiol. Biochim. 88, 255–263.

Carrara, P., Matturri, L., Galbussera, M., Lovati, M.R., Franceschini, G., Sirtori, C.R., 1984. Pantethine reduces plasma cholesterol and the severity of arterial lesions in experimental hypercholesterolemic rabbits. Atherosclerosis 53, 255–264.

Castro, M., Lamas, L., Herrera, E., 1972. Thyroid function, plasma insulin, glucose and ketones and in vitro hepatic gluconeogenesis in rats under chronic low iodine intake. Acta Endocrinol. 69, 1–12.

Cighetti, G., Del Puppo, M., Paroni, R., Fiorica, E., Galli Kienle, M., 1987. Pantethine inhibits cholesterol and fatty acid synthesis and stimulates carbon dioxide formation in isolated rat hepatocytes. J. Lipid Res. 28, 152–161.

Cighetti, G., Del Puppo, M., Paroni, R., Galli Kienle, M., 1988. Modulation of HMG-CoA reductase activity by pantetheine/pantethine. Biochim. Biophys. Acta 963, 389–393.

Domínguez, M.C., Herrera, E., 1976. The effect of glucose, insulin and adrenaline on glycerol metabolism in vitro in rat adipose tissue. Biochem. J. 158, 183–190.

Edens, N.K., Leibel, R.L., Hirsch, J., 1990. Mechanism of free fatty acid re-esterification in human adipocytes in vitro. J. Lipid Res. 31, 1423–1431.

Farina, R., Lovati, M.R., Raucci, G., Sirtori, C.R., 1982. Effects of pantethine on different models of experimental hyperlipidemia in rodents: a comparison with clofibrate. Pharmacol. Res. Commun. 14, 499–510.

Field, F.J., Kam, N.T.P., Mathur, S.N., 1990. Regulation of cholesterol metabolism in the intestine. Gastroenterology 99, 539–551.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fredikson, G., Tornqvist, H., Belfrage, P., 1986. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. Biochim. Biophys. Acta 876, 288–293.

Garland, P.B., Randle, P.J., 1962. A rapid enzymatic assay for glycerol. Nature 196, 987–988.

Grahn, M.F., Davies, J.I., 1980. Lipolytic agents as regulators of fatty acid esterification in rat adipose tissue (proceedings). Biochem. Soc. Trans. 8, 362.

Hajjar, D.P., Minick, C.R., Fowler, S., 1983. Arterial neutral cholesteryl esterase: a hormone-sensitive enzyme distinct from lysosomal cholesteryl esterase. J. Biol. Chem. 258, 192–198.

Halvorsen, O., 1983. Effects of hypolipidemic drugs on hepatic CoA. Biochem. Pharmacol. 32, 1126–1128.

Hammond, V.A., Johnston, D.G., 1985. Measurement of substrate cycling in human adipocytes: a comparison of non-isotopic and isotopic techniques. Clin. Sci. 69 (12), 15.

Hammond, V.A., Johnston, D.G., 1987. Substrate cycling between triglyceride and fatty acid in human adipocytes. Metabolism 36, 308–313.

Hsu, J.-C., Tanaka, K., Inayama, I., Ohtani, S., 1992. Effects of pantethine on lipogenesis and CO_2 production in the isolated hepatocytes of the chick (*Gallus domesticus*). Comp. Biochem. Physiol. 102A, 569–572.

Kameda, K., Abiko, Y., 1980. Stimulation of fatty acid metabolism by pantethine. In: Cavallino, D., Gaull, G.E., Zappia, V. (Eds.), Natural Sulfur Compounds — Novel Biochemical and Structural Aspects. Plenum, New York, pp. 443–452.

Khoo, J.C., Mahoney, E.M., Steinberg, D., 1981. Neutral cholesterol esterase activity in macrophages and its enhancement by cAMP-dependent protein kinase. J. Biol. Chem. 256, 12659–12661.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Maggi, G.C., Donati, C., Criscuoli, G., 1982. Pantethine: a physiological lipomodulating agent, in the treatment of hyperlipidemias. Curr. Ther. Res. 32, 380–386.

Morisaki, N., Matsuoka, N., Saito, Y., Kumagai, A., 1983. Effects of pantethine and its metabolites on fatty acid oxidation in rat liver mitochondria. Tohoku J. Exp. Med. 141, 33–39.

Palacín, M., Lasunción, M.A., Herrera, E., 1988. Utilization of glucose, alanine, lactate, and glycerol as lipogenic substrates by periuterine adipose tissue in situ in fed and starved rats. J. Lipid Res. 29, 26–32.

Prieto, J.C., Laburthe, M., Rosselin, G., 1979. Interaction of vasoactive intestinal peptide with isolated intestinal epithelial cells from rat. 1. Characterization, quantitative aspects and structural requirements of binding sites. Eur. J. Biochem. 96, 229–237.

Prieto, J.C., Laburthe, M., Hui Bon Hoa, D., Rosselin, G., 1981. Quantitative studies of vasoactive intestinal peptide (VIP) binding sites and VIP-induced adenosine 3′;5′-monophosphate production in epithelial cells from duodenum, jejunum, ileum, caecum, colon and rectum in the rat. Acta Endocrinol. 96, 100–106.

Ranganathan, S., Jackson, R.L., Harmony, A.K., 1982. Effect of pantethine on the biosynthesis of cholesterol in human skin fibroblasts. Atherosclerosis 44, 261–273.

Robins, S.J., Fasulo, J.M., Collins, M.A., Patton, G.M., 1985. Cholesterol exchange and synthesis in the live rat. J. Lipid Res. 26, 1230–1240.

Shinomiya, M., Matsuoka, N., Shirai, K., Morisaki, N., et al., 1980. Effect of pantethine on cholesterol ester metabolism in rat arterial wall. Atherosclerosis 36, 75 80.

Shirai, K., Matsuoka, N., Saito, Y., Kumagai, A., Okuda, H., 1979. Effects of pantethine on cholesteryl ester synthesis in the arterial wall of rats on high cholesterol diet. Tohoku J. Exp. Med. 128, 355–358.

Sirtori, C.R., Donati, C., 1986. Pantethine: a review of experimental and clinical studies on its mode of action and efficacy in the treatment of hyperlipidaemia. In: Fears, R. (Ed.), Pharmacological Control of Hyperlipidaemia. Prous, Barcelona, pp. 401–407.

Soley, M., Chieri, R., Herrera, E., 1983. Liver glucose, glycogen and lipid synthesis in fed and 24-hour fasted rats soon after a pulse of [3-^{14}C]pyruvate through the portal vein. Int. J. Biochem. 15, 45–49.

Somogyi, M., 1945. Determination of blood sugar. J. Biol. Chem. 160, 69–73.

Watanabe, T., Manna, H., Suga, T., 1981. Effects of some hypolipidemic drugs on biochemical values and on hepatic peroxisomal enzymes of normolipemic rat. J. Pharmacobiodyn. 4, 743–750.

Wittwer, C.T., Graves, C.P., Peterson, M.A., Jorgensen, E., Wilson, D.E., Thoene, J.G., Wyse, B.W., Windham, C.T., Hansen, R.G., 1987. Pantethine lipomodulation: evidence for cysteamine mediation in vitro and in vivo. Atherosclerosis 68, 41–49.