Theoretical Analysis of the Possible Mechanisms of Interferon Induction for Priming and Blocking

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Interferon (IFN) has been shown to be an important factor in the organism's resistance to viral infections, justifying the use of IFN and its inducers as therapeutic and preventive preparations in viral infections and other pathological states. However, many mechanisms of the induction and action of IFN, as well as its role in the regulation and maintenance of the antiviral state have not been sufficiently studied.

The efficacy of IFN induction is known to depend upon the preliminary treatment of cells or animals with IFN. Pretreatment with low doses of IFN causes an increase of its endogenous production in response to the subsequent induction of its synthesis. This phenomenon has been termed priming. The opposite effect - blocking - is also a result of preliminary treatment with IFN, but, in contrast to the case with priming, the effect of hyporesponsiveness is achieved with high doses of IFN [18]. These data attest to the presence of a certain relationship between the mechanisms of induction and action of IFN. Currently, however, there are no satisfactory explanations of the nature of these phenomena.

In the present study a theoretical analysis of the regularities of IFN induction during priming and blocking was carried out with regard to the functioning of the system as a whole. For this purpose, the data available in the literature on the regulation and functioning of the IFN-1 system were generalized in the framework of a certain conceptual model, which was then formalized and studied with computerized statistics.

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RESULTS

The proposed model of the regulation and functioning of the IFN system attributes the regularities of IFN synthesis for priming and blocking to the mechanisms realized inside the cell during the induction of the antiviral state. This is very important for the functioning of the system as a whole, because it attests to the presence of possible additional IFN-mediated mechanisms providing for effective control of IFN production: enhancement of IFN synthesis in the case of low concentrations of IFN in the medium, and, vice versa, restriction of its excessive activation when high concentrations are used. As a result, such regulation must maintain the optimal antiviral protection when IFN acts upon the cell.

According to antigenic and physicochemical characteristics, as well as with respect to the cell sources, induction pathways, efficacy, and mechanisms of action, all the currently known IFN are assigned to three classes (IFN-α, IFN-β, and IFN-γ), which are encoded by a superfamily comprising 24 α genes, 1 β gene, and 1 γ-gene [1]. IFN-α genes may be grouped in two subfamilies: the IFN-αI subfamily comprises at least 15 loci (including 1 pseudogene), whereas the IFN-β subfamily is represented by 6 or 7 genes, including 5

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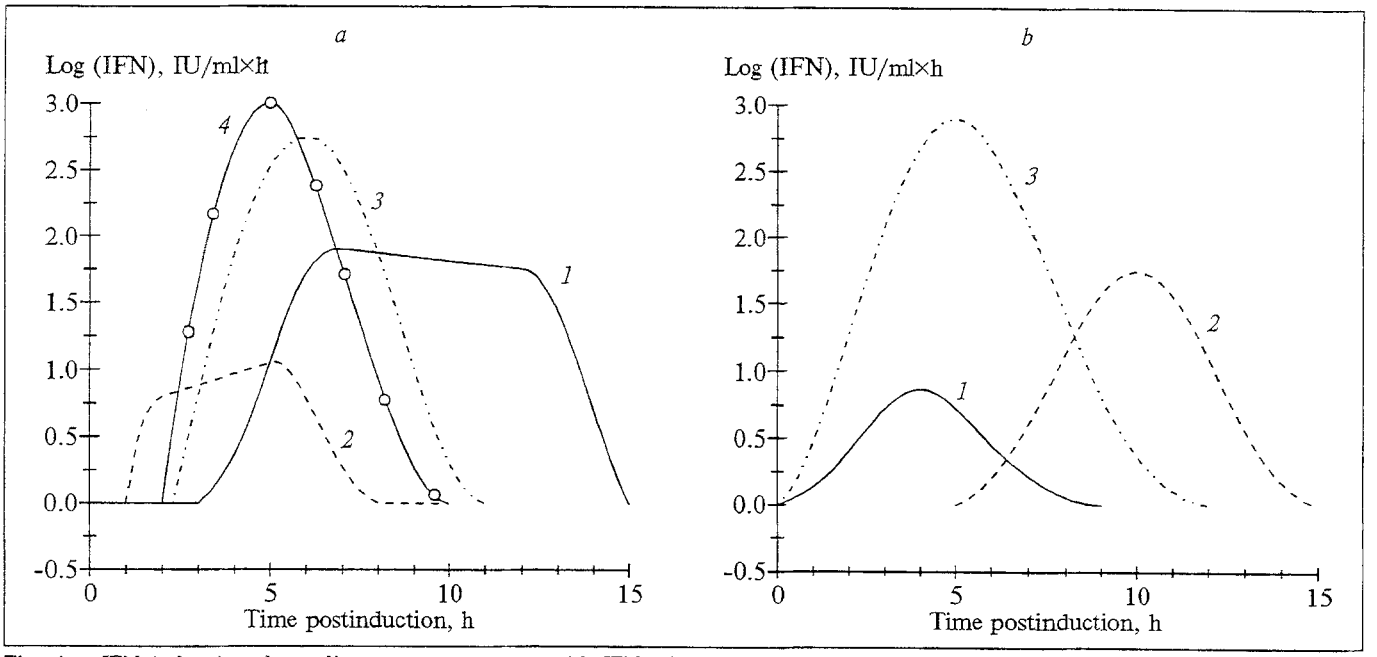

Fig. 1. IFN induction depending on pretreatment with IFN. *a*) calculated results; *b*) experimental results. *1*) normal conditions; *2*) blocking (pretreatment with 1000 IU/ml); *3*) priming (pretreatment with 1 IU/ml); *4*) priming (pretreatment with 1000 IU/ml + cycloheximide).

pseudogenes. IFN-α and IFN-β genes are localized on human chomosome 9, while the IFN-γ gene is situated on chromosome 12. Acid-resistant IFN-α and IFN-β (IFN-α/β) relate to type I, and acid-labile IFN-γ to type II.

Even though the spectra of biological effects of types I and II IFN largely overlap, just the functions of type I IFN will be taken into account during simulation of the induction and antiviral effect of IFN. This is because priming and blocking have been shown only for IFN-α/β, whose mechanisms of induction are entirely different from those of IFN-γ.

In fact, double-stranded RNA (dsRNA) of viral or synthetic origin are the classical inducers of IFN-α/β. Different types of cells produce IFN during viral infection [18]. In contrast to type I IFN, IFN-γ is produced just by the immunocompetent cells responding to specific antigenic or mitogenic stimulation [18]. During viral infections, IFN is produced by the natural killers [7] and by two types of T cells: T helpers and cytotoxic T lymphocytes [21].

The current conceptual model of genetic regulation of IFN-α/β synthesis is largely speculative and is based on a number of premises. The expression of IFN genes is regulated at different levels [15] (Fig. 1). After induction, the transcription of IFN genes, which does not require protein synthesis, rapidly escalates. Activation of the transcription of IFN genes results from their derepression caused by degradation of the specific repressor. Activation of IFN synthesis also depends on the presence of a regulator protein (a specific nuclease destroying IFN mRNA) in the cell. The lower levels of repressor and regulator protein during IFN induction are due to the degradation of their mRNA under the influence of dsRNA-dependent nuclease (Fig. 1).

Following IFN-α or β induction with the aid of dsRNA, the first amounts of IFN are detected as soon as after 4-5 h, and the maximum production is observed 10-12 h after inducer was added to the medium [18]. The rate of inactivation of IFN mRNA then begins to surpass the rate of its synthesis, this resulting in a drop of IFN production and in its complete cessation 18-20 h after the beginning of induction, even if dsRNA is constantly present in the medium. This state of the cells is termed hyporesponsiveness and is associated with the *de novo* synthesis of one more short-lived repressor (designated as inhibitor in Fig. 1) which irreversibly inactivates IFN mRNA [9]. Accumulation of repressor occurs either coincidentally with or following the formation of IFN; therefore, its gene (as well as the IFN gene) is also considered to be activated by dsRNA. IFN synthesis has been shown to be regulated by this repressor at the posttranscription level [6] (Fig. 1).

Currently, specific regulator elements located at the 5' end of the IFN-α and IFN-β genes and necessary for the expression of these genes have been identified. These elements comprise: a TATAA box; a 46 nucleotide-containing segment

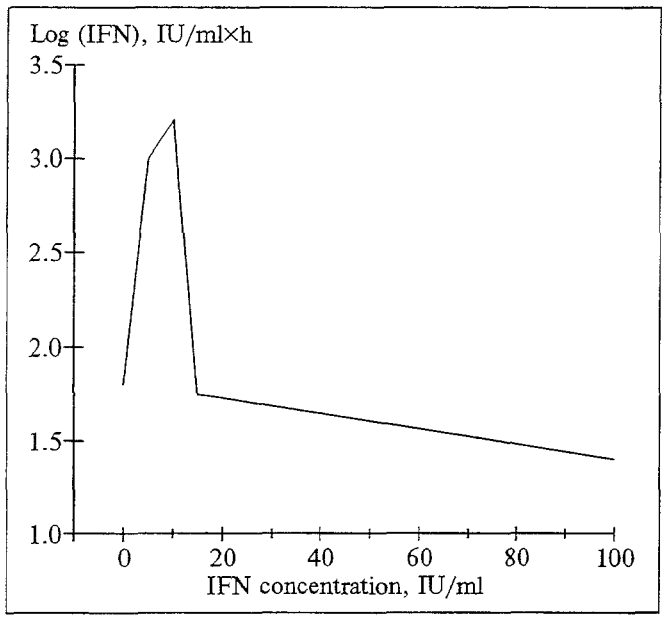

Fig. 2. IFN production vs.its concentration during pretreatment.

[4] necessary for maximum IFN induction under the influence of viruses (virus reponsive element, VRE) and highly conservative for human IFN-α and IFN-β [10]; an enhancer element responsible for constitutive transcription and containing a negative regulator [4]; a zone necessary for maximum IFN induction [3]; and a cluster of three regulatory elements exerting both positive (PRDI and PRDII) and negative (NRD) effects upon IFN gene transcription [8].

There is evidence that the regulation of the expression of IFN genes is also controlled at the posttranscription level. At the 3' end of the untranslated region of IFN mRNA, a consensus sequence comprising the UUAUUUAU repeat, which controls the stability of this RNA, has been identified.

The effect of IFN on the cells is mediated via its interaction with specific cell receptors. At the same time, IFN-α and -β have common receptors other than IFN-γ receptors [2]. The number of receptors in the cell varies from 200 to 6000 for IFN-α/β and IFN-γ, and the dissociation constant constitutes 10^{-11}-10^{-9} M [14].

In all, some 25 IFN-induced proteins have been shown to be synthesized in cells treated with different types of IFN, the functional properties of these proteins determining the spectrum of biological effects of IFN [19]. Approximately half of these proteins are induced by both IFN-α/β and IFN-γ, whereas the other half are induced only by IFN-γ. However, the functions of many IFN-induced proteins are still to be studied.

Among the proteins exhibiting antiviral activity, only two have been thoroughly studied: protein kinase and 2',5'-oligoadenylate synthetase (OAS). Induction of these proteins by IFN attends the development of the antiviral state in virus-infected cells [16]. Both proteins exhibit an enzyme activity; however, they remain inactive until the cells become infected with viruses [14], or, more precisely, until after the formation of dsRNA or the addition of synthetic dsRNA to the system. The OAS- and protein kinase-based mechanisms of inhibition of protein synthesis are summarized in Fig. 2.

Oligoadenylates with unusual 2',5'-phosphodiester linkage, which are synthesized by OAS, activate latent endoribonuclease L, catalyzing degradation of both virus and cell RNA. At the same time, endoribonuclease destroys not only mRNA, but also rRNA, this leading to inhibition of elongation and to a decrease in the rate of protein synthesis [17]. It must be taken into account that the dsRNA necessary for activation of OAS must comprise some 60 base pairs, since the oligomers shorter that 50-60 bp fail to provide for the synthesis of 2',5'-oligoadenylates [12]. In man only one gene coding for OAS, situated on chromosome 11, has been identified.

The induction of OAS and endonuclease depends on the type of IFN, as well as on the type and the state of the cell. The level of OAS varies from 10-fold in human HeLa cells to 10,000-fold in chick embryo cells [14]. The levels of latent endonuclease in most types of cells treated and not treated with IFN differ less than twofold. However, under certain conditions of cell culture, IFN may cause a 10-20-fold increase of the level of this enzyme.

It should be mentioned that 2',5'-oligoadenylates synthesized in IFN-treated cells are relatively unstable and rapidly degrade, this leading to reversed transformation of activated 2',5'-oligoadenylate-dependent endonuclease to an inactive state. One more enzyme (phosphodiesterase), which catalyzes hydrolysis of 2',5'-oligonucleotides, is required for the functioning of the 2',5'-oligoadenylate system. 2',5'-oligoadenylate esterase has been shown to be present in both IFN-treated and IFN-untreated cells. However, the level of this enzyme in some cell lines (such as mouse fibroblasts and human Daudi cells) treated with IFN increases approximately 2-4-fold [14].

The function of protein kinase is phosphorylation of the small subunit of the P1/eIF-2α initiation factor of protein synthesis, leading to the inhibition of translation initiation. Activation of protein kinase causes an approximate 5-10-fold increase of the level of phosphorylated proteins

(such as P1 and eIF-2) in IFN-treated cells as compared to untreated cells [14]. In addition, along with protein kinase, cell phospoprotein phosphatase participates in the phosphorylation of protein P1 and of the initiation factor eIF-2.

Thus, two pathways of inhibition of protein synthesis may be distinguished in IFN-treated cells: one of them is associated with activation of protein kinase, while the other involves activation of OAS and endoribonuclease. Both these pathways are shown in Fig. 2 and were taken into account during simulation of the antiviral effect of IFN.

However, it should be noted that the role of these enzymes in the formation and development of the antiviral state is not equal. The first pathway of the suppression of protein synthesis, associated with activation of protein kinase and phosphorylation of ribosome protein P1 and initiation factor, obviously plays the crucial role in regulating the translation of viral mRNA in IFN-treated cells, whereas in the absence of marked degradation of mRNA IFN-dependent phosphorylation of protein P1 and eIF-2 factor and inhibited translation of viral mRNA were observed [11]. Possibly, this is due to the fact that protein kinase and OAS are active for different dsRNA levels in the cell: protein kinase is active at a low concentration of dsRNA (10^{-8}-10^{7} g/ml) and is inactive at a high concentration (10^{-5} g/ml); on the other hand, OAS exhibits maximum activity at a high concentration of dsRNA (10^{-5} g/ml) [20].

The processes underlying the antiviral activity of IFN, notably, those associated with the induction of protein kinase and OAS, may provide the basis for IFN-induced priming and blocking. Theoretically, under certain conditions both these biochemical mechanisms could underpin priming and blocking as well. The only question is whether these conditions can be realized in the cell during induction of these states.

For priming, IFN induction is known to be independent og the synthesis of cell protein [18], and hence does not require the involvement of the factors determining the efficacy of translation. Therefore, one may assume that the situation during priming is similar to that obtaining in the cell during IFN superinduction resulting from the use of inhibitors of macromolecular syntheses. In fact, under certain conditions, the inhibitors of transcription and translation may cause IFN superinduction, raising its production more than 10-fold [18]. The mechanism of superinduction may be explained by the fact that over the period of action of inhibitors, short-lived repressors controlling IFN expression (Fig. 1) and/or their mRNA are

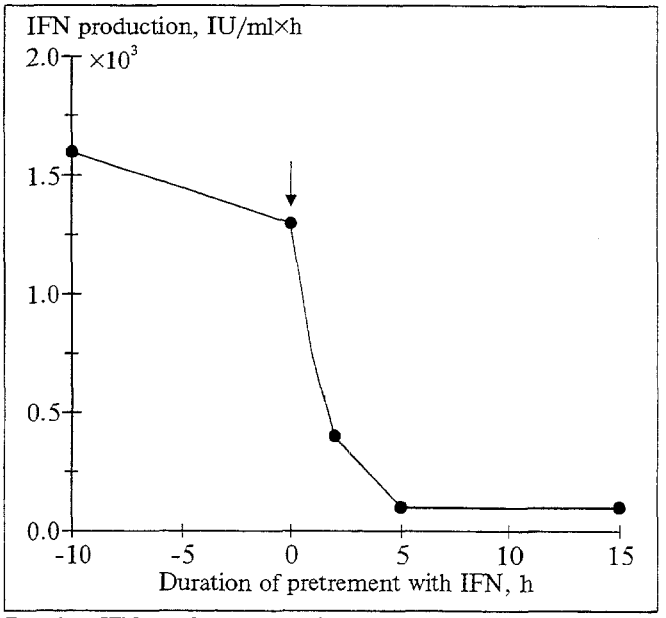

Fig. 3. IFN production vs. duration of pretreatment of cells with IFN.

inactivated, whereas more stable IFN mRNA is accumulated and then translated, resulting in IFN formation.

If the same mechanism lies at the basis of priming, then, under these conditions, IFN induction may be principally provided for by IFN-induced protein kinase, because its dsRNA-activated form inhibits protein synthesis in the cell. However, this speculation is unlikely to be true for normal conditions. Indeed, in accordance with the standard scheme of superinduction, cycloheximide is administered in a concentration 95% higher than that necessary for inhibition of protein synthesis [18]. Hence, during priming, i.e., treatment of the cells with low doses of IFN, the activity of protein kinase must be at least comparable to the effect of cycloheximide. However, it is hardly possible for the level of protein kinase induced during priming to entirely suppress protein synthesis in the cell.

The study of alternative possibilities led us to the assumption that the processes associated with the activation of OAS and endonuclease L underlie IFN induction during priming.

In fact, activation of OAS and endonuclease L during priming actually enhances IFN induction for the standard scheme, since in this case endonuclease L raises the degradation rate of mRNA of repressor and regulator protein destroyed (under normal conditions) only by short-lived dsRNA-dependent nucleases (Fig. 1). In addition, the IFN mRNA formed under these conditions is less sensitive to endonuclease L-induced degradation than is the mRNA of repressor and regulator protein,

because inactivation of regulator protein leads to a prolongation of the half-life of IFN mRNA. As a result, IFN synthesis starts earlier in primed cells than in those not pretreated with IFN.

The situation for blocking proves to be otherwise. Indeed, pretreatment of cells with high doses of IFN markedly raises the concentration of protein kinase and OAS. After the addition of dsRNA inducer to the medium, there is a certain excess of these enzymes over dsRNA, resulting in such conditions that both OAS and protein kinase, which must cause degradation of any mRNA and suppression of the synthesis of any proteins, including IFN, are activated in the cell.

However, in accordance with the above model, in order to cause a virtual suppression of protein synthesis in the cell, protein kinase must be of greatest importance in blocking. Otherwise, blocking will not be attainable, because the effect of OAS and endonuclease L for the treatment of cells with high doses of IFN is governed by the same regularities as for priming resulting in an increase of the rate of IFN mRNA synthesis over its degradation rate. Precisely because of this, IFN induction at the early stage of blocking begins at the same time as for priming, i.e., approximately 2 h earlier than in the cells not treated with IFN. This is connected with the fact that the translation inhibition resulting from phosphorylation of e1F-2α initiation factor lasts longer than the processes leading to activation of IFN expression.

The possible participation of OAS and protein kinase in the regulation of IFN expression for priming and blocking was also analyzed with the aid of a mathematical model. The results showed that the model reproduced all the main regularities of IFN induction which were established for pretreatment of the cells with low and high doses of IFN, as well as with cycloheximide (Fig. 3). Even though this is not direct evidence of the participation of OAS and protein kinase in the maintenance of priming and blocking, such a possibility is not ruled out by our findings, which do not point up any discrepancies in the mechanisms of induction and action of IFN described by the conceptual model (Figs. 1 and 2). In addition, the model study permitted us to obtain a number of adjunct functional characteristics of the IFN system, depending on the conditions of IFN induction.

For instance, the calculations of the efficacy of synthesis of endogenous IFN in cells pretreated with different doses of exogenous IFN may serve as an example (Fig. 2). The dose-effect curve exhibits a markedly pronounced "threshold" pattern, evidence that the changes of IFN production for priming and blocking closely fit the "all or nothing" rule. Here, the model suggests that priming occurs for quite a narrow range of IFN concentrations inducing the maximum response.

The time interval between the addition of IFN and of inducer to the medium may be one of the parameters optimized for priming. In accordance with the model, the time of cell treatment with IFN does not have any marked effect upon its production, provided IFN is added prior to the treatment with inducer. The model allows for priming to occur even when IFN is added after the treatment of cells with inducer. However, the rate of IFN synthesis in the latter case drops markedly, virtually no priming being induced by IFN added to the cells 3-5 h after the addition of inducer (Fig. 3).

Analysis of the limiting stages of IFN induction for priming and blocking showed that mechanisms related to the induction and action of protein kinase and OAS may underlie these phenomena. At the same time, the most likely mechanism of blocking is associated with activation of protein kinase, whereas priming may be explained just by the activation of OAS and ribonuclease L. Even though there is no direct relationship between priming and the antiviral activity of IFN, nevertheless, the former is of crucial importance for the realization of this activity, because priming markedly increases the production of endogenous IFN, possibly enhancing greatly the efficacy of its antiviral effect.

The results lend themselves to experimental verification and can be used when planning studies aimed at optimization of the use of IFN.

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Anticonvulsive Activity of Glutapyrone, a New Type of Derivative of Amino Acid-Containing 1,4-Dihydropyridines

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Screening new drugs with anticonvulsive and antiepileptic effects is of great importance, since the diversity of clinical manifestations of epilepsy calls for equally diverse actions of drug preparations.

A new compound, synthesized at the Latvian Institute of Organic Synthesis, disodium salt (2-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridyl-4-carboxamide) of glutaric acid, comprises structures of both 1,4-dihydropyridine (DHP) and glutamic acid bound as sodium salt to the 4th position of the DHP ring. This compound differs greatly from classical DHP in its being highly soluble in water, in its low toxicity (LD_{50}>8000 mg/kg, i.p.), and in its exhibiting a pronounced and prolonged antiarrhythmic [9] and stress-protective effect [7].

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The present study is devoted to an investigation of the effect of glutapyrone on $^{45}Ca^{2+}$ uptake by synaptosomes of the cerebral cortex of rats, on acute corasole-generalized seizures, and on arecoline and nicotine tremor.

MATERIALS AND METHODS

The experiments were carried out on 211 male Wistar rats weighing 210-260 g and on 300 male Icr:Icl mice weighing 18-25 g. The animals were kept in the vivarium under the usual conditions and on a standard diet. $^{45}Ca^{2+}$ uptake by the synaptosomes of the cerebral cortex was studied according to Pellmar and Wilson's method [8] slightly modified by us [3]. Acute generalized epileptic activity was provoked in the rats by intraperitoneal injections of corasole in a dose of 80 mg/kg. The effects observed were visually recorded over 60 min. The latent time of the first seizure manifestations and the latent time and duration of the clonic and tonic phases (falling of the animal

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