ANTICONVULSANT PROPERTIES OF ENOMELANIN

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A pathogenetic link in the chain of development of epileptic activity (EA) is disturbance of regulation of lipid peroxidation (LPO) of brain neuronal membranes [12]. During audiogenic seizures [6], focal EA induced by penecillin application [7, 8], and also primary generalized EA evoked by injection of bemegride [12], LPO is activated in the cerebral cortex. Administration of antioxidants prevents the activation of LPO and largely inhibits EA [7-10, 12]. However, not all types of EA are equally inhibited after normalization of LPO by the antioxidants α-tocopherol and ionol. The generalized form of EA is the most resistant [9, 10, 12].

A new preparation, enomelanin (from pressed grapes) has recently been obtained [4] and, besides antioxidant properties [5], it also has the ability to catalyze the electron transport reaction, to change the NAD/NADH concentration ratio [2], to activate energy homeostasis of the cell, to bind selectively and transport metal ions [3], to perform the functions of photo- and radioprotectors in the body [13], and to convert the energy of excited states reversibly through photon-phonon conversion transitions [11]. The stress-protective action of enomelanin has been demonstrated on models of acute and chronic exposure to stress [1]. With this combination of properties, enomelanin may be expected to have definite anticonvulsant actions.

The aim of this investigation was to study the antiepileptic properties of enomelanin on various models of EA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 170-210 g. The antiepileptic action of enomelanin was tested on models of primary generalized and focal EA. Primary generalized EA was induced by intraperitoneal injection of metrazol in a dose of 80 mg/kg. The electrocorticogram (ECoG) was recorded by means of electrodes applied epidurally in the region of the frontal and occipital poles of the hemispheres. The experiments began 1.5-2 h after the end of anesthesia (pentobarbital), which was used during the operation. The ECoG and the animals' movements were recorded on an EEG-4P-02 encephalograph immediately after injection of metrazol, with simultaneous visual observation. The intensity of the seizures was estimated in points: 0 point) no seizures, 1 point) convulsive starting movements, 2 points) clonic convulsions, 3 points) marked clonicotonic convulsions with the animal falling onto its side, and with clear tonic extension, 4 points) clonicotonic convulsions leading to death of the animal. The latent periods of onset of the first seizure manifestation and mortality of the animals were determined.

Focal EA was induced by application of a 1% solution of the sodium salt of penicillin to the exposed surface of the sensomotor cortex of one hemisphere. A determinant focus appeared in the zone of application and a "mirror" focus of EA, synchronized with the determinant focus, appeared in the corresponding symmetrical part of the opposite hemisphere. The ECoG was recorded on an 8-channel RM-86M polygraph.

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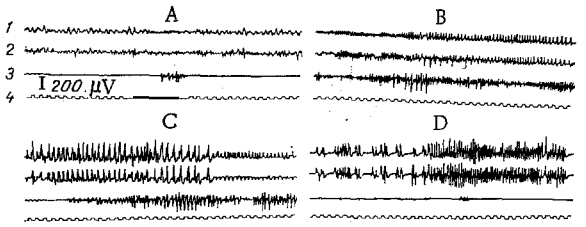

Fig. 1. EA evoked by intraperitoneal injection of metrazol. A) Control; B, C, D) 1, 4, and 7 min, respectively, after injection of metrazol. 1) ECoG (left); 2) ECoG (right); 3) actogram; 4) time marker, 1 sec. Continuous line indicates photic stimulation with frequency of 50 Hz.

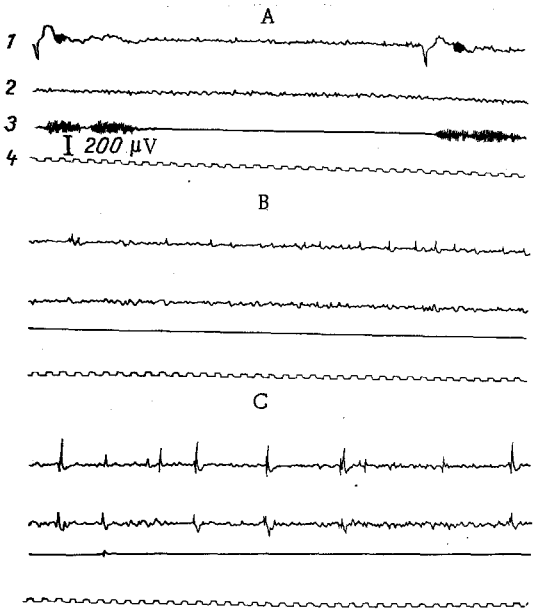

Fig. 2. Effect of preliminary injection of enomelanin on EA evoked by intraperitoneal injection of metrazol. A, B, C) EA 1, 8, and 25 min, respectively, after injection of metrazol. Enomelanin was injected intraperitoneally 4.5 h before metrazol in a dose of 50 mg/kg. Remainder of legend as to Fig. 1.

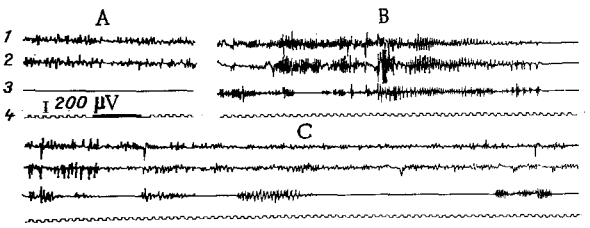

Fig. 3. Effect of enomelanin, injected after development of a clonicotonic seizure. A) Control, B) clonicotonic fit. Remainder of legend as to Fig. 1.

Enomelanin was injected intraperitoneally as a 1% solution in distilled water in doses of 10 to 100 mg/kg.

EXPERIMENTAL RESULTS

Model of Primary Generalized EA. Synchronized spindle-like activity (Fig. 1) appeared on the ECoG 7-10 sec after injection of metrazol (Fig. 1), and this was followed by the formation

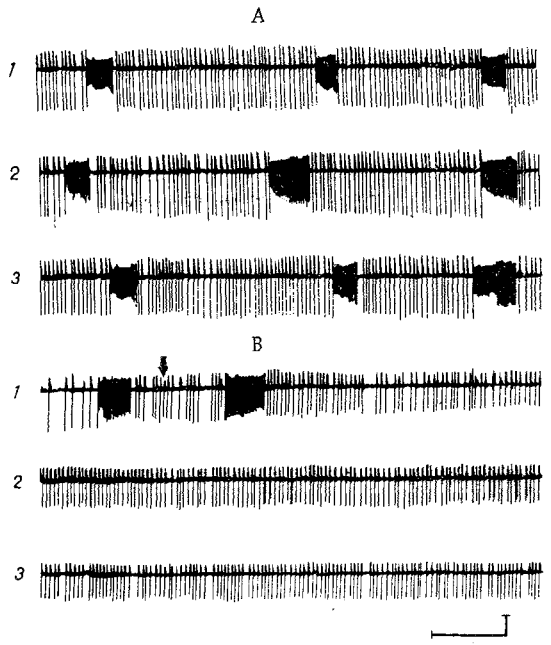

Fig. 4. Effect of enomelanin on focal EA evoked by penicillin application. A) Control; B) injection of enomelanin in a dose of 100 mg/kg against the background of EA (arrow). 1, 2, 3) 15, 30, and 60 min, respectively, after penicillin application.

of seizure potentials, accompanied by clonic seizures. The first intact seizure discharge was accompanied by a generalized convulsion, with the animal falling onto its side (Fig. 1B), and after the end of the convulsion the animal stood up on its paws and froze in that posture for 15-30 sec. The second ictal discharge also was accompanied by a convulsion which progressed into a terminal stage: the seizures died away and were replaced by small twitchings of individual muscle groups (Fig. 1C). An ictal seizure discharge was then recorded with a high-frequency component, but in the absence of any motor components of the convulsion (Fig. 1D), and the animal died.

The maximal protective effect of the preparation was observed when it was given in a dose of 50 mg/kg, 4.5 h before metrazol (Table 1). Single seizure potentials appeared, against the background of enomelanin, 70-110 sec after injection of metrazol (Fig. 2A) and they were accompanied by tremor and by mild clonic convulsions. Interictal discharges of low amplitude were formed after 7-10 min (Fig. 2B), they increased in amplitude, and reached a maximum after 20-25 min. Reduction of clonic convulsions and tremor was observed (Fig. 2B, C). A similar protective effect of enomelanin was observed in 50% of cases. In the remaining animals generalized seizures, less intensive than in the control rats were observed; they appeared after a longer latent period (236 ± 53.1 sec) than in the control (76.2 ± 9.4 sec).

In the next series of experiments enomelanin was injected intraperitoneally in a dose of 50 mg/kg (into 15 animals) after the first generalized convulsion (Fig. 3C). Immediately after the injection of enomelanin, ictal discharges were transformed into interictal, and in seven animals comparatively severe myoclonus and tremor were observed (Fig. 3C).

In five animals single myoclonic contractions occurred, and after 5-7 min their behavior returned to normal, while three animals developed a tonicoclonic fit which terminated fatally.

Model of Focal Epilepsy. Application of penicillin to the sensomotor cortex led to the appearance of a characteristic pattern of EA on the ECoG: to begin with, single interictal discharges (IID) were observed on the ECoG 2-6 min after application, accompanied by clonic spasms of the corresponding muscle groups, and changing quickly into an epileptiform fit (EF), one of which succeeded another regularly for almost 2 h (Fig. 4A).

Injection of enomelanin in a dose of 100 mg/kg in the stage of marked seizure activity (15-25 min after penicillin application) caused rapid inhibition of EF (seven animals): after injection of the compound not more than one or two EF were recorded on the ECoG, and after 8-10 min the IID also ceased, after which the ECoG was virtually indistinguishable from normal

TABLE 1. Effect of Enomelanin on Clonicotonic Convulsions

Experimental conditions	Dose, mg/kg	Time between injection of enomelanin and of metrazol, h	Number of animals in series	Latent period of first seizure manifestations, sec	Severity of seizures, points	Number of animals dying in series
Metrazol	80	—	25	46,7±2,4	3,9±0,06	23
Enomelanin + metrazol	25	4,5	10	84±11,5*	3,7±0,15	7
Enomelanin + metrazol	50	2	10	64,2±3,75**	3,7±0,15	7
Enomelanin + metrazol	50	4,5	20	105,7±9,5**	2,9±0,25**	5
Enomelanin + metrazol	100	4,5	10	77,6±6,3**	3,2±0,25*	4

Legend: *$P < 0.05$, **$P < 0.001$.

for 40-90 min (Fig. 4B). Depression of EA was observed simultaneously in the determinant and "mirror" foci, after which EF reappeared in the foci (five animals). Injection of enomelanin caused total suppression of EA in the foci in two rats, and it did not recur.

In another series of experiments (nine animals) enomelanin was injected in the same dose 1.5 h after penicillin application to the cerebral cortex, in the phase of stabilization of EA, before it began to gradually decline. Under these conditions, after injection of enomelanin EA disappeared even more rapidly, and did not recur.

When injected beforehand, enomelanin had a definite protective effect against primary generalized metrazol seizures, but when injected after clonicotonic seizures had already developed, it weakened them considerably in most cases. By contrast with the fat-soluble antioxidants, whose anticonvulsant action on a model of focal epilepsy was usually not manifested until 12-18 h after their intraperitoneal injection [7-10, 12], enomelanin could effectively suppress this form of EA only a few minutes (and sometimes virtually at once) after its injection.

The results thus indicate that enomelanin possess a marked anticonvulsant action, which is evidently not entirely determined by its antioxidative properties.

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