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EFFECT OF COMBINED TREATMENT WITH DIURETICS AND GABAPENTIN ON CONVULSIVE THRESHOLD IN MICE

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Abstract: Research data show that diuretics can have anticonvulsant properties. This study examined effects of ethacrynic acid, a loop diuretic, and hydrochlorothiazide, a thiazide-type diuretic, on the anticonvulsant activity of gabapentin, a newer antiepileptic drug, in the maximal electroshock seizure threshold test in mice. Diuretics were administered intraperitoneally (*i.p.*) both acutely (single dose) and chronically (once daily for seven days). Electroconvulsions were produced by an alternating current (50 Hz, 500 V, 0.2 s stimulus duration) delivered *via* ear-clip electrodes by a generator. Additionally, the influence of combined treatment with the diuretics and gabapentin on motor performance in the chimney test has been assessed. In the current study, ethacrynic acid at the chronic dose of 12.5 mg/kg and the single dose of 100 mg/kg did not affect the anticonvulsant activity of gabapentin. Similarly, hydrochlorothiazide (100 mg/kg), both in acute and chronic experiments, had no effect on the gabapentin action. On the other hand, in the chimney test, the combined treatment with ethacrynic acid (100 mg/kg) and gabapentin (50 mg/kg) significantly impaired motor performance in mice. Based on the current preclinical findings, it can be suggested that the diuretics should not affect the anticonvulsant action of gabapentin in epileptic patients. However, the combination of ethacrynic acid with gabapentin may cause neurotoxicity

Keywords: Diuretics, gabapentin, maximal electroshock seizure threshold

In the kidney, loop diuretics (furosemide, ethacrynic acid, bumetanide) reach the lumen of the nephron at the proximal tubule, and they increase natriuresis by interfering with the reabsorption of filtered sodium in the thick ascending limb of the loop of Henle (1). Thiazide diuretics (chlorothiazide, hydrochlorothiazide) increase salt and water excretion primarily by inhibiting mechanisms for electroneutral sodium and chloride transport by distal convoluted tubule cells (2). Loop diuretics are widely used in clinical practice including the treatment of heart failure, renal insufficiency, nephrotic syndrome, cirrhosis and hypertension (3). The greatest use of thiazide diuretics has been as antihypertensive agents (3). It is likely that e.g., hypertension may appear in patients with epilepsy and such a comorbidity will require using both antiepileptics and diuretics in the treatment. Indeed, recent epidemiological studies show that hypertension appears more frequently in people with epilepsy as compared with the general population (4). It is commonly known that simultaneous administration of two or more drugs can lead to pharmacokinetic and/or pharmacodynamic interactions, which may influence efficacy and/or toxicity of antiepileptic drugs in patients. Therefore, studying such potential interactions seems important in animal models of seizures.

There are reports showing that diuretics can have anticonvulsant properties. It has been suggested that treatment with chlorothiazide or furosemide is associated with a decreased risk for unprovoked seizure, and is functionally anticonvulsant (5). Experimental studies on animals seem to confirm anticonvulsant activity of diuretics. Both furosemide and chlorothiazide can suppress the occurrence of maximal electroshock-induced seizures in a dose-dependent manner in mice (5). Moreover, furosemide exhibited the anticonvulsant action in kainic acid-induced status epilepticus in rats (6). In another study, ethacrynic acid, furosemide and bumetanide suppressed sound-triggered convulsions in post-ischemic audiogenic seizure-prone rats while

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mannitol was ineffective, suggesting that the mechanism is not through diuresis (7). Further, ethacrynic acid and furosemide have been documented to potentiate the protective activity of valproate in the maximal electroshock seizure (MES) test (8, 9). The combination of ethacrynic acid and topiramate has been also effective in this model of seizures (10).

Considering the above-mentioned anticonvulsant activity of diuretics, we sought to evaluate the effects of ethacrynic acid, a loop diuretic, and hydrochlorothiazide, a thiazide-type diuretic, on the antiseizure action of gabapentin, a novel second-generation antiepileptic drug (11). Gabapentin is used in the adjunctive treatment of partial seizures, with or without secondary generalization (12). Monotherapy with this drug seems to be also effective in refractory partial seizures (13). Additionally, the randomized trials of gabapentin proved its effectiveness in the treatment of neuropathic pain (14) and in migraine prophylaxis (15). In order to evaluate the interactions between diuretics and gabapentin, we used the maximal electroshock seizure threshold test (MEST test) in mice (16).

EXPERIMENTAL

Animals

Male Swiss mice (weighing 20-26 g, eight weeks of age) purchased from a licensed dealer were used in the current study. The mice had free access to food and tap water. They were exposed to a 12 h light/dark cycle and were kept in cages placed in a room with temperature of 21 ± 1°C, relative humidity 50-60%. The experimental groups, consisting of 8 animals, were chosen by means of a randomized schedule. All experimental procedures were approved by the Local Ethics Committee for Animal Experiments.

Drugs

Ethacrynic acid (Ethacrynic acid, MP Biomedicals, Solon, OH, USA), hydrochlorothiazide (Hydrochlorothiazidum, Polpharma S.A., Starogard Gdański, Poland) and gabapentin (Neurontin, Parke-Davis, Freiburg, Germany) were used in the current study. Ethacrynic acid, hydrochlorothiazide and gabapentin were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water. All drugs were injected intraperitoneally (*i.p.*) in a volume of 5 mL/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered 120 min (hydrochlorothiazide), 60 min (gabapentin) or 30 min (ethacrynic acid) prior to electroconvulsions. Diuretics were administered acutely (single dose) or chronically, once daily for seven days. Diuretics were given *i.p.* at doses and pretreatment times prior to electroconvulsions based on previous reports (5, 7, 10).

Maximal electroshock seizure threshold test (MEST test)

Electroconvulsions were produced by a generator (Rodent Shocker, Type 221, Hugo Sachs, Freiburg, Germany). The alternating current (50 Hz, 500 V) was delivered *via* ear-clip electrodes and the stimulus duration was 0.2 s. Full tonic extension of both hind limbs was taken as the endpoint. The convulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) required to produce tonic hindlimb extension in 50% of the animals tested. To calculate the convulsive threshold, at least three groups of mice (eight animals per group) were challenged with electroshocks of various intensities. An intensity-response curve was calculated with a computer, based on a percentage of animals convulsing in experimental groups. This experimental procedure was performed for hydrochlorothiazide following acute (100 mg/kg) and chronic (100 mg/kg) treatment. Ethacrynic acid was evaluated for an acute dose of 100 mg/kg and chronic maximum dose of 12.5 mg/kg as the higher chronic dose of 25 mg/kg of ethacrynic acid caused 18% lethality in mice (10).

Chimney test

Motor performance was evaluated with the chimney test of Boissier et al. (17). The animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm in length). Motor impairment was indicated as the inability of mice to climb backward up the tube within 60 s. Times at which mice were injected with diuretics alone or in combination with gabapentin in the chimney test were the same as in the MEST test.

Statistics

Calculations of median current strengths (CS_{50}s) with their 95% confidence limits were carried out by means of computer log-probit analysis according to Litchfield and Wilcoxon (18). The obtained 95% confidence limits were transformed to standard errors of the mean (SEM) according to a method described previously (19). The effects of tested drugs alone or combined on the convulsive threshold were analyzed using one-way ANOVA and the *post hoc* Dunnett's test for multiple comparisons. Statistical verifications of groups treated with gabapentin + a diuretic *versus*

gabapentin groups alone were performed with a method according to Litchfield and Wilcoxon (18). Group differences were considered statistically significant at $p < 0.05$. GraphPad InStat software was used for statistical analysis.

RESULTS

MEST test

Gabapentin at 12.5 mg/kg did not affect the threshold for electroconvulsions (CS_{50}) in mice,

Table 1. Effect of combined treatment with ethacrynic acid (EA) and gabapentin (GBP) on the convulsive threshold in mice.

Drug (mg/kg)	CS_{50} [mA] ± SEM				
		n		n	p
			Acute treatment EA (100)		
Vehicle	6.0 ± 0.38	16	6.7 ± 0.67	24	NS
GBP (12.5)	6.7 ± 0.56	24	7.7 ± 0.85	32	NS
GBP (25)	8.7 ± 0.37 **	16	8.1 ± 0.78	16	NS
GBP (50)	11.7 ± 0.65 **	16	11.3 ± 0.62 **	16	NS
	F (3, 68) = 21.814, p < 0.0001		F (3, 84) = 17.066, p < 0.0001		
			Chronic treatment EA (12.5)		
Vehicle	6.1 ± 0.52	32	6.5 ± 0.54	24	NS
GBP (12.5)	7.5 ± 0.52	16	5.6 ± 0.82	24	NS
GBP (25)	8.0 ± 0.54	16	9.0 ± 0.43	24	NS
GBP (50)	12.2 ± 0.92 **	24	11.7 ± 1.05 **	24	NS
	F (3, 84) = 5.037, p = 0.0029		F (3, 92) = 13.385, p < 0.0001		

Drugs were administered *i.p.*, ethacrynic acid 30 min and gabapentin 60 min prior to the test. Data are shown as median current strengths (CS_{50} in mA) with SEM values. n – number of animals at those current strengths, whose convulsant effects ranged between 4 and 6 probit according to Litchfield and Wilcoxon (18). SEM – standard error of the mean of CS_{50} values; F – F-statistics from one-way ANOVA; p – probability value. **p < 0.01 *vs.* respective control value (ANOVA/Dunnett's test); NS (not significant) *vs.* respective control group (Litchfield and Wilcoxon method).

Table 2. Effect of combined treatment with hydrochlorothiazide (HCTZ) and gabapentin (GBP) on the convulsive threshold in mice.

Drug (mg/kg)	CS_{50} [mA] ± SEM				
		n		n	p
			Acute treatment HTZ (100)		
Vehicle	6.0 ± 0.38	16	6.0 ± 0.63	24	NS
GBP (12.5)	6.7 ± 0.56	24	8.6 ± 0.85	24	NS
GBP (25)	8.7 ± 0.37 **	16	9.3 ± 0.25	16	NS
GBP (50)	11.7 ± 0.65 **	16	14.8 ± 1.80 **	32	NS
	F (3, 68) = 21.814, p < 0.0001		F (3, 84) = 17.066, p < 0.0001		
			Chronic treatment HCTZ (100)		
Vehicle	6.1 ± 0.52	32	6.3 ± 0.24	16	NS
GBP (12.5)	7.5 ± 0.52	16	7.7 ± 0.56	24	NS
GBP (25)	8.0 ± 0.54	16	8.7 ± 0.57	24	NS
GBP (50)	12.2 ± 0.92 **	24	13.0 ± 0.90 **	24	NS
	F (3, 84) = 17.066, p = 0.0001		F (3, 84) = 18.652, p < 0.0001		

Drugs were given *i.p.*, hydrochlorothiazide 120 min and gabapentin 60 min prior to the test. Data are presented as median current strengths (CS_{50} in mA) with SEM values. n – number of animals at those current strengths, whose convulsant effects ranged between 4 and 6 probit according to Litchfield and Wilcoxon (18). SEM – standard error of the mean of CS_{50} values; F – F-statistics from one-way ANOVA; p – probability value. **p < 0.01 *vs.* respective control value (ANOVA/Dunnett's test); NS (not significant) *vs.* respective control group (Litchfield and Wilcoxon method).

whereas gabapentin at 25 and 50 mg/kg raised the CS_{50} significantly ($p < 0.01$, ANOVA/Dunnett's test) (Table 1 and 2). The combinations of gabapentin (50 mg/kg) and ethacrynic acid (acute 100 mg/kg and chronic 12.5 mg/kg) increased the thresholds ($p < 0.01$, ANOVA/Dunnett's test), however, they did not differ from the CS_{50} values for gabapentin (50 mg/kg) alone treated mice ($p > 0.05$, Litchfield and Wilcoxon method) (Table 1). As shown in Table 2, also the combinations of gabapentin (50 mg/kg) and hydrochlorothiazide (acute 100 mg/kg and chronic 100 mg/kg) raised the thresholds ($p < 0.01$, ANOVA/Dunnett's test) that were not significantly higher compared to the CS_{50} values for gabapentin (50 mg/kg) alone groups ($p > 0.05$, Litchfield and Wilcoxon method).

Chimney test

Gabapentin (50 mg/kg) and the studied diuretics (ethacrynic acid and hydrochlorothiazide) at single doses up to 100 mg/kg, did not affect motor performance of mice in the chimney test. However, the combination of gabapentin (50 mg/kg) and ethacrynic acid (100 mg/kg) impaired motor coordination ($p < 0.01$, Fisher's exact probability test). The combined treatment with gabapentin (50 mg/kg) and hydrochlorothiazide (100 mg/kg) was not significant statistically, although a tendency towards impaired performance in this test was visible (Table 3).

DISCUSSION

Gabapentin significantly elevates the threshold for electroconvulsions in the MEST test (20), although it seems ineffective in the maximal electroshock seizure model (MES test) in mice (21). Both tests are regarded to be experimental models of tonic-clonic seizures in humans (16). Accordingly, gabapentin significantly raised the threshold for electroconvulsions in the current study but its anticonvulsant action was not affected by ethacrynic acid or hydrochlorothiazide. On the other hand, combined treatment with gabapentin and ethacrynic acid produced significant impairment in motor performance while co-administration of gabapentin with hydrochlorothiazide showed a strong tendency to it.

The exact molecular mechanism(s) of the anticonvulsant action of gabapentin remains to be elucidated, however, some potential mechanisms could be considered in this respect. Gabapentin inhibits Ca^{2+} voltage-gated channels through interaction with the $\alpha_2\delta$ subunit (22) and interacts with several enzymes in metabolic pathways of glutamate and GABA (11). It does appear to increase the synthesis of GABA (23) and decrease glutamate concentration (11). Moreover, gabapentin increases the hyperpolarization-activated cation current (I_h) in dendrites and therefore, the drug additionally reduces neuronal excitability (24). Recently, it has been found that gabapentin selectively activates presynaptic $GABA_B$ heteroreceptors, but not $GABA_B$ autoreceptors, decreasing neurotransmitter release (25). The mechanisms of anticonvulsant action of diuretics are not clear. Ethacrynic acid inhibits synaptic excitability likely by the neuronal blockade of K^+-Cl^- co-transport (KCC), especially KCC2 co-transporter (26). In the case of thiazides, an inhibition of the carbonic anhydrase might be responsible for their anticonvulsant properties (5).

Table 3. Effect of combined treatment with diuretics and gabapentin on motor performance in the chimney test.

Drug (mg/kg)	n	Percentage of mice impaired (%)
Control	8	0
EA (50)	8	0
EA (100)	8	0
HCTZ (100)	8	0
GBP (50)	8	0
EA (100) + GBP (50)	8	75 **
EA (50) + GBP (50)	8	12.5
HCTZ (100) + GBP (50)	8	37.5

Data are presented as percentage of animals that failed to perform in the chimney test. Drugs were injected at single doses *i.p.*, hydrochlorothiazide (HCTZ) 120 min, gabapentin (GBP) 60 min and ethacrynic acid (EA) 30 min before the test. n – number of animals. **$p < 0.01$ *vs.* control group (Fisher's exact probability test).

As described above, gabapentin and diuretics act through different mechanisms of action related with their anticonvulsant activity. It is thought that drugs with diverse mechanisms of action may complete their own activities and, thus, produce a synergistic interaction (27). However, it was not present here, at least in terms of the anticonvulsive action of the studied drugs. A possibility exists that there might be pharmacokinetic interactions between gabapentin and diuretics, considering that the antiepileptic drug is excreted as an unchanged drug *via* the kidneys (28). However, this possibility seems unlikely because there was an impaired motor coordination in animals injected with gabapentin + ethacrynic acid. Also, a tendency towards the same direction was observed in mice administered with gabapentin + hydrochlorothiazide.

It has been reported that gabapentin may impair motor functions both in humans and animal models. For example, severe myopathy can be an unexpected adverse reaction to gabapentin therapy in patients (29). In the rat rota-rod model, gabapentin induced significant motor impairment (30). In this study, mice injected with 50 mg/kg gabapentin alone performed the chimney test correctly. It is not surprising since gabapentin at doses up to 1000 mg/kg was documented to be ineffective in this test (31). However, gabapentin when administered with other drugs can disturb motor performance in the chimney test. Co-administration of tiagabine (2 mg/kg) with gabapentin (37.5 mg/kg) impaired motor coordination in mice (32). In another study, gabapentin dose-dependently (at the dose range 5–35 mg/kg) potentiated motor-impairment produced by baclofen, which was attributed to gabapentin-induced increase in GABAergic activity (31). At the moment, it is not clear why combined treatment with gabapentin and ethacrynic acid disturbed motor performance of mice. It is noteworthy that ethacrynic acid, similarly to gabapentin, affects GABAergic and glutamatergic systems (33, 34) that are involved in modulation of motor functions (35). Therefore, it would be of interest to perform neurochemical studies on this issue. In respect of ethacrynic acid activity in the chimney test, nonspecific mechanisms should be also considered e.g., a fall in blood pressure of mice performing the task.

In conclusion, it is suggested that the use of tested diuretics in epileptic patients receiving gabapentin is presumed neutral upon its anticonvulsant action. However, the combination of ethacrynic acid with gabapentin may cause neurotoxic effects.

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