Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 65 (2003) 807–812
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Involvement of nitric oxide/cyclic GMP signaling pathway in the regulation of fatty acid metabolism in rat hepatocytes

Javier García-Villafranca, Alberto Guillén, José Castro*

Departamento de Bioquímica y Biología Molecular I, Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain

Received 27 July 2002; accepted 22 November 2002

Abstract

The role of nitric oxide (NO)/guanosine 3′,5′-cyclic monophosphate (cGMP) signaling pathway in the regulation of fatty acid metabolism was investigated in rat hepatocytes. Treatment with NO donors, which are known to activate soluble guanylyl cyclase, inhibited in parallel fatty acid synthesis *de novo* and acetyl-CoA carboxylase activity. This effect was mimicked by 8-Br-cGMP and abolished by KT5823, a selective inhibitor of cGMP-dependent protein kinase (PKG). Furthermore, specific and hydrolysis-resistant activators of PKG, and inhibitors of Ca^{2+} release from endoplasmic reticulum, were also effective in inhibiting both fatty acid-synthesizing activities. These results suggest that this biological action of NO is regulated by a signaling cascade involving soluble guanylyl cyclase, cGMP, and PKG, and may be mediated, at least in part, by inhibition of Ca^{2+} release from endoplasmic reticulum. In addition, 8-Br-cGMP was able to stimulate fatty acid oxidation by two different mechanisms: the relieving of malonyl-CoA-dependent inhibition by lowering levels of this product of acetyl-CoA carboxylase, and a malonyl-CoA-independent stimulation of carnitine palmitoyltransferase I. Taken together, results of this study suggest that NO/cGMP signaling pathway is endowed with regulatory properties in fatty acid metabolism, and may have a physiological role in the control of this metabolism in liver.
© 2002 Elsevier Science Inc. All rights reserved.

Keywords: Nitric oxide; Cyclic GMP; Fatty acid metabolism; Acetyl-CoA carboxylase; Carnitine palmitoyltransferase I; Hepatocyte

1. Introduction

NO has been recognized as an important physiological mediator in a large number of biological actions, in virtually every organ and tissue. In liver, increased NO levels result from stimulated expression of the inducible form of NO synthase in hepatocytes and Kupffer cells [1,2]. Oxidative stress, endotoxin, and inflammatory cytokines are potent inducers of NO synthase expression in hepatocytes [3,4]. It is well known that many of the biological actions of NO, but not all, are mediated by the direct activation of soluble guanylyl cyclase and the consequent increase in intracellular cGMP levels [5]. This may modulate PKG, cGMP-gated ion channels, cGMP-regulated phosphodiesterases, and, under certain conditions, cyclic AMP-dependent protein kinases [5]. In fact PKG is considered the principal transduction mediator of cGMP. PKG type I, including α and β splicing variants, and type II have been characterized in mammals [5,6]. In addition, NO can also exert direct effects by covalent modification by *S*-nitrosylation of thiol groups in proteins [7].

Among the many metabolic effects of NO, it has been demonstrated its role in modulation of multiple pathways of glucose metabolism, mainly in skeletal muscle [8–10] and liver [11–13]. In contrast to this knowledge, studies on the role of NO/cGMP signaling in hepatic fatty acid metabolism are really scarce and apparently contradictory. Thus, it has been reported that feeding rats with an inhibitor of NO synthase induced hyperlipidemia and lowered hepatic fatty acid oxidation [14]. However, in other study a NO donor was shown to inhibit ketogenesis in isolated rat hepatocytes by a cGMP-independent mechanism [15]. On the other hand, it has been reported in rat heart that 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) activated acetyl-CoA carboxylase (ACC) [16]. Therefore, in this study we went further to investigate the possibility of a role of NO/cGMP signaling pathway in the regulation of fatty acid metabolism

* Corresponding author. Tel.: +34-913944157; fax: +34-913944672.
E-mail address: jcastro@bbm1.ucm.es (J. Castro).

Abbreviations: NO, nitric oxide; cGMP, guanosine 3′,5′-cyclic monophosphate; PKG, cGMP-dependent protein kinase; 8-Br-cGMP, 8-bromo-guanosine 3′,5′-cyclic monophosphate; SNAP, (±)-*S*-nitroso-*N*-acetylpenicillamine; ACC, acetyl-CoA carboxylase; CPT-I, carnitine palmitoyltransferase I.

0006-2952/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0006-2952(02)01623-4

in rat hepatocytes. We examined in detail the rates of the different fatty acid-metabolizing pathways and the activities of two key enzymes in this metabolism, namely ACC in fatty acid synthesis [17] and carnitine palmitoyltransferase I (CPT-I) in fatty acid oxidation [18]. In addition, it is currently thought that the NO/cGMP pathway controls a number of cellular processes by modulating intracellular Ca^{2+} levels, *via* type I PKG-dependent phosphorylation of several key regulatory proteins involved in Ca^{2+} fluxes [5]. Thus, we also studied the possible contribution of this mechanism to the effects of cGMP on fatty acid synthesis in isolated rat hepatocytes.

2. Materials and methods

2.1. Isolation and incubation of hepatocytes

Hepatocytes were isolated from male Wistar rats (225–250 g) by the collagenase perfusion method [19]. To minimize glycogenolysis, all the buffers used in the isolation procedure contained 10 mM glucose [20]. Because lipogenesis is markedly depressed just after hepatocyte isolation, cells were incubated for 15 min at 37° in a gyratory metabolic shaker [20], and then filtered through nylon mesh. Cell viability always exceeded 90%, as determined by trypan blue exclusion. All the animal protocols followed the guidelines of the Spanish Ministry of Health.

Two milliliters of hepatocyte suspension (4–6 mg cellular protein/mL) in Krebs–Henseleit bicarbonate buffer, supplemented with 10 mM glucose and 1% (w/v) defatted and dialyzed BSA, were incubated with the additions indicated in the experiments at 37° for 30 min, with constant shaking under an atmosphere of O_2/CO_2 (19:1). Additions used were: (±)-*S*-nitroso-*N*-acetylpenicillamine (SNAP); sodium nitroprusside (SNP); 8-Br-cGMP; 8-(4-chlorophenylthio)-guanosine 3′,5′-cyclic monophosphate (8-*p*CPT-cGMP); Sp-isomer of β-phenyl-1, N^2-etheno-8-bromo-guanosine 3′,5′-cyclic monophosphorothioate (Sp-8-Br-PET-cGMPS); 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate (TMB-8); 2-aminoethoxydiphenylborate (2-APB); rat atrial natriuretic peptide (rANP); KT5823, and 1,4-dihydro-5-(2-propoxyphenyl)-1,2,3-triazolo[4,5-*d*]pyrimidine-7-one (zaprinast). The cellular protein was determined according to Lowry *et al.* [21].

2.2. Rates of fatty acid synthesis, oxidation, and esterification

For the determination of the rate of fatty acid synthesis, reactions were started by the addition of [1-^{14}C]acetate (0.1 Ci/mol; 3 mM final concentration) to hepatocyte incubations. After 30 min reactions were stopped and total fatty acids were extracted [20]. The rate of fatty acid oxidation was determined by adding to hepatocyte incubations [1-^{14}C]palmitate or [1-^{14}C]octanoate (0.05 Ci/mol; 0.4 mM final concentration) bound to albumin. After 20 min reactions were stopped and oxidation products were extracted and quantified as previously described [22]. For the determination of the rate of fatty acid esterification, reactions were started by the addition to cell incubations of albumin-bound [1-^{14}C]palmitate (0.05 Ci/mol; 0.4 mM final concentration), and carried out for 30 min. Triacylglycerols and phospholipids were isolated by thin-layer chromatography and quantified [22].

2.3. Enzymatic assays

ACC activity was determined in digitonin-permeabilized hepatocytes as the incorporation of radiolabeled acetyl-CoA into fatty acids in a reaction coupled to the fatty acid synthase reaction [20]. To measure enzyme activity, 100 μL of hepatocyte suspension was added to 100 μL of prewarmed digitonin-containing assay medium. The final assay mixture contained 63 mM HEPES buffer (pH 7.5), 5 mM glucose, 1.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM citrate, 2.5 mM EGTA, 1.25 mM $CaCl_2$, 22.5 mM $NaHCO_3$, 70.5 mM NaCl, 2 mM ATP, 0.5 mM NADPH, 0.44 mM dithioerythritol, 0.93% BSA (defatted and dialysed), 0.062 mM butyryl-CoA, 64 μg digitonin/mg cellular protein, 3.2 mU of rat liver fatty acid synthase purified as previously reported [23], and 0.062 mM [1-^{14}C]acetyl-CoA (4 Ci/mol). The reaction was carried out for 2 min and then, samples were processed as described [20].

CPT-I activity was determined in digitonin-permeabilized hepatocytes as the tetradecylglycidate-sensitive incorporation of radiolabeled L-carnitine into palmitoylcarnitine [24]. To measure enzyme activity, 100 μL of hepatocyte suspension, previously preincubated for 20 min in the absence or in the presence of 5 μM TDGA, was added to 100 μL of prewarmed digitonin-containing assay medium. The final assay mixture contained 12.5 mM Tris–HCl (pH 7.4), 70 mM sucrose, 5 mM glucose, 32.5 mM KCl, 12.5 mM $NaHCO_3$, 60 mM NaCl, 0.5 mM KH_2PO_4, 0.5 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1 mM EDTA, 1 mM dithioerythritol, 50 μM palmitoyl-CoA, 0.5% BSA (defatted and dialysed), 40 μg digitonin/mg cellular protein, and 0.5 mM L-[Me-^{14}C]carnitine (1 Ci/mol). After 20 or 40 s, reactions were stopped and samples were processed as previously reported [25].

2.4. Statistical analysis

Determination of the rates of metabolic pathways and enzymatic assays were carried out in triplicate with hepatocyte incubations also done in triplicate. Results in figure and tables are the means ± SD of the number of rats indicated in every case. Statistical analysis was performed by Mann–Whitney's non-parametric test, using the software package Statgraphics.

3. Results

NO is a short-lived free radical and, therefore, several types of synthetic donors that slowly release this mediator have been currently used in the study of NO-dependent biological actions. In the present work, the effects of NO donors and cGMP-related modulators on the main pathways of fatty acid metabolism were examined in intact hepatocytes. In addition, the effects on ACC and CPT-I activities were determined in digitonin-permeabilized hepatocytes [25,26]. Determination of enzyme activities in a permeabilized-cell system is essential to preserve the short-term changes induced by diverse cellular modulators, specially when these effects are supposed to be mediated by a mechanism of labile modification.

When hepatocytes were treated with SNAP or SNP, two NO donors, ACC activity and the rate of fatty acid synthesis were inhibited in parallel (Table 1). This effect was concentration and time dependent. A direct effect of NO donors on ACC can be discarded since enzyme activity was unchanged by adding these modulators to the enzyme assay mixture without previous incubation (not shown). Hepatocytes were also incubated with 8-Br-cGMP, a phosphodiesterase-resistant cGMP analog. The cGMP analog produced a concomitant decrease in ACC activity and the rate of fatty acid synthesis to a higher extent than that produced by NO donors. This decrease was concentration dependent (Fig. 1), reaching a maximal effect when cells were incubated with 0.1 mM 8-Br-cGMP for 30 min. Moreover, maximal concentrations of SNAP and 8-Br-cGMP showed a non-additive effect (Table 1). These results led to the assumption that effects of NO donors could be mediated by soluble guanylyl cyclase and PKG activation. In this way, the inhibition produced by SNAP was completely abolished by KT5823, a highly specific inhibitor of PKG (Table 1). In addition, when zaprinast, an inhibitor of

Table 1
Effects of diverse NO donors and cGMP-related modulators on acetyl-CoA carboxylase activity and the rate of fatty acid synthesis

Additions	Parameter	
	Acetyl-CoA carboxylase activity (% of control)	Fatty acid synthesis (% of control)
None	100	100
0.5 mM SNAP	73 ± 1*	77 ± 4*
0.1 mM SNP	80 ± 2*	78 ± 3*
0.1 mM 8-Br-cGMP	51 ± 3*	45 ± 6*
0.5 mM SNAP + 0.1 mM 8-Br-cGMP	55 ± 5*	43 ± 3*
0.1 mM KT5823	101 ± 3	103 ± 4
0.5 mM SNAP + 0.1 mM KT5823	100 ± 6	98 ± 2
0.1 mM zaprinast	72 ± 2*	71 ± 6*
0.4 μM rANP	103 ± 4	99 ± 1

Hepatocytes were incubated in the presence of indicated additions. After 30 min, samples were removed to determine the activity of ACC and the rate of fatty acid synthesis. Results are expressed as percent of controls and represent means ± SD of nine different animals. Control values (100%) from incubations without additions were: ACC activity, 0.72 ± 0.05 nmol product/min mg cellular protein; rate of fatty acid synthesis, 29.8 ± 1.8 nmol acetyl units/hr mg cellular protein.

* Significantly different from incubations with no additions, $P < 0.01$.

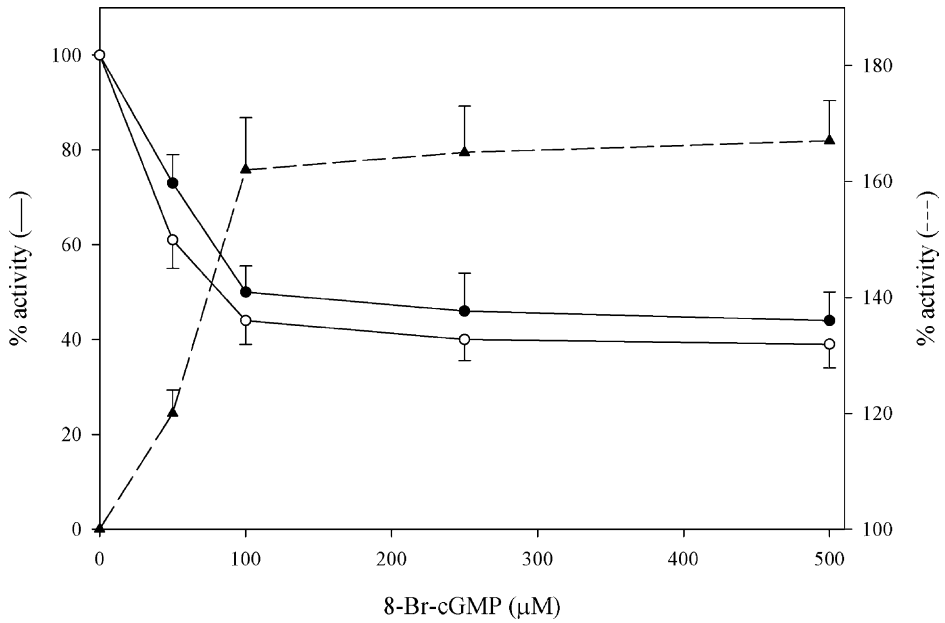

Fig. 1. Effect of 8-Br-cGMP on fatty acid metabolism, showing inhibition (continuous line) of ACC (●) and fatty acid synthesis (○), and stimulation of palmitate oxidation (broken line, ▲). Hepatocytes were incubated in the presence of increasing concentrations of 8-Br-cGMP. After 30 min, samples were removed to determine the activity of ACC and the rates of fatty acid synthesis and palmitate oxidation. Results are expressed as percent of controls and represent means ± SD of four different animals. Control values (100%) in the absence of 8-Br-cGMP were: ACC activity, 0.70 ± 0.06 nmol product/min mg cellular protein; rate of fatty acid synthesis, 32.2 ± 2.1 nmol acetyl units/hr mg cellular protein; rate of palmitate oxidation, 40.1 ± 5.0 nmol palmitate converted into product/hr mg cellular protein.

cGMP-specific phosphodiesterase, was added to hepatocyte incubations, the extent of the inhibition on the fatty acid synthesis pathway was similar to that obtained with NO donors. However, rANP, an activator of membrane-bound guanylyl cyclase A, was not able to inhibit ACC nor the rate of fatty acid synthesis (Table 1).

Biological actions of NO are complex, hence, we decided to use cGMP analogs for studying the role of cGMP signaling pathway on hepatic fatty acid metabolism. Table 2 shows the effects of hepatocyte incubation with 8-*p*CPT-cGMP or Sp-8-Br-PET-cGMPS, two non-hydrolysable cGMP analogs considered to act as potent and selective activators of protein kinase G. Both cGMP analogs were as effective as 8-Br-cGMP in the inhibition of ACC activity and the rate of fatty acid synthesis. It is known the implication of calcium in cGMP signaling. Thus, we assayed the possible modifications of the 8-Br-cGMP effects described above when hepatocytes were isolated and incubated in the absence of extracellular calcium. In these conditions, a significant alteration of the effect of 8-Br-cGMP on ACC and fatty acid synthesis was observed (not shown). In order to gain a better understanding of the involved mechanism, we incubated hepatocytes with TMB-8 or 2-APB, two inhibitors of Ca^{2+} release *via* inositol 1,4,5-trisphosphate (IP_3) receptors. These two Ca^{2+}-release inhibitors mimicked the effect of 8-Br-cGMP on ACC, although they showed a lower effect on fatty acid synthesis (Table 2).

Modulation of ACC is also essential for the control of long-chain fatty acid oxidation in hepatocytes, because malonyl-CoA is a potent inhibitor of CPT-I [18,27]. Thus, we found that incubation of hepatocytes with 8-Br-cGMP induced a concentration-dependent stimulation of palmitate oxidation (Fig. 1). With the aim to study cGMP effects on the fatty acid oxidation pathway, hepatocytes were treated with 8-Br-cGMP and then, CPT-I activity and the rates of palmitate and octanoate oxidation were measured (Table 3). In a 20-s permeabilization assay, the activity of CPT-I was increased by approx. 170%. This assay time is not long enough to allow malonyl-CoA depletion in digitonin-permeabilized hepatocytes [28], and it should reflect the conditions of measurement of palmitate oxidation in intact hepatocytes. In this way, the rate of exogenous palmitate oxidation was increased by 8-Br-cGMP in a similar extent to the activation of CPT-I, whereas oxidation of octanoate, which may enter mitochondria independently of carnitine [18,27], was not affected. It is thought that after 40 s of permeabilization, hepatocytes are depleted of malonyl-CoA [28] and, therefore, CPT-I activity assayed for 40 s should reflect an effect of 8-Br-cGMP independent of malonyl-CoA regulation. Thus, CPT-I showed a less marked (approx. 130%) but significant activation. Finally, the effect of the cGMP analog on palmitate esterification was also studied in intact hepatocytes (Table 3). Incubation with 8-Br-cGMP induced an inhibition of the rate of palmitate esterification to triacylglycerols (approx. 50%) or phospholipids (approx. 25%). These results were in agreement with the finding that exogenous palmitate was preferentially oxidized to ketone bodies due to activation of CPT-I by 8-Br-cGMP.

Table 2
Effects of cGMP analogs and calcium-release inhibitors on acetyl-CoA carboxylase activity and the rate of fatty acid synthesis

Additions	Parameter	
	Acetyl-CoA carboxylase activity (% of control)	Fatty acid synthesis (% of control)
None	100	100
0.1 mM 8-Br-cGMP	51 ± 3*	45 ± 6*
0.1 mM 8-*p*CPT-cGMP	50 ± 4*	47 ± 4*
15 μM Sp-8-Br-PET-cGMPS	48 ± 5*	45 ± 2*
0.1 mM TMB-8	64 ± 6*	74 ± 3*
0.5 mM 2-APB	50 ± 2*	70 ± 5*
0.1 mM 8-Br-cGMP + 0.1 mM TMB-8	38 ± 5*	40 ± 3*
0.1 mM 8-Br-cGMP + 0.5 mM 2-APB	36 ± 5*	47 ± 2*

Hepatocytes were incubated in the presence of indicated additions. After 30 min, samples were removed to determine the activity of ACC and the rate of fatty acid synthesis. Results are expressed as percent of controls and represent means ± SD of nine different animals. Control values (100%) from incubations without additions were: ACC activity, 0.72 ± 0.05 nmol product/min mg cellular protein; rate of fatty acid synthesis, 29.8 ± 1.8 nmol acetyl units/hr mg cellular protein.

* Significantly different from incubations with no additions, $P < 0.01$.

Table 3
Effects of 8-Br-cGMP on CPT-I activity and the rates of fatty acid oxidation and palmitate esterification

Parameter	Additions	
	None	0.1 mM 8-Br-cGMP
CPT-I activity for 40 s (N = 10)	0.87 ± 0.07	1.18 ± 0.09*
CPT-I activity for 20 s (N = 6)	0.25 ± 0.03	0.42 ± 0.05*
Palmitate oxidation (N = 9)	42.6 ± 6.2	73.2 ± 6.3*
Octanoate oxidation (N = 4)	70.0 ± 2.5	69.2 ± 4.1
Palmitate esterification (N = 6)		
Triacylglycerols	38.3 ± 4.6	18.4 ± 0.8*
Phospholipids	18.3 ± 1.4	13.4 ± 0.6*

Hepatocytes were incubated in the absence or in the presence of 0.1 mM 8-Br-cGMP. After 30 min, samples were removed to determine CPT-I activities for 20 and 40 s, the rates of palmitate and octanoate oxidation and the rate of palmitate esterification. Rate of fatty acid oxidation is expressed as nm fatty acid converted into product/hr/mg cellular protein. Rate of palmitate esterification is expressed as nm palmitate converted into lipid/hr/mg cellular protein. Activities of CPT-I are expressed as nanomoles of product per minute per milligram cellular protein. Data are means ± SD of the number of animals given in parentheses.

* Significantly different from incubations with no additions, $P < 0.01$.

4. Discussion

The present study provides the first demonstration that NO/cGMP signaling pathway is endowed with regulatory

properties in fatty acid metabolism in rat hepatocytes. NO donors and cGMP analogs inhibited in parallel fatty acid synthesis and ACC activity. In addition, cGMP analogs stimulated CPT-I and CPT-I-dependent fatty acid oxidation, with an inverse reduction in the rate of fatty acid esterification. The parallel inhibition of ACC and fatty acid synthesis *de novo* is in agreement with the general view that ACC is a key regulatory enzyme of the fatty acid-synthesizing process in the liver [17]. Nevertheless, it is noteworthy that it has been reported ACC activation by 8-Br-cGMP in rat heart [16]. Unfortunately, this is a poorly studied field in order to compare these contradictory data, but they could be related to the different degrees of expression of the two ACC isoforms in both tissues. ACC-α is the dominant isoform in liver and other tissues capable of high rates of fatty acid biosynthesis *de novo*, and is considered the rate-limiting enzyme in this pathway. However, the minor component of ACC in liver, ACC-β, is the predominant isoform expressed in heart and other tissues that perform little or no fatty acid biosynthesis, and in which malonyl-CoA is required predominantly for the regulation of mitochondrial fatty acid oxidation as physiological inhibitor of CPT-I [17].

The inhibition of ACC and fatty acid synthesis by NO donors was mimicked by zaprinast, a selective inhibitor of cGMP-specific phosphodiesterase, and by 8-Br-cGMP (Table 1) and other metabolically stable analogs of cGMP (Table 2), suggesting that these actions of NO were mediated by the heme-dependent activation of soluble guanylyl cyclase [5]. This hypothesis was supported by the finding that the addition of atrial natriuretic peptide, an activator ligand of membrane-bound guanylyl cyclase A [5], showed no effect (Table 1). In addition, the more likely hypothesis concerning the downstream target of cGMP in these effects is the involvement of PKG. This is supported by the observation that the effect of NO donor SNAP was blunted by KT5823, a selective inhibitor of PKG (Table 1), whereas specific and hydrolysis-resistant activators of PKG, such as 8-*p*CPT-cGMP and Sp-8-Br-PET-cGMPS, were also effective inhibitors of both ACC activity and fatty acid synthesis (Table 2). In fact, the magnitude of the inhibition by NO donors is lower than that produced by incubation with cGMP analogs, but similar to the inhibition exerted by zaprinast (Table 1). The stronger effect of the incubation with these phosphodiesterase-resistant derivatives of cGMP must be due to the fact that they would reach more elevated and persistent intracellular concentrations, compared to physiological levels of cGMP. Thus, there was no additivity of the effects of SNAP and 8-Br-cGMP that produced the same inhibition as 8-Br-cGMP alone.

Furthermore, to gain insight into the mechanism of this signaling by cGMP, we analyzed the assumption that cGMP could be acting by modulating intracellular Ca^{2+} levels, possibly by type I PKG-dependent phosphorylation of substrate proteins involved in the control of Ca^{2+} fluxes [5,6]. Thus, we found that the effects of cGMP were mimicked by TMB-8 and 2-APB (Table 2), inhibitors of Ca^{2+} release from endoplasmic reticulum [29]. cGMP has been shown to influence intracellular Ca^{2+}-dependent responses in a number of different cell types. One mechanism involves phosphorylation of IP_3 receptor by activated PKG [29]. This mechanism inhibits Ca^{2+} release from IP_3-sensitive stores in different cell types [30–32]. In hepatocytes, contradictory reports have been published about the effect of cGMP on intracellular Ca^{2+} signaling, describing either enhancement [33] or inhibition [34] of agonist-induced increase of intracellular Ca^{2+}. At this point the present study is not conclusive, but these data afford indirect evidence to suggest that the inhibition of ACC activity and fatty acid synthesis by cGMP may be mediated, at least in part, by the inhibition of Ca^{2+} release from IP_3-sensitive stores, although additional targets of PKG signaling could also be involved.

Regulation of ACC also affects the activity of CPT-I, but our data led us to conclude that stimulation of CPT-I induced by 8-Br-cGMP involved two different mechanisms: a malonyl-CoA-dependent mechanism, by inhibition of ACC and decrease of malonyl-CoA concentration, and a malonyl-CoA-independent stimulation of CPT-I, persisting after cell permeabilization and cytosolic leakage. It is interesting to underline that the existence of a related type of malonyl-CoA-independent regulation of CPT-I activity was first proposed in the stimulation by AMP-activated protein kinase [28], and demonstrated to involve interactions between mitochondria and cytoskeletal components (reviewed in [35,36]). Studies are in progress investigating this mechanism involved in cGMP-mediated activation, and preliminary results have shown that this stimulation of CPT-I activity was lost after cell homogenization and preparation of mitochondria (not shown). It is noteworthy that this is also a characteristic of malonyl-CoA-independent mechanisms described previously [28,35,36].

In conclusion, our results show that an NO/cGMP signaling pathway mediates regulatory effects on key enzymes of fatty acid synthesis and oxidation, and allow us to suggest that it may be involved in physiological control of fatty acid metabolism in liver. NO donors and cGMP analogs inhibit in parallel fatty acid synthesis and ACC activity. These effects seem to be dependent on activation of PKG, and are mediated, at least in part, by the inhibition of Ca^{2+} release from IP_3-sensitive stores. In addition, cGMP appears to be involved in activation of CPT-I by malonyl-CoA-dependent and -independent mechanisms. This opens the possibility that *in vivo* an NO/cGMP pathway may have a role in the control of lipid metabolism in liver. This knowledge could be of interest for a better understanding of the regulation of hepatic metabolism and the mechanisms involved in the modulation of response to liver injury from diverse insults which increase NO production.

Acknowledgments

This work was supported by grants from Spanish Comisión Interministerial de Ciencia y Tecnología (SAF97-0142) and Universidad Complutense (PR181/96-6760). Javier García-Villafranca was the recipient of a fellowship from Universidad Complutense.

References

[1] Jones BE, Czaja MJ. Mechanisms of hepatic toxicity. III. Intracellular signaling in response to toxic liver injury. Am J Physiol 1998;275: G874–8.
[2] Li J, Billiar TR. Nitric oxide. IV. Determinants of nitric oxide protection and toxicity in liver. Am J Physiol 1999;276:G1069–73.
[3] Duval DL, Sieg DJ, Billings RE. Regulation of hepatic nitric oxide synthase by reactive oxygen intermediates and glutathione. Arch Biochem Biophys 1995;316:699–706.
[4] Nussler AK, Geller DA, Sweetland MA, Di Silvio M, Billiar TR, Madariaga JB, Simmons RL, Lancaster Jr JR. Induction of nitric oxide synthesis and its reactions in cultured human and rat hepatocytes stimulated with cytokines plus LPS. Biochem Biophys Res Commun 1993;194:826–35.
[5] Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev 2000;52:375–413.
[6] Lohmann SM, Vaandrager AB, Smolenski A, Walter U, De Jonge HR. Distinct and specific functions of cGMP-dependent protein kinases. Trends Biochem Sci 1997;22:307–12.
[7] Stamler JS. Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell 1994;78:931–6.
[8] Roberts CK, Barnard RJ, Scheck SH, Balon TW. Exercise-stimulated glucose transport in skeletal muscle is nitric oxide dependent. Am J Physiol 1997;273:E220–5.
[9] Young ME, Radda GK, Leighton B. Nitric oxide stimulates glucose transport and metabolism in rat skeletal muscle in vitro. Biochem J 1997;322:223–8.
[10] Higaki Y, Hirshman MF, Fujii N, Goodyear LJ. Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. Diabetes 2001;50: 241–7.
[11] Borgs M, Bollen M, Keppens S, Yapp SH, Stalmans W, Vanstapel F. Modulation of basal hepatic glycogenolysis by nitric oxide. Hepatology 1996;23:1564–71.
[12] Sprangers F, Sauerwein HP, Romijn JA, van Woerkom GM, Meijer AJ. Nitric oxide inhibits glycogen synthesis in isolated rat hepatocytes. Biochem J 1998;330:1045–9.
[13] Monti LD, Valsecchi G, Costa S, Sandoli EP, Phan CV, Pontiroli AE, Pozza G, Piatti PM. Effects of endothelin-1 and nitric oxide on glucokinase activity in isolated rat hepatocytes. Metabolism 2000; 49:73–80.
[14] Khedara A, Kawai Y, Kayashita J, Kato N. Feeding rats with the nitric oxide synthase inhibitor, L-N^{ω}Nitroarginine, elevates serum triglyceride and cholesterol and lowers hepatic fatty acid oxidation. J Nutr 1996;126:2563–7.
[15] Nomura T, Ohtsuki M, Matsui S, Sumi-Ichinose C, Nomura H, Hagino Y. Nitric oxide donor NOR3 inhibits ketogenesis from oleate in isolated rat hepatocytes by a cyclic GMP-independent mechanism. Pharmacol Toxicol 1998;82:40–6.
[16] Depre C, Gaussin V, Ponchaut S, Fischer Y, Vanoverschelde J-L, Hue L. Inhibition of myocardial glucose uptake by cGMP. Am J Physiol 1998;274:H1443–9.
[17] Kim K-H. Regulation of mammalian acetyl-coenzyme A carboxylase. Ann Rev Nutr 1997;17:77–99.
[18] McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997;244:1–14.
[19] Beynen AC, Vaartjes WJ, Geelen MJH. Opposite effects of insulin and glucagon in acute hormonal control of hepatic lipogenesis. Diabetes 1979;28:828–35.
[20] Bijleveld C, Geelen MJH. Measurement of acetyl-CoA carboxylase activity in isolated hepatocytes. Biochim Biophys Acta 1987;918: 274–83.
[21] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[22] Guzmán M, Castro J. Zonal heterogeneity of the effects of chronic ethanol feeding on hepatic fatty acid metabolism. Hepatology 1990; 12:1098–105.
[23] Linn TC. Purification and crystallization of rat liver fatty acid synthetase. Arch Biochem Biophys 1981;209:613–9.
[24] Declercq PE, Falck JR, Kuwajima M, Tyminski H, Foster DW, McGarry JD. Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. I. Use of inhibitors. J Biol Chem 1987;262:9812–21.
[25] Guzmán M, Geelen MJH. Activity of carnitine palmitoyltransferase in mitochondrial outer membranes and peroxisomes in digitonin-permeabilized hepatocytes. Biochem J 1992;287:487–92.
[26] Guzmán M, Castro J. Zonation of fatty acid metabolism in rat liver. Biochem J 1989;264:107–13.
[27] Zammit VA. Carnitine acyltransferases: functional significance of subcellular distribution and membrane topology. Prog Lipid Res 1999;38:199–224.
[28] Velasco G, Geelen MJH, Guzmán M. Control of hepatic fatty acid oxidation by 5′-AMP-activated protein kinase involves a malonyl-CoA-dependent and a malonyl-CoA-independent mechanism. Arch Biochem Biophys 1997;337:169–75.
[29] Wilcox RA, Primrose WU, Nahorski SR, Challiss RAJ. New developments in the molecular pharmacology of the *myo*-inositol 1,4,5-trisphosphate receptor. Trends Pharmacol Sci 1998;19:467–75.
[30] Geiger J, Nolte C, Butt E, Sage SO, Walter U. Role of cGMP and cGMP-dependent protein kinase in nitrovasodilator inhibition of agonist-evoked calcium elevation in human platelets. Proc Natl Acad Sci USA 1992;89:1031–5.
[31] Komalavilas P, Lincoln TM. Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase. J Biol Chem 1994;269:8701–7.
[32] Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, Wang G-X, Allescher H-D, Korth M, Wilm M, Hofman F, Ruth P. Regulation of intracellular calcium by a signalling complex of IRAG, IP_3 receptor and cGMP kinase Iβ. Nature 2000;404:197–201.
[33] Rooney TA, Joseph SK, Queen C, Thomas AP. Cyclic GMP induces oscillatory calcium signals in rat hepatocytes. J Biol Chem 1996; 271:19817–25.
[34] Dufour J-F, Turner TJ, Arias IM. Nitric oxide blocks bile canalicular contraction by inhibiting inositol trisphosphate-dependent calcium mobilization. Gastroenterology 1995;108:841–9.
[35] Guzmán M, Velasco G, Geelen MJH. Do cytoskeletal components control fatty acid translocation into liver mitochondria? Trends Endocrinol Metab 2000;11:49–53.
[36] Kerner J, Hoppel C. Fatty acid import into mitochondria. Biochim Biophys Acta 2000;1486:1–17.