the gradient on the basis of the degree of *in vivo* antibody coating and aggregation.

The authors also concentrated enough core particles to analyze the HCV native nucleocapsid protein on SDS-PAGE and ELISA. The molecular weight of the core protein was determined to be approximately 26,000 Da (p26). The native core antigen polypeptide occurring in the circulation of an infected host appeared significantly larger than that predicted from residues 1 through 191 of the putative HCV core gene translational product. This could be due to a different cleavage site *in vivo* than that predicted or to a posttranslational modification. To address this disparity and to speculate on its biological significance will require the isolation of larger quantities of native particles and their associated proteins.

This study provides our first confident glimpse at the long-sought particle associated with NANB/HCV infection. Although the quality of the EM picture is not optimal and although evidence for the positive identification of the HCV virion is only partial, considering the low concentration of viral particles in the circulation, this study is a monumental achievement. With this breakthrough in hand, progress toward final characterization of the HCV virion should be rapid.

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CAN THE LIVER BE INDUCED TO SAY NO?

Hortelano S, Genaro AM, Boscá L. Phorbol esters induce nitric oxide synthase activity in rat hepatocytes: antagonism with the induction elicited by lipopolysaccharide. J Biol Chem 1992;267:24937-24940.

ABSTRACT

The incubation of primary cultures of rat hepatocytes with lipopolysaccharide (LPS) or biologically active phorbol esters promotes the release of nitric oxide to the incubation medium. This process is the result of the induction of the Ca^{2+}- and calmodulin-independent form of nitric oxide synthase. Both the release of nitric oxide to the incubation medium and the expression of nitric oxide synthase activity exhibited a lag period of about 45-60 min after cell stimulation. Exposure of hepatocytes to both stimuli produced an antagonistic effect on nitric oxide release, with a half-maximal inhibition obtained with 14 nM phorbol 12,13-dibutyrate at saturating concentration of LPS. Incubation of cells with α-phorbol 12,13-didecanoate failed to counteract the effect of LPS or to induce nitric oxide synthase, suggesting that activation of protein kinase C was involved in this process.

COMMENTS

Nitric oxide (NO) has been implicated in a variety of physiological processes including smooth muscle relaxation, immune regulation, penile erection, platelet inhibition and neurotransmission (1). The discovery of the role of NO in these processes has been a rapidly evolving story. Prior to reports in 1987 that NO was synonymous with endothelium-derived relaxing factor, the concept that a gas could act as a second messenger was not well accepted (2). The key to establishing the significance of NO in biological systems was the demonstration that the enzymatic machinery for its generation existed in a variety of tissues. NO is produced by nitric oxide synthase (NOS). Two types of NOS have been identified to date: they are "constitutive" and "inducible" forms (3, 4). Both types utilize L-arginine and NADPH as substrates and are located in the soluble fraction of the cell. The constitutive form is Ca^{2+} and calmodulin dependent, whereas the inducible form does not require Ca^{2+} or calmodulin for its function but requires tetrahydrobiopterin as a cofactor. Interferon-γ, cytokines and bacterial lipopolysaccharide (LPS) activate the inducible form of NOS (5, 6). The two types of NOS exhibit differential sensitivity to the inhibitory actions of L-arginine analogs: N^G-monomethyl-L-arginine is significantly more potent than N^G-nitro-L-arginine in inhibiting the inducible form of NOS, whereas the opposite is true for the constitutive form of the enzyme (1).

In a variety of tissues NO activates soluble guanylate cyclase (7). Elevation of cyclic GMP is associated with smooth muscle relaxation in the cardiovascular system and gastrointestinal tract (8, 9).

Hepatocytes demonstrate specific responses to NO, including inhibition of mitochondrial respiration, control of glycogenolysis and inhibition of protein synthesis in cultures of Kupffer and hepatocyte cells (10-12). Kupffer cells demonstrate some constitutive NOS activity, but none is detectable in hepatocytes studied with immunological techniques (13).

An important second messenger system in hepatocytes involves phosphoinositide turnover resulting in generation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 triggers mobilization of an intracellular pool of calcium, and DAG activates protein kinase C (PKC). Phorbol esters mimic the action of DAG to activate PKC. Activation of PKC is associated with

inhibition of agonist-mediated stimulation of cytosolic calcium oscillations in hepatocytes (14).

Hortelano et al. demonstrated that phorbol esters and LPS promote expression of the inducible form of NOS in cultured hepatocytes. The constitutive form of NOS was not detectable in the cultured hepatocytes. Exposure of hepatocytes to both agents had an inhibitory effect on NO release. This is an interesting series of experiments that set the stage for future studies. Much of the work on NO has focused on the role of the constitutive form of NOS because it represents an ideal system for generating an autocrine/paracrine messenger—(a) NO has a short half-life, degrading within 5 to 10 sec to nitrite and nitrate; (b) it is membrane permeant and (c) it is a potent stimulator of cyclic GMP by way of activation of the soluble form of guanylate cyclase.

A fundamental issue in the inducible form of NOS is clarification of its physiological role. The authors suggest that the inducible form of NOS participates in the liver's response to bacteremia and sepsis on the basis of reports that LPS and various cytokines induce this form of the enzyme (5, 6).

It will be interesting to examine the signal-transduction pathways that link LPS, cytokines and interferon-γ to activation of the inducible form of NOS. Do these agents stimulate the release of NO by directly activating the inducible form of NOS or, perhaps, indirectly by stimulating PKC, which in turn activates NOS? If these agents act by means of PKC, then their effect on NOS activity and NO release should be antagonized by inhibitors of PKC such as H-7 or staurosporine. This issue could also be approached by examining the effect of these agents after long-term treatment of hepatocytes with phorbol esters to down-regulate PKC activity. Pertinent to this issue is the observation that neuronal NOS contains multiple sites phosphorylated by protein kinases, including PKC and protein kinase A (3). It will also be of interest to determine whether substances such as angiotensin II and vasopressin that activate PLC in hepatocytes also stimulate the inducible form of NOS. Because these substances also stimulate free cytosolic calcium levels, it will be important to evaluate the potential role of $[Ca^{2+}]_{cyto}$ as a regulator of inducible NOS activity under basal and stimulated conditions.

The authors observed that concurrent treatment of hepatocyte cultures with phorbol esters and LPS antagonized NO release. Again, it will be important to identify the pathway(s) that mediates this action. At least two isoforms of PKC have been identified in hepatocytes (15). Does the same isoform of PKC mediate the stimulatory and inhibitory actions of PKC on inducible NOS activity, or are distinct isoforms involved? Alternatively, "cross-talk" among different second messenger systems may mediate the "dual" actions of PKC. For example, synergism between the α_1-adrenergic response, glucagon and cyclic AMP and their antagonism by insulin and diacylglycerol occurred in the modulation of cytosolic $[Ca^{2+}]$ oscillations in hepatocytes (14). In platelets, both NO and 8-Br-cyclic GMP prevented Ca^{2+} mobilization and platelet aggregation induced by receptor-mediated agonists by interfering with signal transduction at a point proximal to PLC activation (16). This suggests that NO autoregulates its own production by means of negative feedback on PKC activation. Finally, it will be important to determine whether these kinds of interactions occur in the time frame required for activation of inducible NOS in hepatocytes.

From the observations of Hortelano and others, it appears that the liver can be induced to "say NO." It is safe to say that we have not heard the last about NO as a potentially important regulator of hepatocyte function.

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CORRECTION

James M. Crawford's name was inadvertently printed as "James J. Crawford" in his February 1993 Hepatology *Elsewhere* commentary titled "Push Me–pull you: The Challenge of Endocytic Sorting." The journal and publisher regret the error.