Pentoxifylline Potentiates Antiepileptic Activity of Diazepam on the Model of Treatment-Resistant Focal Epilepsy

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Kindling-syndrome was modeled in male Wistar rats using repeated corazole administration (25-30 mg/kg intraperitoneally). The effects of pentoxifylline (25 and 100 mg/kg intraperitoneally) and diazepam (0.05 and 1 mg/kg intraperitoneally) were studied in 3 weeks after kindling development under conditions of acute observation on the model of penicillin-induced foci in cerebral cortex. An anticonvulsant effect of combined treatment with diazepam and pentoxifylline in the doses, ineffective if administered separately (0.05 and 25 mg/kg intraperitoneally), was demonstrated.

Key Words: pentoxifylline; resistant convulsive syndrom; corazole kindling; diazepam

Pentoxifylline (PTF) is capable of reducing the synthesis and release of proinflammatory cytokines TNF-α and IL-1β [4] and produces an anticonvulsant effect in the model of pharmacological kindling in rats [1]. Increased production of the specified cytokines represents a pathogenic mechanism of pharmacological resistance of kindling-provoked convulsions [1,5]. Under conditions of kindling epileptogenesis, foci produced by administration of penicillin disturbing GABAergic inhibition [7] are also resistant to antiepileptic drugs [1,2].

Here we studied the dynamics of activity of epileptic foci created in the frontal cortex of rats with resistant kindling syndrome under conditions of individual or combined application of PTF and diazepam.

MATERIALS AND METHODS

Acute experiments were performed on male Wistar rats weighing 180-250 g kindled by repeated daily administration of corazole in a subthreshold dose (25-30 mg/kg intraperitoneally, 21 injections). Only the animals with generalized clonic-tonic seizures after the last 3 injection were included into the experiments. These animals were observed in 3 weeks after corazole withdrawal, which allows modeling of a pharmacologically resistant epileptic syndrome [1,2,5].

The rats narcotized with ketamine (100 mg/kg intraperitoneally) were fixed in a stereotaxis SEG-5, the left and right frontal cortex was exposed, and the active electrode was placed there. The indifferent electrode was fixed to the nasal bones. In 30 min, the animals were administered with d-tubocurarine (0.15 mg/kg intravenously; “Orion”) and jet ventilation was started. The sites of dissection and compression were infiltrated with novocaine (0.25%).

Electric activity of brain structures was recorded in 1.5 h after the start of operative intervention using a DX-5000 computer system (Kharkov) at a sampling rate of 256 imp/sec; the data were visualized on the monitor and recorded on a hard disk for subsequent
offline processing using Matlab 7.0 software. Signal frequency range was 0.5-40.0 Hz.

Foci of epileptic activity (EA) were formed by applying filter paper soaked in fresh benzylpenicillin sodium salt solution (30,000 U/ml) on the brain surface [2].

PTF (Sigma-Aldrich) was injected intraperitoneally in doses of 25 and 100 mg/kg, diazepam (Gedeon Richter) was used in doses of 0.05 and 1.0 mg/kg. Control animals received physiological saline under similar conditions. Drug injections were administered against the background of generation of frequency- and amplitude-stable spike activity in the foci.

The severity of focal EA was estimated by frequency—amplitude properties of spike potentials and focus lifetime [2].

The results (latency and power of bioelectric activity) were processed using one-way ANOVA and Newman–Keuls test; the severity of convulsions was estimated using Kruskal–Wallis test.

RESULTS

Formation of foci induced by penicillin solution application onto cerebral cortex in control rats was followed by the appearance of interictal spikes with a latency of 2.5–5.5 min. Firing frequency increased to 25-45 per minute and firing amplitude up to 1.5-2.0 mV within 5-10 min. Frequency- and amplitude-stable EpA was recorded in the foci over 15-25 min and then spike amplitude and frequency gradually decreased over 30-50 min. The lifetime of EpA foci in control animals was 57-89 min (71.6±10.5 min at average).

In 30 min after administration of 100 mg/kg PTF, firing rate in the foci was 18.7±2.4 per minute and was below the control by 19.3% (p<0.05; Fig. 1, a). The difference between the groups remained significant during the following 30 min (Fig. 1, a). In 50 min after application of 100 mg/kg PTF, spike amplitude was below the control by 18.4%: 1.42±0.30 mV (p<0.05; Fig. 1, b). Lifetime of EpA foci generated under conditions of PTF application (100 mg/kg) was 45-80 min (mean 65.3±8.4 min) and did not differ from the same index in the control group (p>0.05).

On minute 30 of diazepam application (1 mg/kg), the spike generation rate in the foci significantly decreased by 27.8% in comparison with the corresponding parameter in control (p<0.05; Fig. 2, a). Significant difference between groups persisted over the next 30 min and constituted 37.9% by the 60th minute (p<0.05; Fig. 2, a). Diazepam (1 mg/kg) decreased spike amplitude by 21.5% on minute 40 in comparison with the control (p<0.05; Fig. 2, b). Significant difference between the groups persisted to the end of observation and amounted to 24.8% by the 60th minute (p<0.05; Fig. 2, b). Diazepam application did not change the lifetime of penicillin-induced foci: it was below the corresponding parameter in the control after administration of 0.05 and 1 mg/kg diazepam by 7.5 and 12.1%, respectively (p>0.05).

Combined application of diazepam and PTF in ineffective individual doses (0.05 and 25 mg/kg, respectively) produced a decrease of spike generation frequency by 22.8% as early as in 20 min in comparison with the corresponding parameter in the control (16.3±1.5 spikes per minute, p<0.05; Fig. 3). Significant decrease of firing amplitude (by 26%) was observed on minute 30 (p<0.05). At the same time, significant difference between groups persisted to the end of observation time, and duration of existence of foci reduced down to 47.9±5.8 min (with fluctuations from 27.5 to 62 min, p<0.05).

![Fig. 1](image-url). Frequency (a) and amplitude (b) of penicillin-induced EpA foci in cerebral cortex of intact rats under conditions of PTF administration in doses of 25 (light bars) and 100 mg/kg (dark bars). Here and in Figs. 2 and 3: *p<0.05 in comparison with the corresponding parameter in the control (100%).