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ORIGINAL ARTICLE

Interaction between diuretics and tiagabine in the maximal electroshock seizure threshold test in mice

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Abstract: It has been reported that loop diuretics can affect the antiseizure action of valproate (VPA) and topiramate (TPM) in mice. This study evaluated the effect of ethacrynic acid (EA), a loop diuretic, and hydrochlorothiazide (HCTZ), a thiazide-type diuretic, on the anticonvulsant activity of tiagabine (TGB), a newer antiepileptic drug, in the maximal electroshock seizure threshold test. The threshold for electroconvulsions is regarded as an experimental model of tonic-clonic seizures in humans. Diuretics were administered intraperitoneally (i.p.) at single doses (acute treatment) or once daily for seven days (chronic administration). EA at subthreshold doses of 12.5 mg/kg (chronic) and 100 mg/kg (acute) did not affect the anticonvulsant activity of TGB. Similarly, HCTZ at the subthreshold dose of 100 mg/kg, both in acute and chronic experiments, remained without effect on the anticonvulsant action of TGB. From the preclinical point of view, the use of tested diuretics in epileptic patients receiving TGB is presumed neutral upon its anticonvulsant potency.

Key words: diuretics, tiagabine, maximal electroshock seizure threshold test

INTRODUCTION

Loop and thiazide diuretics are commonly prescribed medications. The site of effect of loop diuretics is the thick ascending limb of the loop of Henle in the kidney. They inhibit the Na^+-K^+-$2Cl^-$ reabsorptive pump, causing a diuresis of NaCl and KCl [1]. Thiazide diuretics block electroneutral NaCl reabsorption at the distal convoluted tubule, connecting tubule, and early collecting duct, evoking especially a NaCl diuresis [2]. Diuretics are used in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis [3].

Both clinical and experimental studies show that diuretics, especially loop diuretics, can possess anticonvulsive potency. For example, furosemide (FUR), a loop diuretic, suppressed spontaneous spiking and stimulation-evoked discharges in patients who were intractable to existing antiepileptic drugs [4]. FUR and chlorothiazide, a thiazide-type diuretic, were protective for the first unprovoked convulsions in adult patients [5]. In animal models, FUR and chlorothiazide suppressed the occurrence of maximal electroshock-induced seizures (MES) in a dose-dependent manner in mice [5]. In another study, ethacrynic acid (EA) and other loop diuretics prevented the occurrence of sound-triggered convulsions in post-ischemic audiogenic seizure-prone rats [6]. Further, EA and FUR potentiated the protective activity of some antiepileptics in the MES test in mice [7, 8]. The exact molecular mechanism(s) of the anticonvulsant action of diuretics remains to be elucidated. Loop diuretics including EA, block the potassium-chloride transporter KCC2 in neuronal membranes [6, 9]. KCC2 is a neuronal-specific isoform [10] compared to KCCA1 transporter highly expressed in the kidney [11]. The KCC2 blockade has been suggested as providing the anticonvulsant activity of loop diuretics [12, 13], whereas the carbonic anhydrase inhibition might be involved in the antiseizure action of thiazides [5].

Taking into consideration presented above anticonvulsant effects of diuretics, we decided to evaluate the effects of EA, a loop diuretic, and hydrochlorothiazide (HCTZ), a thiazide-type diuretic commonly used in clinical practice as an antihypertensive agent, on the antiseizure action of tiagabine (TGB), a novel second-generation antiepileptic drug [14]. It is noteworthy that among the tested second-generation antiepileptics (lamotrigine [LTG], oxcarbazepine [OXC] and topiramate [TPM]) with EA in the MES test, so far EA has been demonstrated to enhance the anticonvulsant action of TPM [7]. Because TGB is ineffective in the MES test in mice [14], to assess the antiseizure action of TGB combined with diuretics we used the threshold for electroconvulsions that is regarded as an experimental model of tonic-clonic seizures in humans, such as the MES test [15].

MATERIAL AND METHODS

Animals. Male Swiss mice, weighing 20-26 g, were purchased from a licensed dealer. The animals were housed in colony cages with free access to food (chow pellets) and tap water in a room with a 12 h light/dark cycle (temperature 21±1 °C, relative humidity 50-60%). The experimental groups consisting of 8 animals were made up at random. The experimental procedures were carried out between 09:00 -15:00 and each mouse was used only once. The experimental procedures run in this study were approved by the Local Ethics Committee for Animal Experiments.

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Drugs. The following drugs were used: ethacrynic acid (MP Biomedicals, Solon, OH, USA), hydrochlorothiazide (Hydrochlorothiazidum, Polpharma S.A., Starogard Gdański, Poland) and tiagabine (Gabitril, Cephalon, Maisons-Alfort, France). All the drugs were suspended in a 1% solution of Tween 80 (Sigma, St.Louis, MO, USA) in distilled water. EA, HCTZ and TGB were injected intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. The treatment times are shown in the Tables. In this study, diuretics were administered at single doses (acute treatment) or once daily for seven days (chronic treatment). The route of administration, doses and pretreatment times of diuretics before testing of their anticonvulsant activity, were based upon earlier reports [5, 6, 7].

Maximal electroshock seizure threshold test (MEST test). Electroconvulsions in mice were produced with ear-clip electrodes and an alternating current delivered by a generator (Rodent Shocker, Type 221, Hugo Sachs, Freiburg, Germany). 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 hind limb 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 acute (100 mg/kg) and chronic (100 mg/kg) treatment with HCTZ. Similarly, EA was tested for acute dose of 100 mg/kg. The maximum dose of chronic EA was 12.5 mg/kg. It has been previously demonstrated that EA at a higher dose of 25 mg/kg caused 18% lethality in mice [7].

Statistics. The results obtained in the MEST test were analyzed as follows: median current strengths (CS_{50}) were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [16], followed by the method transforming 95% confidence limits to SEM, as described previously [17]. The influence of drugs on the convulsive threshold was analyzed using the log-probit method for single comparisons, and next, if significance occurred with Litchfield and Wilcoxon method [16], by one-way ANOVA and the *post hoc* Dunett's test for multiple comparisons. Group differences were considered statistically significant at $P < 0.05$.

RESULTS

As shown in Tables 1 and 2, TGB (administered alone i.p., 15 min prior to the MEST test) at 2 mg/kg did not affect the threshold for electroconvulsions (CS_{50}) in mice. TGB (4 and 6 mg/kg) raised the threshold significantly ($P < 0.05$ and $P < 0.01$, respectively, ANOVA/Dunnett's test) (Tables 1 and 2). The combinations of TGB (6 mg/kg) with acute EA (100 mg/kg i.p., injected 30 min before the MEST test) or acute HCTZ (100 mg/kg i.p., administered 120 min prior to electroconvulsions) increased the CS_{50} values ($P < 0.01$ and $P < 0.05$, respectively, ANOVA/Dunnett's test); however, they were not significantly higher than the CS_{50} values for TGB (6 mg/kg) alone treated groups ($P > 0.05$, Litchfield and Wilcoxon method [16]) (Tables 1 and 2). Moreover, the

Table 1 Influence of combined treatment with ethacrynic acid and tiagabine in the MEST test.

Drug (mg/kg)	CS_{50} [mA] ± S.E.M.	n		n	P
			Acute treatment EA (100)		
Vehicle	6.2 ± 0.280	16	6.7 ± 0.666	24	NS
TGB (2)	6.3 ± 0.463	24	7.4 ± 0.418	16	NS
TGB (4)	7.7 ± 0.537	24	8.5 ± 0.255	16	NS
TGB (6)	9.7 ± 0.567 **	24	10.3 ± 0.441 **	16	NS
	F (3, 84) = 10.519, P < 0.0001		F (3, 68) = 8.749, P < 0.0001		
			Chronic treatment EA (12.5)		
Vehicle	5.7 ± 0.632	24	5.8 ± 0.375	16	NS
TGB (2)	6.2 ± 0.406	16	6.1 ± 0.442	24	NS
TGB (4)	7.5 ± 0.281 *	16	7.0 ± 1.027	32	NS
TGB (6)	8.7 ± 0.230 **	16	8.3 ± 0.434	16	NS
	F (3, 68) = 7.768, P < 0.0002		F (3, 84) = 1.475, P = 0.2271		

Table data are median current strengths (CS_{50} in mA) with S.E.M. values. Drugs were given i.p.: 30 min (EA) and 15 min (TGB) prior to electroconvulsions. n – number of animals at those current strengths, whose convulsant effects ranged between 4-6 probit (16 and 84%) according to Litchfield and Wilcoxon [16]. **P < 0.01; *P < 0.05 vs. respective control value (ANOVA/Dunnett's test); NS vs. respective control group (Litchfield and Wilcoxon method). S.E.M. – standard error of the mean; NS – not significant; EA – ethacrynic acid; TGB – tiagabine.

Table 2 Influence of combined treatment with hydrochlorothiazide and tiagabine in the MEST test.

Drug (mg/kg)	CS_{50} [mA] ± S.E.M.	n		n	P
			Acute treatment HCTZ (100)		
Vehicle	6.2 ± 0.280	16	5.6 ± 0.823	24	NS
TGB (2)	6.3 ± 0.463	24	8.0 ± 0.996	32	NS
TGB (4)	7.7 ± 0.537	24	9.0 ± 0.621	16	NS
TGB (6)	9.7 ± 0.567 **	24	10.1 ± 0.607 *	8	NS
	F (3, 84) = 10.519, P < 0.0001		F (3, 76) = 3.131, P = 0.0305		
			Chronic treatment HCTZ (100)		
Vehicle	5.7 ± 0.632	24	6.3 ± 0.241	16	NS
TGB (2)	6.2 ± 0.406	16	6.8 ± 0.429	8	NS
TGB (4)	7.5 ± 0.281 *	16	7.3 ± 0.440	24	NS
TGB (6)	8.7 ± 0.230 **	16	8.2 ± 0.634	24	NS
	F (3, 68) = 7.768, P = 0.0002		F (3, 68) = 2.402, P = 0.0752		

Table data are shown as median current strengths (CS_{50} in mA) with S.E.M. values. Drugs were administered i.p.: 120 min (HCTZ) and 15 min (TGB) prior to electroconvulsions. n – number of animals at those current strengths, whose convulsant effects ranged between 4-6 probit (16 and 84%) according to Litchfield and Wilcoxon [16]. **P < 0.01; *P < 0.05 vs. respective control value (ANOVA/Dunnett's test); NS vs. respective control group (Litchfield and Wilcoxon method). S.E.M. – standard error of the mean; NS – not significant; HCTZ – hydrochlorothiazide; TGB – tiagabine.

combinations of TGB (6 mg/kg) with chronic EA (12.5 mg/kg) or HCTZ (100 mg/kg) also raised the thresholds (statistically not significant, ANOVA/Dunnett's test) that did not significantly differ from the CS_{50} values for TGB (6 mg/kg) alone groups ($P > 0.05$, Litchfield and Wilcoxon method [16]).

DISCUSSION

The purpose of this study was to determine the effect of combined treatment of diuretics, ethacrynic acid (EA) and hydrochlorothiazide (HCTZ), with a novel antiepileptic drug tiagabine (TGB) on the convulsive threshold. TGB is licensed as an adjunctive drug for the treatment of partial seizures with secondary generalization [18]. TGB inhibits neuronal and glial uptake of GABA, leading to the enhancement and prolongation of GABA-mediated synaptic events [19]. TGB is a potent drug in antagonizing tonic convulsions induced by pentylenetetrazol (PTZ), 6,7-dimethoxy-4-ethyl-b-carboline-3-carboxylate (DMCM) and sound-induced seizures in DBA/2 mice [14]. TGB also blocks PTZ-induced clonic convulsions; however, it is ineffective in the MES test [14]. On the other hand, TGB (at doses higher than 2 mg/kg) significantly elevates the threshold for electroconvulsions [20]. Similarly, TGB (4 and 6 mg/kg) significantly raised the convulsive threshold in the current study while at the dose of 2 mg/kg it remained without effect on the threshold.

It has been earlier demonstrated that EA enhanced the anticonvulsant action of TPM in the MES test [7]. It is known that TPM is an antiepileptic drug sharing multiple mechanisms of action including potentiation of GABA inhibition. It potentiates inhibitory neurotransmission mediated by GABA through a novel binding site on the $GABA_A$-receptor complex [21]. Moreover, the antielectroshock activity of valproate (VPA), another antiepileptic with multiple mechanisms of action, has been also enhanced by EA (unpublished data). One of the mechanisms of VPA is interaction with the GABAergic neurotransmitter system. VPA elevates brain GABA levels and potentiates GABA responses, possibly by enhancing synthesis and inhibiting degradation of this neurotransmitter [22]. On the other hand, the combination of EA or HCTZ with phenobarbital (PB), a drug which exerts its pharmacological effects by allosteric activation of the $GABA_A$ receptor [23], was not protective against electroconvulsions (unpublished data). As shown here, no significant interaction between EA or HCTZ with TGB, a GABA enhancer [18], was observed. It would be of interest to examine the combined treatment with tested diuretics and vigabatrin (VGB), another GABA enhancer [18], as a further study on interaction between GABA-mimetic drugs and diuretics. VGB is an irreversible inhibitor of GABA-T, the intracellular enzyme responsible for the catabolism of GABA, and its administration in animals or humans results typically in an increased synaptic GABA level [18].

In conclusion, EA and HCTZ, both in acute and chronic experiments, did not affect the protective activity of TGB against seizures induced by electroconvulsive shock. From the preclinical point of view, the use of tested diuretics in epileptic patients receiving TGB should not disturb its anticonvulsant potency.

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