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ELECTROPHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS OF THE ANTICONVULSANT ACTION OF A 3-HYDROXYPYRIDINE DERIVATIVE

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UDC 615.272.4.014.425:547.823].017:615.213].015.4:[612.825.1.014.423+612.822.1

KEY WORDS: antioxidants; convulsions; 3-HPD.

A promising class of antioxidants, among which some biologically active compounds have been synthesized, is that consisting of 3-hydroxypyridine derivatives (3-HPD) [8, 9]. 3-HPD have a broad spectrum of psychotropic effects, the most important of which are anxiolytic, antistressor, and antihypoxic [1, 7]. It has been shown, in particular, that 3-HPD prevent the development of convulsions induced by metrazol, strychnine, bicuculline, thiosemicarbazide, and maximal electric shock, and can reduce epileptiform activity (EPA) in experimental cobalt and penicillin epilepsy [1, 3, 6, 7].

The mechanism of the anticonvulsant effect of these antioxidants is not yet clear. Accordingly, the aim of this investigation was to study the mechanisms of the anticonvulsant action of 3-HPD from electrophysiological and biochemical aspects.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-220 g. Primary generalized EPA was induced by intramuscular injection of various doses of bemegride. In the experiments of series I rats with chronically implanted electrodes were used. Electrical activity was studied in the sensomotor cortex, dorsal hippocampus, and lateral hypothalamus. Methods of electrode implantation and of EEG recording were described previously [5]. The EEG was recorded in unrestrained animals on a "Neurograph" electroencephalograph and the data were processed on a BAS-161 neurocomputer (O.T.E. Biomedica, Italy). The animals were divided into three groups: 1) 3-HPD (50 mg/kg) were injected 15 min after bemegride (10 mg/kg) in the presence of frank EPA; 2) 3-HPD in the same dose were given 30-40 min before injection of bemegride; 3) control — only bemegride was given.

In the experiments of series II the effect of 3-HPD on lipid peroxidation (LPO) in the animal brain was studied on a model of primary generalized EPA. In the experiments of this series bemegride was injected intramuscularly in a dose of 25 mg/kg. To assess the convulsions the latent period (LP) of the first twitch of single muscles and LP of the first generalized fit, arising after injection of bemegride, were recorded. The state of LPO in the animals'

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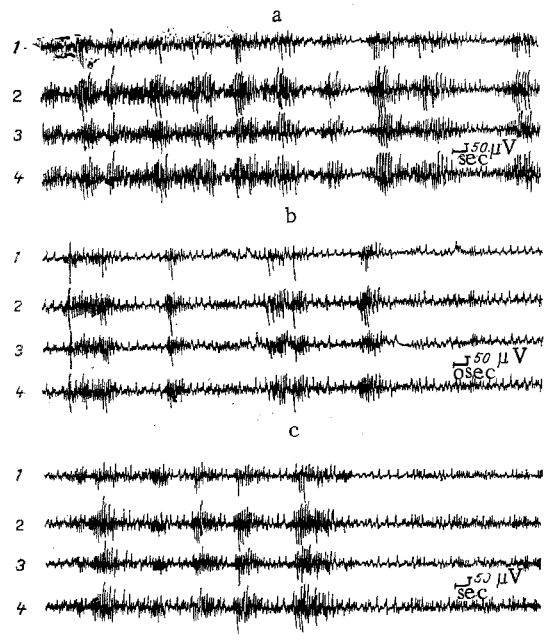
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 12, pp. 663-665, December, 1986. Original article submitted February 10, 1986.

 0007-4888/86/0012-1644$12.50 © 1987 Plenum Publishing Corporation

TABLE 1. Effect of 3-HPD on General Seizure Activity of Brain Structures Induced by Bemegride

Substance	Duration of discharges in 1 min	Number of discharges in 1 min	Duration of one discharge
Bemegride (10 mg/kg)	23,56 16,42—30,70	11,33 7,97—14,69	2,08 1,20—2,95
3-HPD (50 mg/kg) + bemegride (10 mg/kg)	12,61* (7,57—17,66)	5,4* (2,88—7,93)	2,3 (1,7—2,96)

Legend. *P = 0.05.



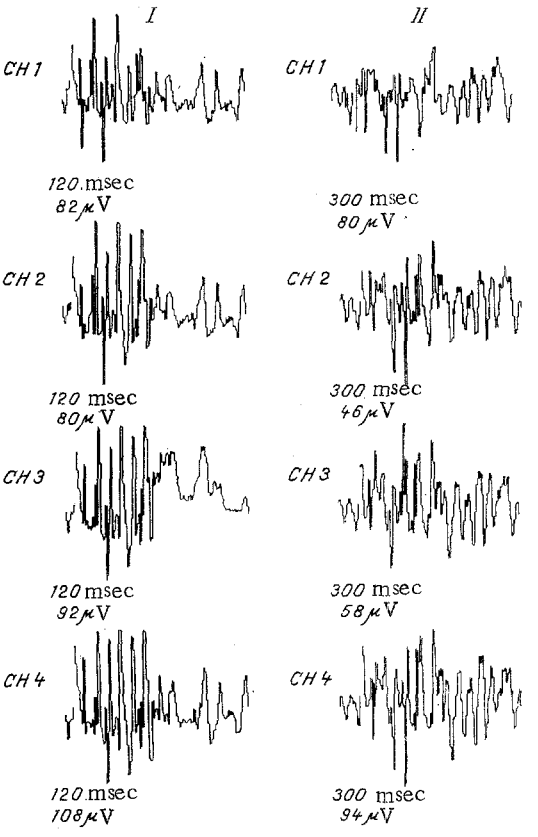


Fig. 1 Fig. 2

Fig. 1. Effect of 3-HPD on generalized EPA of rat brin induced by bemegride (10 mg/kg). ECG recorded from: 1) right and 2) left sensomotor cortex; 3, 4) from lateral hypothalamus and dorsal hippocampus, respectively. a) 10 min after injection of bemegride; b) 15 min after injection of 3-HPD preceded by bemegride; c) 30 min after injection of 3-HPD alone.

Fig. 2. Effect of 3-HPD on EPA of rat brain induced by bemegride. Separate paroxysms isolated from ECG of right (1) and left (2) sensomotor cortex and also of lateral hypothalamus (3) and dorsal hippocampus (4). I) Before, II) after injection of 3-HPD.

brain was determined from the concentrations of products reacting with 2-thiobarbituric acid [5]. In the experiments of this series 3-HPD were injected in a dose of 150 mg/kg 30-40 min before injection of bemegride. The control animals received bemegride only.

The results were subjected to statistical analysis with calculation of the arithmetical mean and confidence interval at the P = 0.05 level.

TABLE 2. Level of LPO Products in Rat Brain Homogenates

Substance	Concentration of LPO product in brain homogenates, relative units	Degree of change
Control	0,195 (0,175—0,215)	1
3-HPD (150 mg/kg)	0,182 (0,152—0,212)	0,93
Bemegride (25 mg/kg)	0,452 (0,412—0,492)	2,31
3-HPD + bemegride	0,193 (0,183—0,203)	0,98

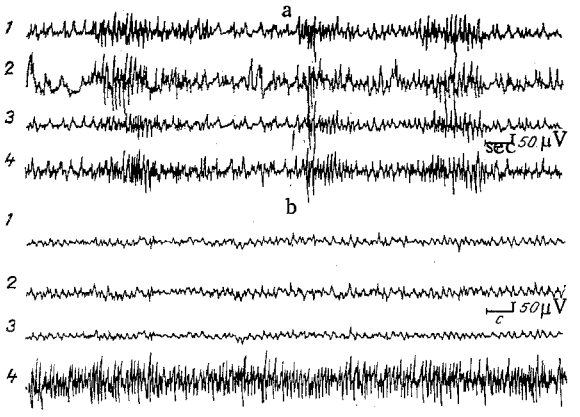


Fig. 3. Effect of preliminary injection of 3-HPD on development of EPA induced by bemegride. a) EEG after injection of bemegride; b) bemegride injected 40 min after 3-HPD. Remainder of legand as to Fig. 1.

EXPERIMENTAL RESULTS

Bemegride in a dose of 10 mg/kg caused distinct EPA on the EEG of all regions of the rat brain investigated. Discharges of EPA appeared synchronously in all structures with the same duration of the individual discharges, and with the greatest amplitude in the dorsal hippocampus (Fig. 1).

The epileptiform discharges were characterized by high-amplitude (up to 250 μV) waves with a frequency of 7-8 Hz, with pointed crests, alternating with pointed peaks; from 8 to 15 paroxysms were observed during 1 min, each up to 3 msec in duration (Table 1).

Injection of 3-HPD against the background of frank generalized EPA, induced by injection of bemegride, led to marked inhibition of paroxysmal activity in all structures studied. It will be clear from Table 1 that under the influence of 3-HPD the duration of single paroxysms induced by bemegride was unchanged, but there was a marked decrease in the number of convulsions per unit time. This effect took place as early as 10-15 min after injection of 3-HPD and it was most clearly noted 30-40 min after injection of the antioxidant. Incidentally, the hydroxypyridine did not disturb synchronization of onset of paroxysms induced by bemegride. For instance, Fig. 2 shows that the beginning of the discharges was the same for all structures, both after injection of bemegride and under the influence of 3-HPD. The decrease in amplitude of the potentials under these circumstances was very small.

When 3-HPD were given 30-40 min before injection of bemegride the antioxidant prevented the development of EPA almost completely in the sensory cortex and lateral hypothalamus, and the EEG showed only single reduced discharges of "pointed" waves. Meanwhile, many high-amplitude "pointed" waves could be seen on the EEG recorded from the dorsal hippocampus (Fig. 3).

In the next series of experiments the physiological parameters were compared with the results of the biochemical experiments. Intramuscular injection of bemegride (25 mg/kg) was found to produce the characteristic pattern of primary generalized EPA. After a definite LP the animals exhibited single spasms of muscles of the head, neck, and upper limbs, but later these infrequent spasms of single groups of muscles soon increased in frequency and changes into a fully developed and generalized episode of EPA.

Preliminary injection of 3-HPD (150 mg/kg) caused abrupt suppression of this form of EPA. Paroxysmal manifestations were completely prevented in 37.5% of the animals, and in the remaining rats LP of the first seizure discharges was considerably increased: 5.1 (3.0-6.3) min in the control, 8.7 (6.3-11.64) min in the experiment.

Comparison of the physiological parameters with the results of the biochemical experiments showed a considerable increase in the quantity of LPO products in brain homogenates obtained from rats at the height of seizure activity induced by bemegride.

Preliminary injection of 3-HPO led to normalization of LPO and lowered the level of LPO products, when raised by the action of bemegride, to the control values (Table 2).

EPA was inhibited by 3-HPD principally in the cortex and hypothalamus, and to a lesser degree in the hippocampus.

If these results are analyzed in the light of the theory of generator mechanisms of neuropathological syndromes [2] it can be postulated that the hippocampus is the determinant structure in the development of primary generalized EPA induced by bemegride, and that through the action of 3-HPD on the hyperactive system EPA is abolished, initially in the dependent, originally weaker, focus, which reflects the general rule of disintegration of a pathological system.

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