GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effects Interferon-2α on Seizures in Corasol-Induced Kindling

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Intracerebroventricular injection of recombinant human interferon-2α (100-500 U/rat) decreased the mean effective dose of corasol inducing clonic convulsions in 50% kindled Wistar rats. This effect was more pronounced in delayed periods of kindling and was accompanied by enhancement of epileptiform signs on EEG.

Key Words: *corasol-induced kindling; interferon-2α; epileptic activity*

The model of corasol kindling (CK) reproduces peculiarities of chronic epileptization of the brain and the most important pathogenetic mechanisms underlying the development of long-lasting changes in the excitability of brain structures [3-5]. A close relationship between the mechanisms of regulation of immunological reactivity and control of the functional state of the nervous tissue was demonstrated [4,7]. For instance, it was shown that IL-1β suppresses kainite-provoked epileptogenesis in various brain structures, while blockade of IL-1β receptors arrests pathologically enhanced excitation of neuronal formations [10].

Some cytokines, *e.g.* IFN-2α, produce pronounced central effects and provoke convulsive activity during long-term treatment under clinical conditions [6]. Here we studied the effect of IFN-2α on the model of experimental kindling syndrome.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-270 g and maintained at 12-h day/night regimen with free access to food and water. The rats were narcotized with nembutal (40 mg/kg intraperitoneally). Nichrome electrodes were implanted into the frontal (AP=1.7, L=2.0, H=1.0) and occipital (AP=-6.3, L=3.0, H=1.0) cortex and ventral hippocampus (AP=-4.3, L=4.5, H, =8.0) and cannulas were implanted into the lateral ventricles (AP=0.8, L=1.5, H=3.5). The indifferent electrode was fixed to the nasal bones. The electrodes and the cannula were fixed to the scull surface with a fast-hardening filling material similar to Noracryl. The animals were used in experiments 7-12 days after surgery.

CK was induced by repeated intraperitoneally injections of corasol in a subconvulsive dose of 30 mg/kg [3,5]. Mild convulsions were then observed after injection of initially subconvulsive dose of the epileptogen and their severity increased after subsequent injections (a total of 21 injections). The animals responding to the last 3 injections by generalized clonic-tonic seizures were used in further experiments. In animals with CK, the mean effective dose of corasol (ED_{50}) inducing clonic seizures (CS) in muscles of the body and extremities in the early period of CK (24 h after the last injection of corasol) and in delayed period of CK (3 weeks after

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the last injection of corasol) were determined. These terms were chosen because the delayed period of CK is considered as a model of pharmacologically resistant form of epileptic syndrome [5].

Electrical activity of brain structures was recorded in a monopolar mode using a DX computer system at 256 pulse/sec polling rate.

Human recombinant IFN Laferon (IFN-2α preparation, Biolek) was used in the experiments. The preparation was injected into the lateral cerebral ventricles of freely moving animals in a volume of 2 μl over 2.5 min with a Hamilton microinjector. Controls received physiological saline. The test dose of corasol was injected 10 min after intracerebroventricular injections.

The data were processed using ANOVA and Kruskal—Wallis tests.

RESULTS

Administration of corasol in a dose of 20 mg/kg in the early phase of developed CK induced CS in 25% rats (Table 1). In 2 rats with CS, generalized clonic-tonic seizures were observed during the subsequent 30 min of observation. After increasing the dose of corasol to 25 mg/kg, the number of rats with CS increased to 80%. In 5 of 8 animals, generalized tonic-clonic seizures were then observed. The calculated ED_{50} was 21.6 mg/kg (Table 1).

In the delayed period, injection of corasol in a dose of 15 mg/kg induced CS in $^1/_3$ animals (Table 1). Three of 4 animals with CS then developed generalized tonic-clonic seizures. Administration of corasol in a dose of 25 mg/kg induced convulsive response in more than 90% rats (Table 1). Generalized tonic-clonic seizures were observed in the majority of animals (8 of 10 animals). ED_{50} of corasol inducing the formation of CS in delayed period of CK was 16 mg/kg, *i.e.* by 25.9% lower than the corresponding parameter in the early period of kindling ($p<0.05$).

ED_{50} of corasol in the early period of CK determined against the background of IFN-2α administration in doses of 200 and 500 U/rat were lower than the corresponding doses determined in the early period of CK without IFN-2α treatment by 15% ($p<0.05$) and 26% ($p<0.01$), respectively (Fig. 1). In the delayed period of CK, ED_{50} of corasol in rats treated with IFN-2α (100 U/rat) was lower than in kindled rats not receiving IFN-2α by 20% ($p<0.05$). After increasing the dose of IFN-2α to 200 and 500 U/rat these differences became more pronounced: 37% ($p<0.01$) and 41% ($p<0.001$), respectively (Fig. 1).

EEG recording revealed the appearance of spike-wave complexes with amplitude of 150-250 μV and frequency of 7-11 sec^{-1} 5.0-7.5 min after administration of corasol in ED_{50} (Fig. 2, *b,* records *3* and *4*). After the next 5-10 min, epileptic spike discharges with an amplitude of 0.3-1.0 mV and a frequency of 25-40 min^{-1} were recorded (Fig. 2, *c*) correlating with clonic jerks of some muscle groups.

Spike potentials with an amplitude of 200-500 μV and frequency of 15-25 min^{-1} were recorded

Dose-Dependent Development of CS Induced by Intraperitoneal Injections of Corasol in the Early Period of CK

Corasol dose, mg/kg	*n*	Number of rats with CS					
		ED_{16}	ED_{50}	ED_{84}	ED_{100}	mg/kg	ED_{50} error
Early period of CK (4 h after the last injection of corasol)							
10	10	0					
15	11	1					
20	12	3	16.8	21.6	26.5	28.9	1.5
25	10	8					
30	10	10					
Delayed period of CK (3 weeks after the last injection of corasol)							
10	9	2					
15	12	4					
20	10	8	9.5	16.0	22.5	25.7	1.6
25	11	10					

Note. ED_{16}, ED_{50}, ED_{84}, ED_{100} are effective dose of corasol inducing CS in 16, 50, 84 and 100% rats, respectively.

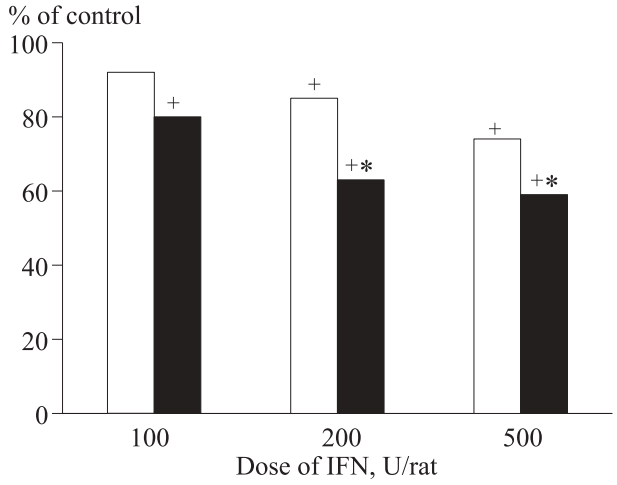

Fig. 1. ED_{50} of corasol inducing CS in rats with CK during the early (light bars) and delayed (dark bars) periods of CK against the background of intracerebroventricular administration of IFN-2α. $p<0.05$ compared to: +control, *corresponding parameter during the early period of CK.

as soon as 2.5-5.0 min after administration of corasol in ED_{50} against the background of IFN-2α (100 U/rat) treatment; during the subsequent 5-10 min the amplitude and frequency of these spikes increased to 1.0-1.2 mV and 3 sec^{-1} (Fig. 2, *d*). Similar discharges were recorded in all studied brain structures during the subsequent 20-40 min (Fig. 2, *e*) and were associated with generalized clonic-tonic seizures, falling down on the side, autonomic disturbances, and depression after seizure termination. In 3 of 9 cases, repeated generalized seizures led to animal death.

Thus, the epileptogenic effects of corasol increased under conditions of central administration of IFN-2α, which manifested in increased seizure severity and more pronounced electrical epileptiform activity. Previously ineffective doses of IFN-2α administered to animals with CK facilitated corasol-induced convulsions, especially in the delayed period of CK.

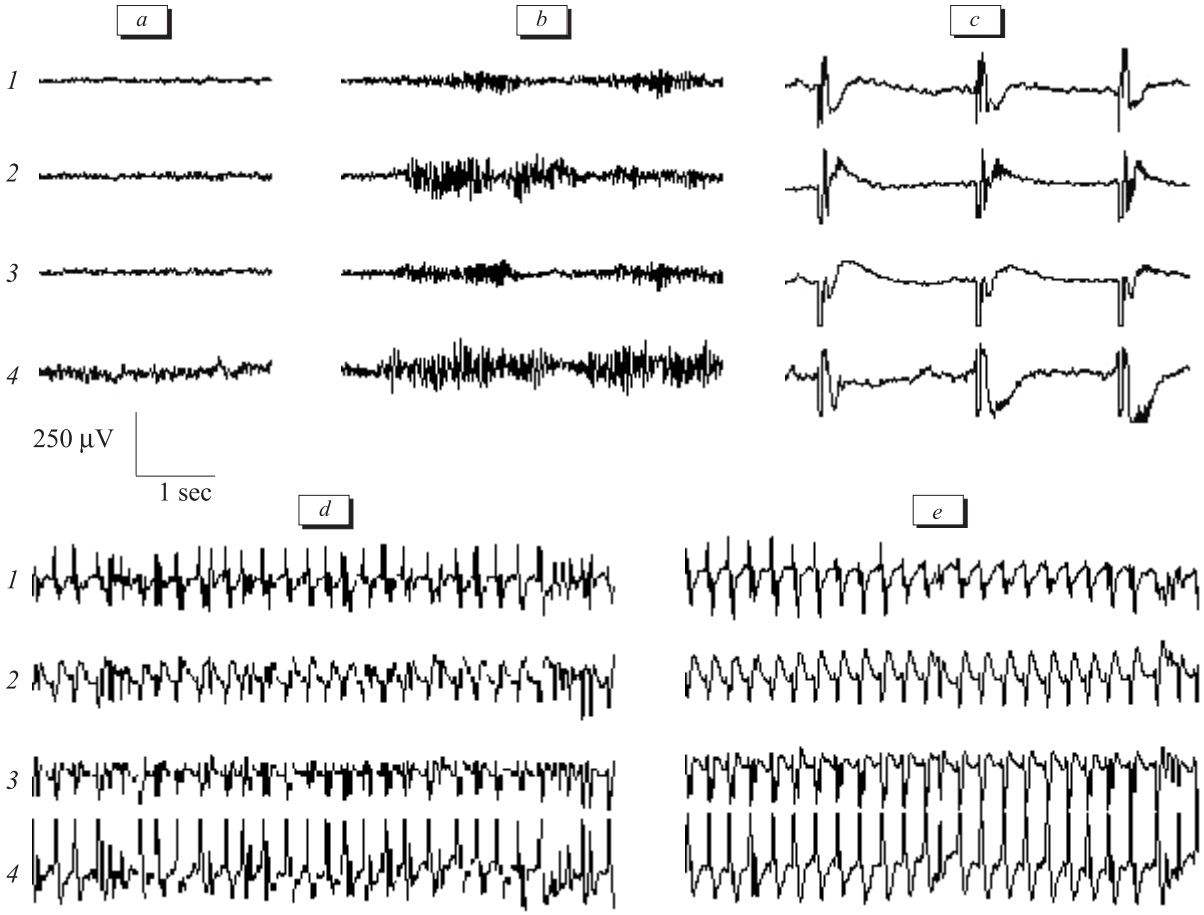

Fig. 2. Electrographic changes induced by corasol in ED_{50} during the early period of CK against the background of IFN-2α treatment. *a*) before treatment; *b*) 7 min after injection of the test dose of corasol (30 mg/kg) and 27.5 min after injection of corasol in ED_{50}; *c*) 17 and 37.5 min after microinjection of physiological saline; *d*, *e*) 8 and 23 min after injection of corasol (22 mg/kg) and 17 and 32 min after injection of IFN-2α (100 U/rat). Electrical activity was recorded in the frontal cortex (*1, 3*) and ventral hippocampus (*2, 4*) of the right (*1, 2*) and left (*3*, *4*) hemispheres.

The more pronounced facilitation of corasol-induced epileptic activity in the delayed period of CK attests to an important role of endogenous IFN system in the pathogenesis of pharmacological resistance, which is probably activated during this period. On the other hand, IFN-2α can decrease the tone of the serotoninergic system of the brain [1] involved into the formation of the anticonvulsive effects [3,5]. Moreover, IFN-2α-induced impairment of the GABAergic inhibitory control can play a role in the observed phenomena [8].

Thus, modulation of immunological reactivity developed during the formation of chronic epileptic syndrome, in our experiments during CK, can led to the development of central effects of cytokines potentiating primary epileptogenic excitation, which is a potential mechanism of epileptogenic effects of kindling.

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