Functional Aspects of Neuroprotective Effects of New Salts and Compositions of Baclofen in the Convulsive Syndrome Caused by Electroshock

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 667-670, May, 2012
Original article submitted March 9, 2011

New salts of baclofen (4-amino-3-(para-chlorophenyl)-butyric acid) and its compositions with organic carbonic acids (citric, succinic, malic, oxalic, nicotinic, glutamic acids and glycine) exhibited neuroprotective and anticonvulsive effects. They reduced the intensity of the convulsive syndrome and postconvulsive psychoneurological disorders in animals exposed to the maximum electroshock and electroconvulsive shock. Analogs of baclofen containing citrate and to a lesser extent those containing glutamate and glycine were significantly more active than the initial substance.

Key Words: *baclofen; electroconvulsive shock; maximum electroshock; neuroprotective effect; anticonvulsive effect*

Baclofen (Lioresal) is a myorelaxant of central action exhibiting anticonvulsive, sedative, anxiolytic, and analgesic effects. Clinical efficiency of baclofen in multiple sclerosis, spinal diseases of infectious, vascular, degenerative, and traumatic origin [5,6] prompted researchers to evaluate the prospects for the use of baclofen and its new derivatives in combined therapy of neurodegenerative diseases. Previous experience gained in the creation of drugs on the basis of mexidol and picamilon and our data on neuroprotective effects of phenibut, tolibut, phenotropil salts and compositions with metabolically active acids [2-4] suggest that the search for new compounds among baclofen salts and compositions more effective and safe than the initial substance is a promising trend. This concept was realized by the development of a series of baclofen salts and compositions with organic carbonic acids (citric, succinic, nicotinic, malic, oxalic, glutamic and glycine), involved in neurochemical processes, constituting the metabolic support of the CNS, and characterized by neurotropic and neurotransmitter effects.

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We studied neuroprotective activity of new salts and compositions of baclofen in convulsive disorders of the CNS.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (200-260 g) kept under standard vivarium conditions.

The cerebroprotective and possible anticonvulsive effects of the substances were evaluated using the maximum electroshock (MES) [1]. Electric stimuli (50 Hz, 150 mA, 0.2 sec) were applied through corneal electrodes. The neuroprotective and anticonvulsive effects of the studied substances were evaluated by the effects on the duration of postconvulsive coma, duration of the clonic and tonic phases, and total duration of convulsions.

Another model used in the study was primary generalized epilepsy induced by electroconvulsive shock (ECS) [1]. The animals were exposed to alternating electric current (50 Hz, 20 mA, 0.5 sec) through corneal electrodes. The effects of the substances on convulsion intensity were evaluated by a 5-point scale [1].

The effects on the animal behavioral profiles were evaluated by the open field test, conditioned passive avoidance (CPA) [1], and extrapolation avoidance (EA) tests. CPA and EA were trained 24 h before ECS, tested 2 h before ECS, and their retention was tested 24 h after ECS. Animal behavior in the open field was tested twice: directly before induction of convulsions (30 min after injection of substances) and 2-3 h after ECS.

Baclofen and its derivatives were injected intraperitoneally 30 min before electroshock in equimolar concentration in single doses of $^1/_{10}$ mol. weight: baclofen, 21 mg/kg; baclofen citrate (I), 41 mg/kg; baclofen succinate (II), 33 mg/kg; nicotinoyl-containing baclofen analog (III), 34 mg/kg; oxalate-containing baclofen analog (IV), 30 mg/kg; malate-containing baclofen analog (V), 35 mg/kg; glutamate-containing baclofen analog (VI), 36 mg/kg; and glycine-containing baclofen analog (VII), 29 mg/kg. Controls were injected with an equivalent volume of saline.

The results were statistically processed by Kruskal–Wallis ranked one-factor analysis, Dunn multiple comparisons test, and χ^2 test.

RESULTS

All the studied compounds (I>VI, VII>II, III, IV, V, and baclofen), significantly reduced the duration of the tonic phase of convulsions, total duration of convulsions, duration of coma, and percent of lethal outcomes in the group in the MES model (Table 1). Substance I (baclofen citrate) exhibited the highest effect and was significantly superior to baclofen by the majority of parameters.

In the ECS model, the initial substance and all its analogs significantly ($p<0.01$, χ^2 test) reduced the intensity of convulsive episode in animals: baclofen (by 34.8%), II (by 36.75%), III (by 31.6%), IV (by 30.88%)<V (by 42.25%), VI (by 47.65%)<I (by 55.6%), VII (by 56.05%).

Since open-field behavior, CPA, and EA of animals in the experimental groups were tested before and after ESC, a group of intact control not exposed to electric stimulation was formed for comparing the possible behavioral changes caused by repeated testing and electroshock. Repeated open-field testing of intact animals showed significant reduction of motor and exploratory activities because of the habituation phenomenon: the development of habituation to the contextual settings and extinction of exploration motivation in the familiar field. After ECS, the animals treated with I>VI>VII>II≥baclofen had less severe psychoneurological disorders: they exhibited less significant reduction of the locomotor, orientation, and exploratory activities in the open field than controls exposed to ECS; the values in animals treated with substance I were comparable with those of intact controls (Fig. 1).

Testing of CPA showed that long latent period before the first venture into the dark section was retained in intact rats; this fact indicated that information about dangerous compartment was fixed in their memory. In EA, the latency of diving (time from the moment when the animals were placed in aversive medium until they found the way out of the stress situation) was shorter in intact controls with every repeated test, because

TABLE 1. Effects of Baclofen Salts and Compositions on the Intensity of Convulsive Syndrome Induced by Maximum Electroshock (*M*±*m*)

Substance	D_{cp}, sec	D_{tp}, sec	TD_c, sec	D_{coma}, sec	NLO, *N*/*n* (%)
Saline (control)	296.62±27.09	43.18±3.54	337.14±32.11	741.03±69.24	87.5
Baclofen	204.21±18.59*	29.24±2.40*	241.20±20.05*	583.15±51.22*	62.5**
Baclofen derivatives					
I	193.09±19.66*	17.25±2.54*+	216.14±26.73*	328.65±7.55*+	37.5**++
II	203.24±20.31*	23.15±1.35*	230.67±32.18*	625.12±57.34*	62.5**
III	198.23±21.07*	24.75±1.49*	226.12±26.48*	639.26±60.11*	62.5**
IV	183.37±24.14*	25.48±2.53*	217.75±28.46*	622.48±55.12*	50**
V	228.45±22.07	30.18±2.66*	264.08±25.15*	612.23±57.04*	62.5**
VI	250.33±20.11	22.32±1.84*	280.04±18.05*	424.18±41.63*+	50**
VII	183.02±16.14*	19.32±1.49*+	212.36±27.44*	526.06±50.14*	50**

Note. D_{cp}: duration of clonic phase of convulsions; D_{tp}: duration of tonic phase of convulsions; TD_c: total duration of convulsions; D_{coma}: duration of coma; NLO: number of lethal outcomes in the group (*N*) *vs*. total number of animals in the group (*n*). *$p<0.05$, **$p<0.01$ in comparison with the control; +$p<0.05$, ++$p<0.01$ in comparison with animals treated with baclofen: Kruskal–Wallis ranked one-factor analysis, Dunn test for multiple comparisons, χ^2 test.

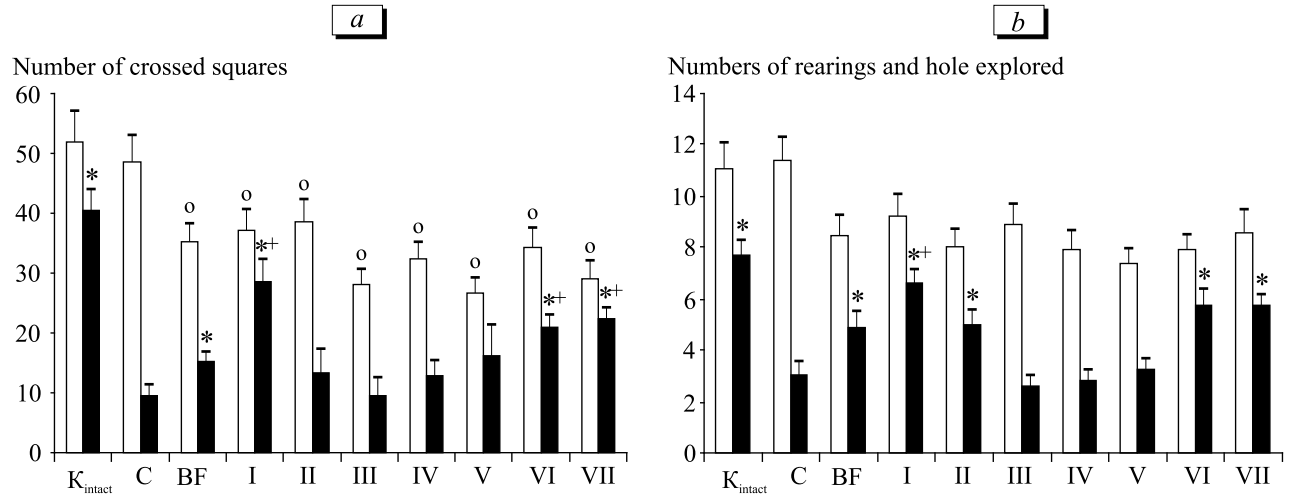

Fig. 1. Effects of new salts and compositions of baclofen on the motor activity (*a*) and orientation and exploratory behavior (*b*) of animals subjected to ECS in the open field test. Light bars: before ECS; dark bars: after ECS. C: control; BF: baclofen; I-VII: baclofen salts and compositions. Here and in Fig. 2: $p<0.05$ in comparison with: *C (after convulsions), °C (before convulsions), +group treated with baclofen. Intact controls were repeatedly tested without electrostimulation.

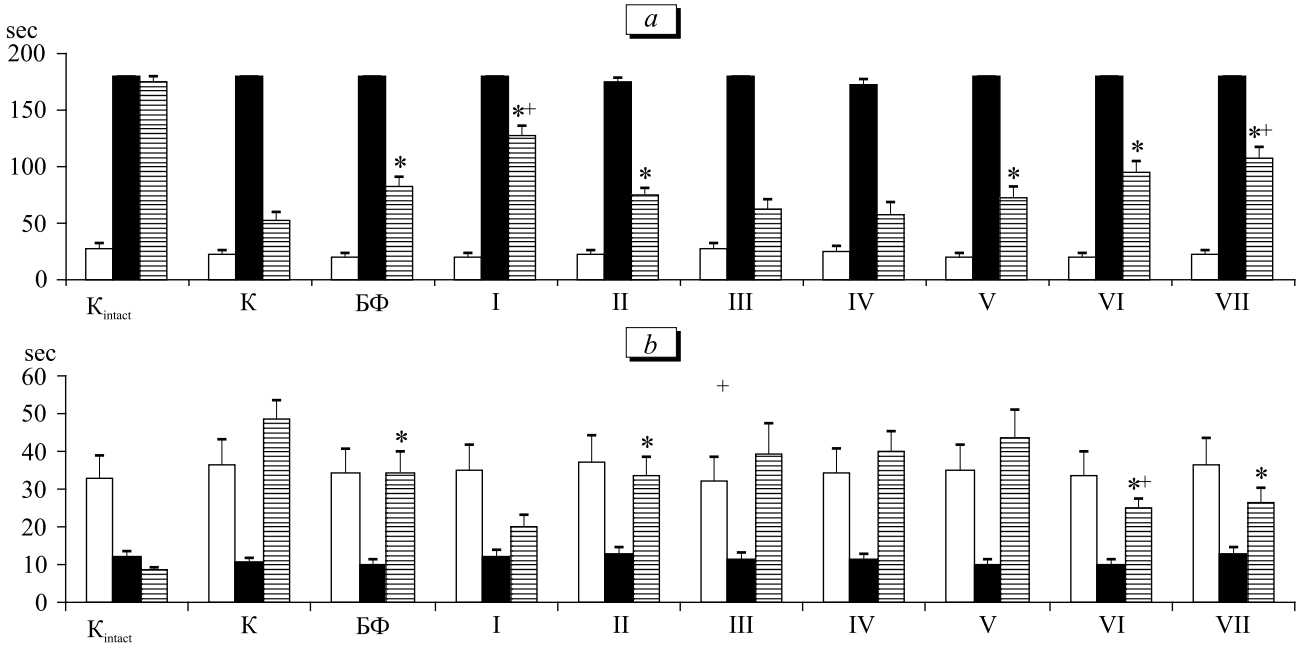

Fig. 2. Effects of baclofen salts and compositions on mnestic function of animals subjected to ECS. *a*) latency of the first venture into the dark section in CPA test (light bars: reflex training; dark bars: reproduction before ECS; cross-hatched bars: after ECS); *b*) latency of diving in EA test (light bars: avoidance training; dark bars: EA performance before ECS; cross-hatched bars: EA performance after ECS).

repeated exposure to this stimulus improved the training. The animals exposed to ECS demonstrated extinction of conditioned behavior. The animals treated with I>VI, VII>II, and baclofen before ECS demonstrated significantly longer latency of the first venture into the dark section in CPA test and reduced latency before diving in EA test in comparison with controls exposed to ECS (Fig. 2), this indicating the neuroprotective and nootropic effects of these substances.

Hence, the studied baclofen salts and compositions with carbonic acids were characterized by anticonvulsive and neuroprotective effects: they reduced the severity of the convulsive syndrome and of the postconvulsive psychoneurological disorders. Baclofen citrate (I) and less so glutamate- and glycine-containing baclofen analogs (VI and VII) were significantly more effective than the original substance.

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