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European Journal of Pharmacology 516 (2005) 212–218

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Influence of aminoglutethimide and spironolactone on the efficacy of carbamazepine and diphenylhydantoin against amygdala-kindled seizures in rats

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Received 11 November 2004; received in revised form 5 January 2005; accepted 28 January 2005

Abstract

Antagonists of steroid receptors may interfere with seizure phenomena. The present study deals with effects of aminoglutethimide and spironolactone on the action of carbamazepine and diphenylhydantoin in amygdala-kindled rats of both genders. Co-administration of the antimineralocorticoid with carbamazepine at their ineffective doses (50 and 15 mg/kg, respectively) led to significant reduction of the seizure and afterdischarge durations. No anticonvulsant effect was observed when spironolactone was combined with diphenylhydantoin.

The concomitant treatment of aminoglutethimide and carbamazepine (both drugs at their subprotective doses of 5 and 15 mg/kg, respectively) resulted in antiseizure activity in respect of all measured parameters, including the afterdischarge threshold, seizure severity, seizure duration and afterdischarge duration. The similar combination of aminoglutethimide with diphenylhydantoin (2.5 mg/kg) significantly shortened the seizure and afterdischarge durations.

The antiseizure effect of tested combinations was not sex-dependent and not reversed by hydrocortisone pretreatment. Pharmacokinetic events may be involved only in the interaction between spironolactone and carbamazepine. Among various chemoconvulsants, bicuculline reversed the action of aminoglutethimide on carbamazepine and diphenylhydantoin. The effect of aminoglutethimide on diphenylhydantoin was also abolished by *N*-methyl-D-aspartic acid and aminophylline. In conclusion, our results suggest that doses of carbamazepine and diphenylhydantoin should be modified in epileptic patients concomitantly treated with aminoglutethimide or spironolactone.

Keywords: Aminoglutethimide; Spironolactone; Carbamazepine; Diphenylhydantoin; Amygdala-kindled seizures

1. Introduction

Some experimental data indicate the influence of several steroid hormones on seizure propagation. Receptors for gluco- and mineralocorticosteroids may be involved in the excitatory events in the central nervous system (Roberts et al., 1993; Roberts and Keith, 1994a). It was observed, for instance, that corticosterone enhances seizure susceptibility in mice (Roberts and Keith, 1994b). Aminoglutethimide, as an inhibitor of fat tissue aromatase and adrenal desmolase, decreases the synthesis of peripheral estrogens and adrenal steroids (cortisone, aldosterone and androgens). The drug is commonly used in hormone-dependent breast cancer in women after menopause and, as a pre-operative treatment in individuals with adrenal gland adenomas. On the other hand, spironolactone is a non-specific antagonist of aldosterone receptors, applied in cases of primary hyperaldosteronism and drug-resistant edemas. It was also reported that seizures might also develop in the course of pheochromocytoma syndrome. Therefore, epileptic patients taking common anticonvulsant therapy may be, from other reasons, treated also with aminoglutethimide or spironolactone (Leiba et al., 2003, Pinheiro et al., 2004). This prompted us to investigate possible interactions between amino-

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doi:10.1016/j.ejphar.2005.01.040

glutethimide, spironolactone and conventional antiepileptic drugs. Since amygdala-kindled seizures in rats are considered as a widely recognized model of human complex partial seizures, the similar pattern of interactions may be observed in epileptic individuals.

In our previous studies, we found that tamoxifen and cyproterone inhibit seizures evoked by maximal electroshock in mice (Borowicz, 2001), but not by amygdala kindling in rats (Borowicz et al., 2004). Moreover, the two sex hormone antagonists enhanced the anticonvulsant action of some antiepileptic drugs in both seizure tests. Recently, we reported that aminoglutethimide, but not spironolactone, increases the antiseizure efficacy of phenobarbital, clonazepam and valproate in amygdala-kindled rats. Spironolactone remained without effect in this respect (Borowicz and Czuczwar, 2004). In the present study we wanted to continue previous investigations expanding them over their effect on the protective action of carbamazepine and diphenylhydantoin. Both conventional sodium channel inhibitors are effective in amygdala-kindled seizures in rats and partial complex seizures with secondary generalization in humans (Löscher et al., 1986; Löscher and Schmidt, 1988).

2. Materials and methods

2.1. Animals and experimental conditions

Male and female Wistar rats (200–250 g) were used throughout the experiments after at least 1 week of acclimatization. They were housed in plastic cages under standard laboratory conditions (ambient temperature of 22±1 °C, natural light–dark cycle). Chow pellets and tap water was freely available. All experiments were done at the same period of time (between 9.00 a.m. and 12.00 a.m.) to minimize the influence of circadian rhythms on seizure susceptibility. The experimental groups consisted of six rats. All animal care and experimental procedures were carried out in accordance with Ethical Committee of Lublin Medical University and with Polish law on animal welfare.

2.2. Surgery and kindling procedure

The rats were anesthetized with pentobarbital (50 mg/kg i.p.) and received stereotaxic implantation of one bipolar electrode in the right basolateral amygdala. The following stereotaxic coordinates for electrode implantation were used: AP-2.2, L-4.8, V-8.5, according to the brain atlas of Paxinos and Watson (1986). All coordinates were measured from bregma. Scull screws served as the indifferent reference electrode. The electrode assembly was attached to the scull by dental acrylic cement. After electrode implantation, the animals were treated topically with an antibiotic (neomycin) for 1 week to prevent infection.

After a post-operative period of 2 weeks, the stimulation of amygdala was initiated. Each stimulus consisted of a 1-s train of 50 Hz, 1-ms biphasic square-wave pulses, with pulse amplitude of 500 μA, and was delivered every 24 h, until at least 10 sequential fully kindled stage 5 was elicited. The afterdischarges from the amygdala were recorded prior to and after the stimulation. The seizure severity (SSv) was assessed according to a modified Racine's scale (Racine, 1972). Rats were given 0 to 5 points depending on their behavior. Animals gained no point when no seizures were observed. Rats received 1 point when they showed immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, or facial clonus. Two points were associated with head nodding accompanied with more severe facial clonus. Animals received three points when they exhibited clonus of one forelimb, while 3.5 when bilateral forelimb clonus without rearing was seen. Four points characterized bilateral forelimb clonus with rearing, whereas 4.5 were attributed to falling on a side without rearing and loss of righting reflex accompanied by generalized clonic seizures. Finally five points were given to rats rearing and falling on the back accompanied by generalized clonic seizures. Seizure duration (SDr) was the duration of limbic seizures (stages 1–2) and motor seizures (stages 3–5). Afterdischarges (ADr) were defined as spikes with a frequency of at least 1 Hz and amplitude at least twice greater than the prestimulation baseline present in the EEG recorded from the site of stimulation. Afterdischarge threshold (ADT) was determined after administration of a test drug or its vehicle by administering a series of stimulations at intervals of 3 min increasing in steps of about 20% of the previously applied current until an afterdischarge of a duration of at least 3 s was evoked. At the ADT current, seizure severity, seizure duration, and afterdischarge duration were recorded and analyzed. Control readings were made 2 days before and 2 days after respective treatments. Subsequently, the means with standard deviation (S.D.) were calculated and used for statistical analysis of obtained data.

2.3. Drugs

Following drugs were employed in the study: diphenylhydantoin sodium, carbamazepine (both from Sigma, St. Louis, MO, USA), aminoglutethimide (Ciba, Switzerland), spironolactone and hydrocortisone (Polfa, Warsaw, Poland), kainic acid, *N*-methyl-D-aspartic acid, bicuculline and aminophylline (all convulsants from Sigma, St. Louis, MO, USA). Aminoglutethimide, spironolactone, diphenylhydantoin and carbamazepine were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA). All remaining drugs were brought into solution with sterile saline (bicuculline with additional drop of glacial acetic acid). The excitatory aminoacid solutions was adjusted with 0.1 N NaOH to pH 7.2, while that of bicuculline solution to 5.5. All drugs were given i.p., aminoglutethimide and spironolactone in a volume of 5 ml/kg (because of high density of their suspensions), while remaining drugs in a volume of 3 ml/kg. Hydrocortisone was injected on 2 consecutive days (twice daily) before convulsive tests. Carbamazepine was administered 30 min, diphenylhydantoin, aminoglutethimide, spironolactone 120 min, bicuculline 15 min, and all remaining drugs 30 min before tests.

2.4. Rotarod test

Motor coordination was assessed with the use of the rotarod test (Dunham and Miya, 1957). Each animal (pretreated 24 h earlier) was placed on a 6-cm diameter rod rotating at 6 rev/min. The time of falling off an animal was noted and the test was run up to 120 s.

Table 1
Effect of carbamazepine and the combinations of carbamazepine with aminoglutethimide and spironolactone in amygdala-kindled seizures in rats

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
CBZ (15)	49.6±4.1	51.2±4.6	5	4.5 (4;5)	38.8±2.6	36.5±3.1	65.4±2.8	64.3±3.1
CBZ (20)	48.2±4.2	59.2±5.2[c]	5	3.5 (3;4)[c]	37.7±2.9	30.3±2.9[c]	64.0±2.1	56.7±2.7[b]
CBZ (15)+AGLD (5)	44.0±4.1	91.5±4.0[a]	5	2.5 (2;3)[c]	45.3±3.3	39.5±3.0[b]	77.2±4.0	71.4±3.8[b]
CBZ (10)+AGLD (5)	44.5±4.3	46.8±4.6	5	4.5 (4;5)	48.3±3.7	46.7±4.0	76.1±3.9	74.0±4.1
CBZ (15)+SPIR (50)	46.3±4.3	48.0±4.2	5	5	47.4±3.3	41.6±3.6[b]	77.8±4.0	72.5±4.1[b]
CBZ (10)+SPIR (50)	44.0±4.3	45.6±4.5	5	5	48.5±3.9	45.9±4.6	79.2±4.3	77.5±4.2

AGLD, and SPIR were administered 120 min, CBZ 30 min before the test. [a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$ vs. respective controls (Student's t-test for paired replicates). AGLD, aminoglutethimide; SPIR, spironolactone; CBZ, carbamazepine; C, control group; ADT, afterdischarge threshold; SSv, seizure severity; SDr, seizure duration; ADr, afterdischarge duration.

2.5. Passive avoidance task

The rats were placed in an illuminated box (40×40×30 cm) connected to a dark box (40×40×30 cm), which was equipped with an electric grid floor. Entrance to the dark box was punished by an electric footshock of 2-s duration (0.2 mA). The animals that did not enter the dark compartment were excluded from the experiment. On the next day (24 h later), the same animals (without treatment) were put into the illuminated box and observed up to 180 s. The time period, an animal entered the dark box, was subsequently noted and the medians with 25 and 75 percentiles were calculated. According to Venault et al. (1986), the step-through passive avoidance task is recognized as a measure of long-term memory.

2.6. Estimation of free plasma levels and brain concentrations of antiepileptic drugs

The animals were administered an antiepileptic with vehicle (control group) or with the respective antihormone (tested group). Samples of blood of approximately 1 ml were obtained from the decapitation wound and collected into Eppendorf tubes at times scheduled for the convulsive test. Brains of rats were homogenized in TDx (Abbott, Irving, TX, USA) buffer in Eppendorf tubes. Samples of blood and brain homogenates were centrifuged at 10 500×g (Abbott centrifuge, Irving, TX, USA) for 3 min and plasma samples of 70 μl were transferred into system MPS-1 (Amicon, Danvers, USA) for separation of free from protein bound microsolutes. Then, the MPS-1 tubes were centrifuged at 3150×g (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min and the filtrate samples of 50 μl were put into Abbott system cartridges. Brain supernatant samples of 70 μl were also put into Abbott system cartridges. Free plasma levels and brain levels of antiepileptic drugs were estimated by immunofluorescence, using an Abbott TDx analyzer (Abbott, Irving, TX, USA) and expressed in μg/ml of plasma or μg/g of wet brain tissue as means±S.D. of least 8 determinations.

2.7. Statistics

The statistical significances of the afterdischarge threshold, seizure and afterdischarge durations in the same group were calculated by Student's t-test for paired replicates. The statistical significances between seizure scores were evaluated by the Wilcoxon signed rank test. The results from the rotarod and passive avoidance tests were compared using the Kruskal–Wallis test followed by Dunn's post-hoc test. Plasma levels of antiepileptics alone and in combination with carbamazepine or diphenylhydantoin were evaluated with Student's t-test for unpaired replicates.

3. Results

In our previous study, we reported that aminoglutethimide, spironolactone, and hydrocortisone (up to 5, 50, and 200 mg/kg, respectively) did not influence seizure parameters in kindled rats. However, aminoglutethimide (10 mg/kg) significantly decreased the seizure severity from 5 to 2.5, seizure duration from 35.7 to 25.0 s, and afterdischarge duration from 62.5 to 32.8 s (Borowicz and Czuczwar, 2004). Bicuculline (2 mg/kg), aminophylline (10

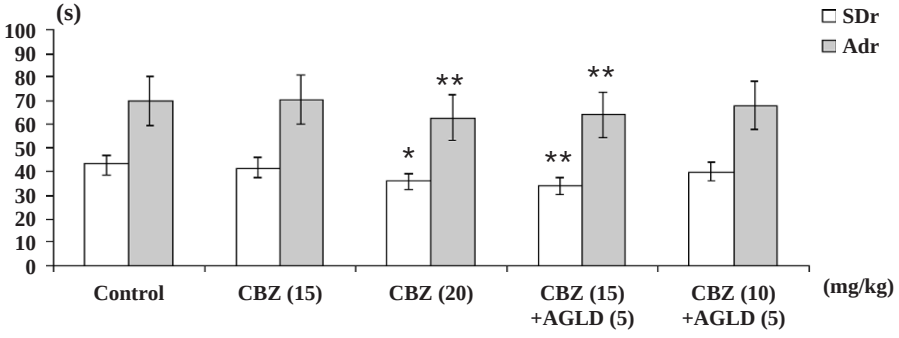

Fig. 1. Effect of carbamazepine (CBZ) and its combinations with aminoglutethimide (AGLD) on seizure duration (SDr) and afterdischarge duration (ADr) in fully kindled rats. SDr and ADr data are means±S.D. (in seconds). AGLD was used at the subprotective doses of 5 and 2.5 mg/kg, whereas PB was administered at various doses (in parentheses). Control readings were made 2 days before and after the respective treatments. *$P<0.05$, **$P<0.01$ vs. averaged control [Wilcoxon signed rank test].

Table 2
Effect of hydrocortisone and some convulsants on the protective effect of aminoglutethimide/carbamazepine combination in amygdala-kindled rats

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
CBZ (15)+AGLD (5)								
+saline	44.0±4.1	91.5±4.0[a]	5	2.5 (2;3)[c]	45.3±3.3	39.5±3.0[b]	77.2±4.0	71.4±3.8[b]
+HC (200)	42.4±3.6	83.0±4.0[a]	5	2.5 (2;3)[c]	46.7±3.4	41.7±3.2[b]	76.2±4.0	66.7±3.6[a]
+BIC (2)	46.3±3.8	46.6±4.4	5	4.5 (4;5)	48.3±3.7	46.5±4.2	75.6±3.6	74.4±4.1
+AMI (10)	42.8±3.6	84.5±4.2[a]	5	3 (2;4)[c]	47.5±3.6	40.2±4.1[b]	74.6±4.0	67.0±4.2[b]
+NMDA (30)	45.7±4.2	86.1±4.6[a]	5	2.5 (2;3)[c]	47.4±3.7	42.8±3.9[b]	78.6±4.1	71.9±4.4[b]
+KA (2.5)	43.4±4.3	85.8±4.4[a]	5	2.5 (2;3)[c]	48.5±4.1	41.7±4.4[b]	79.3±4.2	69.7±3.9[a]

AGLD, aminoglutethimide; CBZ, carbamazepine; HC, hydrocortisone; C, control group; ADT, afterdischarge threshold; SSv, seizure severity; SDr, seizure duration; ADr, afterdischarge duration. [a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$ vs. respective controls (Student's t-test for paired replicates).

mg/kg), *N*-methyl-D-aspartic acid (30 mg/kg) and kainic acid (2.5 mg/kg) did not affect amygdala-kindled seizures. However at higher doses, bicuculline (3 mg/kg), aminophylline (20 mg/kg), *N*-methyl-D-aspartic acid (40 mg/kg) and kainic acid (5 mg/kg) showed significant proconvulsive action, prolonging the seizure and afterdischarge durations (Borowicz and Czuczwar, 2004).

In the present study, the statistical analysis of results obtained from male and female rats indicates the same level of significance in both genders. In order to maintain the clarity of presented data, tables contain findings observed only in males.

3.1. Effect of aminoglutethimide and spironolactone on the action of carbamazepine in amygdala-kindled rats

Carbamazepine (20 mg/kg) significantly shortened the seizure duration and afterdischarge duration from 37.2 to 30.3 s, and from 64.0 to 56.7 s, respectively. The combined treatment of carbamazepine (15 mg/kg) and aminoglutethimide (5 mg/kg) significantly increased the afterdischarge threshold from 44.0 to 91.5 μA. This combination reduced also the seizure severity from 5 to 2.5, the seizure duration from 45.3 to 39.5 s, and the afterdischarge duration from 77.2 to 71.4 s (Table 1, Fig. 1). The concomitant treatment of spironolactone (50 mg/kg) with carbamazepine (15 mg/kg) reduced both seizure and afterdischarge durations, from 47.4 to 41.6 s, and from 77.8 to 72.5 s, respectively (Table 1).

3.2. Effect of hydrocortisone and some convulsants on the aminoglutethimide/carbamazepine combination in amygdala-kindled rats

The aminoglutethimide-induced action on carbamazepine was reversed by bicuculline (2 mg/kg). *N*-methyl-D-aspartic acid (30 mg/kg), kainic acid (2.5 mg/kg), aminophylline (10 mg/kg) and hydrocortisone (200 mg/kg) did not influence the effect of the aminoglutethimide/carbamazepine combination in kindled rats (Table 2).

3.3. Effect of aminoglutethimide and spironolactone on the action of diphenylhydantoin in amygdala-kindled rats

Diphenylhydantoin (5–10 mg/kg) exerted protective efficacy, significantly shortening both seizure and afterdischarge durations. When the drug was applied at the dose of 10 mg/kg, the seizure parameters were changed from 42.0 to 35.3 s and from 72.7 to 67.0 s, respectively. Spironolactone (50 mg/kg) failed to affect the action of diphenylhydantoin in the inactive dose of 2.5 mg/kg against kindled seizures in rats. Conversely, the combined treatment of aminoglutethimide (5 mg/kg) with diphenylhydantoin (2.5 mg/kg) resulted in significant protective effect. The seizure duration was shortened from 48.3 to 43.8 s, and the afterdischarge duration from 76.7 to 69.2 s, respectively (Table 3, Fig. 2).

3.4. Effect of hydrocortisone and some convulsants on the aminoglutethimide-induced action on diphenylhydantoin in amygdala-kindled rats

The aminoglutethimide-mediated effect on diphenylhydantoin was reversed by bicuculline (2 mg/kg), aminophylline (10 mg/kg) and *N*-methyl-D-aspartic acid (30 mg/kg). Kainic acid (2.5 mg/kg) and hydrocortisone (200 mg/kg) remained without effect on the action of aminoglutethimide/diphenylhydantoin combination against kindled seizures in rats (Table 4).

3.5. Rotarod test and passive avoidance task

Aminoglutethimide (5 mg/kg), spironolactone (50 mg/kg), carbamazepine (15 mg/kg), diphenylhydantoin (2.5 mg/kg), and

Table 3
Effect of diphenylhydantoin and its combinations with aminoglutethimide or spironolactone on amygdala-kindled seizures in rats

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
DPH (10)	49.4±3.9	51.2±5.0	5	5	42.0±2.8	35.3±2.8[b]	72.7±2.9	67.0±3.0[b]
DPH (5)	48.2±4.0	47.3±4.4	5	5	42.4±3.1	38.2±3.1[c]	74.8±3.4	65.5±4.2[c]
DPH (2.5)	50.6±4.4	52.0±4.7	5	5	46.8±3.8	45.1±4.1	78.2±3.6	78.0±4.5
DPH (2.5)+AGLD (5)	45.6±4.6	47.2±3.9	5	5	48.3±3.5	43.8±4.1[c]	76.7±4.4	69.2±4.2[b]
DPH (1.25)+AGLD (5)	43.0±4.1	43.9±4.3	5	5	47.4±3.4	46.8±4.5	78.4±4.3	77.1±4.6
DPH (2.5)+SPIR (50)	44.3±4.3	43.0±4.6	5	5	42.2±3.6	41.6±3.9	75.9±4.2	76.2±4.3

AGLD, SPIR and DPH were administered 120 min before the test. [b]$P<0.01$, [c]$P<0.05$ vs. respective controls (Student's t-test for paired replicates). AGLD, aminoglutethimide; SPIR, spironolactone; DPH, diphenylhydantoin; C, control group; ADT, afterdischarge threshold; SSv, seizure severity; SDr, seizure duration; ADr, afterdischarge duration.

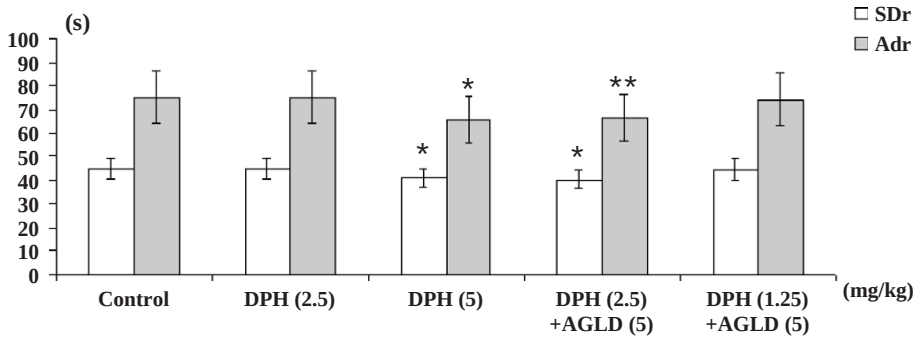

Fig. 2. Effect of diphenylhydantoin (DPH) and its combinations with aminoglutethimide (AGLD) on seizure duration (SDr) and afterdischarge duration (ADr) in fully kindled rats. SDr and ADr data are means±S.D. (in seconds). AGLD was used at the subprotective doses of 5 and 2.5 mg/kg, whereas CLO was administered at various doses (in parentheses). Control readings were made 2 days before and after the respective treatments. *P<0.05, **P<0.01 vs. averaged control [Wilcoxon signed rank test].

the combinations of hormone antagonists with antiepileptic drugs did not produce any adverse effects reflected in motor- and long-term memory performance (Table 5).

3.6. Effect of aminoglutethimide and spironolactone on the plasma levels and brain concentrations of carbamazepine and diphenylhydantoin in rats

Aminoglutethimide (5 mg/kg) did not alter free plasma levels and brain concentrations of either diphenylhydantoin (2.5 mg/kg) or carbamazepine (15 mg/kg). Spironolactone (50 mg/kg) significantly changed free plasma levels and brain concentrations of carbamazepine. The first parameter increased from 2.65 to 3.84 μg/ml, whilst the second one from 3.45 to 4.58 μg/g of wet brain tissue (Table 6).

4. Discussion

The present study demonstrates that aminoglutethimide (an antagonist of adrenal steroid synthesis) exhibits anti-seizure properties, reducing three of four parameters in amygdala-kindled rats, the seizure severity, seizure duration, and afterdischarge duration. Spironolactone (a non-specific antagonist of aldosterone receptors) did not affect amygdala-kindled convulsions in rats. Moreover, the co-administration of aminoglutethimide with carbamazepine or diphenylhydantoin when all components were applied at subprotective doses resulted in significant protective effect. Among chemoconvulsants used in the study, only bicuculline reversed the action of aminoglutethimide/carbamazepine combination. On the other hand, bicuculline, *N*-methyl-D-aspartic acid and aminophylline reversed the action of AGLD/diphenylhydantoin mixture. Aminoglutethimide did not alter free plasma levels or brain concentrations of the two antiepileptic drugs, so a pharmacokinetic interaction is not probable. On the other hand, the benefit interaction between spironolactone and carbamazepine was strongly associated with pharmacokinetic events.

It is worth mentioning that present results are in agreement with the study of Roberts et al. (1993) describing anticonvulsant properties of aminoglutethimide and spironolactone. According to authors, the two drugs prolonged the latency to pentetrazole-induced tonic hindlimb extension. Described effect was not reversed by dexamethasone, a potent agonist of corticosteroid receptor (Roberts et al., 1993). Our experiments revealed that aminoglutethimide much easier than spironolactone interacts with antiepileptic drugs (Borowicz, 2001; Borowicz and Czuczwar, 2004; Borowicz et al., 2004). However, the nature of this phenomenon remains unclear. One should remember that aminoglutethimide was administered 2 h before the seizure test. During this time the drug can reach and block the respective enzymes, but it is too early to affect plasma levels of adrenal steroids. Then, it is not surprising that hydrocortisone did not reverse the anticonvulsant effect of aminoglutethimide. On the other hand,

Table 4
Effect of hydrocortisone and convulsants on the protective effect of aminoglutethimide/diphenylhydantoin combination in amygdala-kindled rats

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
DPH (2.5)+AGLD (5)								
+saline	45.6±4.6	47.2±3.9	5	5	48.3±3.5	43.8±4.1[c]	76.7±4.4	69.2±4.2[b]
+HC (200)	45.7±3.5	47.1±4.0	5	5	48.4±3.8	44.7±3.8[c]	77.8±3.9	69.5±4.1[b]
+BIC (2)	44.3±3.3	43.6±4.2	5	5	47.3±3.5	46.2±4.0	76.5±3.8	75.1±4.2
+AMI (10)	43.8±3.6	44.7±4.1	5	5	48.1±3.8	46.7±4.0	76.3±4.2	74.6±4.2
+NMDA (30)	44.0±3.4	45.0±3.9	5	4.5 (4;5)	46.5±3.6	45.0±3.3	77.5±4.2	78.2±4.1
+KA (2.5)	44.0±3.8	44.8±4.0	5	5	47.5±3.9	42.9±4.0[c]	78.6±4.2	74.2±4.5[c]

AGLD, aminoglutethimide; DPH, diphenylhydantoin; HC, hydrocortisone; C, control group; ADT, afterdischarge threshold; SSv, seizure severity; SDr, seizure duration; ADr, afterdischarge duration. [b]P<0.01, [c]P<0.05 vs. respective controls (Student's t-test for paired replicates).

also the antiseizure action of sex hormone antagonists was not changed by the pretreatment with the respective steroid hormone. The first hypothesis trying to explain such effect assumes that steroid antagonists, with greater affinity to steroid receptors, might replace steroid agonists from their binding sites. The second one suggests that hormone antagonists (independently of steroid receptors) can modulate the function of ionotrpoic or metabotropic membrane receptors. Results of our experiments may be an argument for the involvement of GABA-ergic neurotransmission in the interaction between aminoglutethimide and carbamazepine interaction. Moreover, enhancement of GABA-ergic, glutamatergic and purinergic transmission may contribute to effect of the combined treatment with aminoglutethimide and diphenylhydantoin.

The mechanism of antiseizure activity of carbamazepine and diphenylhydantoin is undoubtedly complex. Both drugs decrease sustained repetitive firing by delaying the recovery from inactivation of sodium channels. It is supposed that the two drugs bind to different types of the alpha-subunit of the "neurotoxin" binding site within sodium channel complex (Willow and Catterall, 1996). Different pattern of interaction between aminoglutethimide and carbamazepine, on one hand, or diphenylhydantoin, on the other hand, emphasizes discrepancies between the two sodium channel inhibitors, believed for a long time to have the same mechanism of action. Returning to the mechanism of action, diphenylhydantoin and carbamazepine are hypothesized to act as non-competitive antagonists or modulatory agents of NMDA receptors (Steppuhn and Turski, 1993). The two antiepileptics may also affect the purinergic neurotransmission. Though carbamazepine (Skerrit et al., 1983) acts as an adenosine A_1 antagonist, diphenylhydantoin is a potent adenosine uptake inhibitor (Phillis, 1984). Bearing it all in mind, the involvement of aforesaid neurotransmission system in the anticonvulsant effect of aminoglutethimide combined with carbamazepine or diphenylhydantoin seems quite probable. However, additional investigations including electrophysiological studies are needed to elucidate the real nature of described interactions.

Carbamazepine and diphenylhydantoin are first choice antiepileptic drugs used in the treatment of complex partial seizures in humans. Therefore, the present results may be of some clinical value. Modification of the antiepileptic drug dosage should be considered in epileptic patients co-treated with aminoglutethimide or spironolactone. Nevertheless, further study based on chronic treatment with both steroid antagonists should be designed to confirm this conclusion.

Table 5
Effect of aminoglutethimide, spironolactone and their combinations with carbamazepine or diphenylhydantoin on motor activity and long-term memory in amygdala-kindled rats

Treatment (mg/kg)	Rotarod (s)	Long-term memory (s)
Vehicle	120 (120; 120)	180 (180; 180)
SPIR (50)	120 (120; 120)	180 (92; 180)
AGLD (5)	120 (120; 120)	180 (72; 180)
CBZ (15)	120 (120; 120)	176 (141; 180)
CBZ (15)+AGLD (5)	120 (120; 120)	152 (100; 180)
CBZ (15)+SPIR (50)	120 (120; 120)	160 (124; 180)
DPH (2.5)	120 (120; 120)	180 (180; 180)
DPH (2.5)+AGLD (5)	118 (108; 120)	165 (131; 180)

Table data are medians (with 25, 75 percentiles) of 6 determinations. Motor impairment was indicated when the animals were unable to perform the rotarod task within 120 s. Long-term memory impairment of the animal was considered when it did not avoid the dark compartment within 180 s. The Kruskal–Wallis test followed by Dunn's post-hoc test was used for statistical analysis of the data. AGLD, aminoglutethimide; SPIR, spironolactone; CBZ, carbamazepine; DPH, diphenylhydantoin.

Table 6
Effect of aminoglutethimide and spironolactone on free plasma levels and brain levels of antiepileptic drugs in rats

Treatment (mg/kg)	Plasma levels	Brain levels
CBZ (15)+saline	2.65±0.71	3.45±0.84
+AGLD (5)	2.44±0.76	3.36±0.78
+SPIR (50)	3.84±0.92[c]	4.58±0.94[c]
DPH (2.5)+saline	0.16±0.02	0.50±0.04
+AGLD (5)	0.17±0.03	0.48±0.04

Presented values are the means (μg/ml of plasma and μg/g of brain wet tissue) of 8 determinations±S.D. Unpaired Student's *t*-test was used for statistical evaluation of the data. [c]$P<0.05$ vs. CBZ alone. AGLD, aminoglutethimide; SPIR, spironolactone; CBZ, carbamazepine; DPH, diphenylhydantoin. For treatment times, see also legend of Table 1.

Acknowledgments

This study was supported by a grant from Lublin Medical University School. The authors appreciate generous gifts of valproate magnesium and phenobarbital sodium from Polfa (Rzeszów, Poland) and Polfa (Kraków, Poland).

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