Is a hypothermic effect of LY300164, valproate and phenobarbital evident in mice?

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Abstract

Purpose: **The aim of this study was to evaluate the influence of LY300164 (an AMPA/kainate receptor antagonist) administered alone or in combination with valproate (VPA) or phenobarbital (PB) on body temperature in mice.**

Material and methods: **The temperature measurements were performed in Albino Swiss mice injected with the respective drugs by using a rectal thermistor thermometer.**

Results: **LY300164, at the dose of 2 mg/kg, did not affect the body temperature of the examined animals. However, the combination of LY300164 (2 mg/kg) with VPA (165 mg/kg) resulted in a significant decrease in body temperature within 60-180 min after their peak of maximum anticonvulsant activity. Moreover, VPA (269 mg/kg) administered alone, evidently produced hypothermic effects at the times between 120-180 min after the peak of the maximum antiseizure effect. In contrast, phenobarbital administered alone or in combination with LY300164 did not affect the body temperature in the mice.**

Conclusions: **Hypothermia induced by LY 300164 combined with VPA may be useful in various central nervous system disease treatments.**

Key words: temperature monitoring, hypothermia, LY300164, valproate, phenobarbital.

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Received 19.04.2004 Accepted 31.07.2004

Introduction

The excessive activity of excitatory amino acid (EAA) neurotransmitters in the brain is thought to be an important factor involving in several central nervous system diseases such as: cerebral ischemia/hypoxia, trauma injuries, epilepsy, Huntington's chorea, Parkinson's disease and Alzheimer's disease [1,2]. There are numerous brain pathologies resulting from the imbalance between excitatory and inhibitory neurotransmitter system functioning in the brain [1,3]. For thirty years, an important role of EAA in epileptogenesis and seizure initiation, amplification or propagation has been documented [4]. Moreover, the neuroprotective effect of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5,7--methylisoxazole-4-propionic acid (AMPA)/kainate receptor antagonists have been demonstrated in several experimental models of epilepsy [5,6]. At present, the AMPA/kainate receptor antagonists seem to be more advantageous than NMDA receptor antagonists as to the acute neurotoxic effects produced by these agents [7].

In experimental and histological studies, it has been found that hypothermia protects the neurons against cerebral ischemia and traumatic brain injury, whereas conversely, hyperthermia exacerbates such brain pathologies [8]. The investigations, after the intracerebroventricular (i.c.v.) administration of NMDA, have revealed that this agent is responsible for the increase in brain temperature of examined rats [9]. This increment was inhibited by NMDA receptor antagonists, such as: MK-801 (dizocilpine) and (±)-2-amino-5-phosphonopentanoic acid [10]. In contrast, AMPA and kainic acid produced a biphasic effects on body temperature of experimental animals: short-lasting hypothermia followed by hyperthermia [11]. So, the suppression of hyperthermia by appropriate drugs might be useful during the treatment of patients with some brain pathologies in which the EAA are involved.

LY300164 {7-acethyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo-[4,5-h][2,3]-benzodiazepine}; (Talampanel®), a non-selective antagonist of AMPA/kainate

receptors, enhanced the anticonvulsant activity of conventional and some novel antiepileptic drugs (AEDs) against maximal electroshock (MES) [12], aminophylline-induced seizures in mice [13] or amygdala-kindling rats [14]. Also, the substance was resistant to the convulsive action of sub-effective doses of aminophylline and strychnine when compared to conventional or novel AEDs (i.e. phenobarbital, valproate, diphenylhydantoin [15] or lamotrigine [16]). Therefore, in the present study we examined the effect of LY 300164 alone or in combination with phenobarbital or valproate on body temperature following the administration of this AMPA/kainate receptor antagonist in mice. The doses of the investigated AEDs and LY 300164 as well as the scheduled times, in which the experiment was performed, were previously determinate by Czuczwar et al. [17].

Material and methods

General

The experiments were conducted on female Swiss mice weighing 20-25 g. The animals were housed in colony cages with food (chow pellets) and tap water ad libitum. The laboratory temperature was 21 ± 1°C and the mice were kept on a natural light-dark cycle. The experimental groups consisting of 10-12 animals were randomly assigned. The procedure of temperature monitoring was carried out between 10:00 a.m. and 2:00 p.m. All experiments were approved by a Local Ethics Committee at the Medical University of Lublin.

Drugs

The AEDs used were: valproate magnesium (ICN Polfa Rzeszów, Poland) and phenobarbital sodium (Polfa, Warsaw, Poland). Valproate and phenobarbital were dissolved in sterile saline. LY 300164 ({7-acethyl-3-(4-aminophenyl)-8,9-dihydro--8-methyl-7H-1,3-dioxolo-[4,5][2,3]-benzodiazepine}; kindly supplied by Eli-Lilly, Indianapolis, IN, USA) was dissolved in sterile saline. All drugs were injected intraperitoneally (i.p.), in a volume of 10 ml/kg; valproate magnesium – 30 min, phenobarbital – 60 min, and LY 300164 – 15 min before the tests.

Measurement of animals' temperature

Before the test examination, the animals were pretrained three times a week to eliminate any handling-evoked variability in their body temperature. During this procedure, all mice received an i.p.-injection of saline. The temperature measurements were performed at a constant room temperature of 21 ± 1°C, and were performed in rectum of the animals with a thermistor thermometer (Elab, Copenhagen, Denmark); the probe was inserted into a depth of 10 mm, and maintained till a stabilization of temperature was achieved. The reference temperature was a mean temperature of three preliminary measurements taken consecutively at 10 min intervals. After the third measure, valproate magnesium and phenobarbital sodium or LY 300164 were administered to animals and the temperature was recorded. The control animals received always the respective amount of saline. Next, the temperature was monitored at 15, 30, 45, 60, 90, 120 and 180 min, after the first recording. Alterations in body temperature were presented as means ± SEM of at least 10 determinations for each time of measurement.

Statistics

Statistical evaluation of data was performed with repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni a posteriori test.

Results

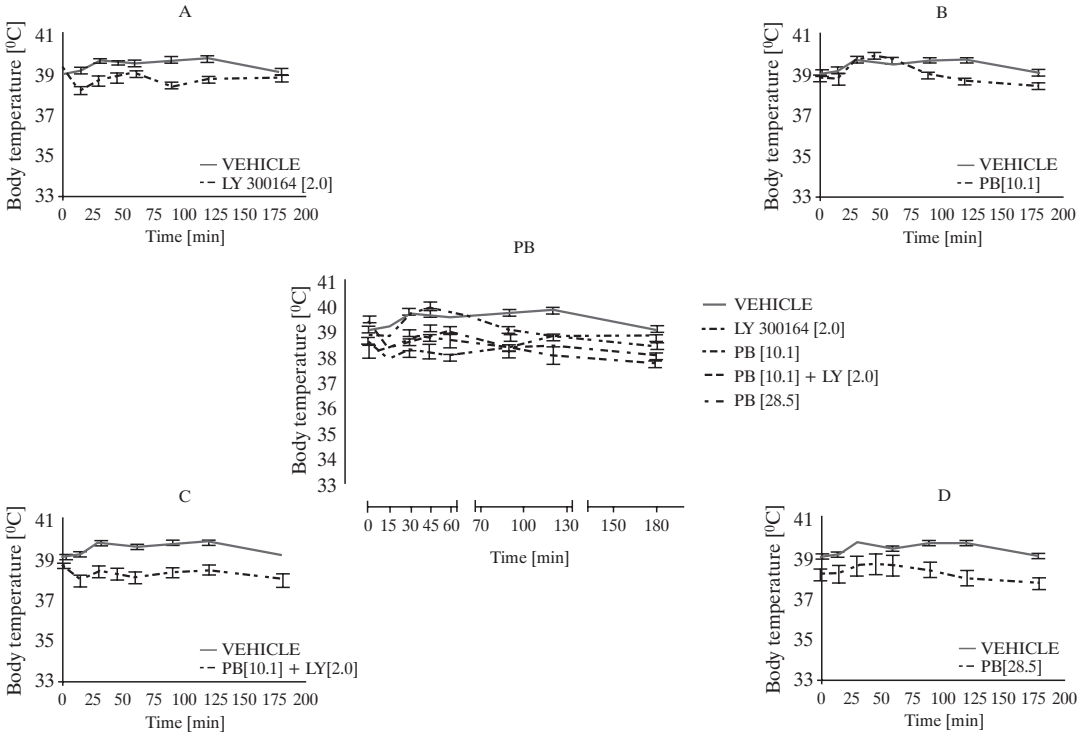
LY 300164 administered singly at the dose of 2 mg/kg did not affect body temperature of animals (*Fig. 1*; *Fig. 2*). Likewise, phenobarbital injected alone (28.5 mg/kg) did not significantly alter the body temperature. The combination of phenobarbital (10.1 mg/kg) with LY 300164 (2 mg/kg) did not influence this parameter in examined animals (*Fig. 1*).

Mean temperatures for LY 300164 and VPA administered alone or in combinations were calculated from 10-12 individual animals per group subjected to the temperature recording in various times after their peak of maximum anticonvulsant effects. The mean body temperature for the combination of VPA (165 mg/kg) and LY 300164 (2 mg/kg) was drastically lowered at the times of 90, 120 and 180 min. after their peak of maximum anticonvulsant activity. The difference between the mean temperature of control animals and those injected with the drug mixture (VPA + LY) achieved 4°C, 4.7°C, and 4.7 °C for 90, 120 and 180 min, respectively (at $P < 0.001$; *Tab. 2*). Simultaneously, the drug mixture decreased body temperature of the animals when compared with LY 300164 and VPA (*Tab. 2*). All remaining comparisons of the body temperature in the mice did not reach statistical significance for both, time-effect and drug-effect relationships with the repeated measures two-way ANOVA (*Tab. 2*; *Fig. 2*).

Discussion

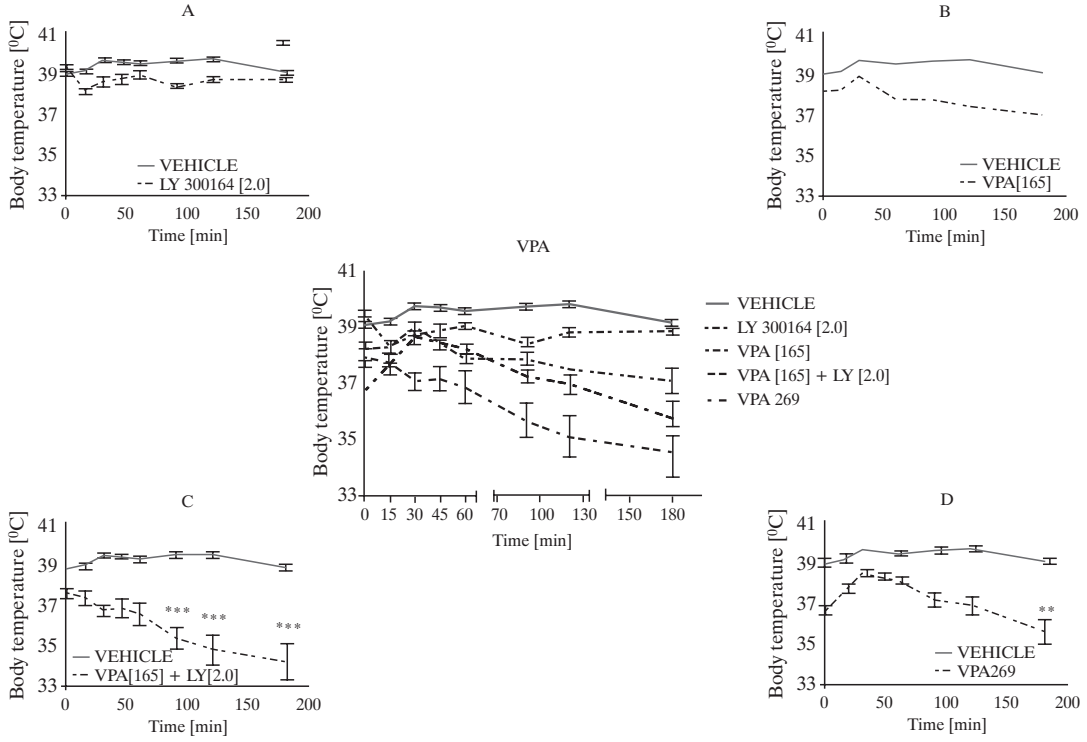
Our findings indicate that LY 300164 co-administered with valproate (at the dose of 165 mg/kg; providing a 50% protection against MES-induced seizures) produced a marked hypothermic effect. Moreover, valproate (269 mg/kg) injected alone, also reduced the body temperature in experimental animals. However, the mixture of valproate with LY 300164 showed longer and more expressed effects than valproate alone. In contrast, no temperature changes between animals administered with phenobarbital alone or in combination with LY 300164 were observed in our study. Since the results in our study were analyzed using two-way repeated measures ANOVA, both, time-dependent and drug-dependent relationships were estimated. Following this analysis, PB and LY 300164 alone or in combination did not significantly alter the body temperature of the animals as compared with control group. We are fully aware of the fact that the obtained results, despite the temperature difference ranging between 1-3°C among the analyzed groups, did not achieve statistical significance which was a result of the inter-group (individual) variance in body temperature after AEDs administration.

Figure 1. **Influence of LY 300164 and phenobarbital on body temperature in mice**



The temperature was monitored by rectal probe several times within the experimental period as follows: 0, 15, 30, 45, 60, 90, 120 and 180 min after the time of peak AED effect. The mice received LY 300164 (2.0 mg/kg) (A), or a combination of LY 300164 with PB (10.1 mg/kg) (C), had not significantly reduced the body temperature. Moreover, the temperature of animals subjected to the i.p. injection of PB (28.5 mg/kg) and (10.1 mg/kg) alone (B,D) did not differ from that of the control (vehicle-treated) mice. PB-phenobarbital

Figure 2. **Influence of LY 300164 and valproate on body temperature in mice**



The temperature was monitored by rectal probe several times within the experimental period as follows: 0, 15, 30, 45, 60, 90, 120 and 180 min after the time of peak AED effect. VPA at the dose of 269 mg/kg produced hypothermic effects at the times ranging between 120-180 min at $^{**}P<0.01$ (D). Moreover, the combined treatment of LY 300164 (2 mg/kg) with VPA (165 mg/kg) resulted in a significant decrease in animals' temperature at the times ranging between 90-180 min at $^{***}P<0.001$ (C). (For more details see also the *Tab. 2*). The body temperature of the mice injected with VPA (165 mg/kg) did not significantly differ from that of the vehicle-treated animals (A; B). VPA-valproate

***Table 1*. Effects of LY 300164, phenobarbital and its combination on body temperature in mice**

Time (min.)	Temperature (°C)			
	Control	LY [2.0]	PB [10.1]	PB[10.1]+LY[2.0]
0	39.1 ± 0.15	39.5 ± 0.10	38.8 ± 0.14	38.6 ± 0.10
15	39.2 ± 0.11	38.2 ± 0.16	38.8 ± 0.26	37.9 ± 0.26
30	39.8 ± 0.05	38.7 ± 0.25	39.8 ± 0.15	38.4 ± 0.35
45	39.7 ± 0.07	38.9 ± 0.23	39.9 ± 0.14	38.2 ± 0.31
60	39.6 ± 0.11	39.1 ± 0.17	39.8 ± 0.08	38.1 ± 0.22
90	39.7 ± 0.11	38.5 ± 0.16	39.0 ± 0.13	38.4 ± 0.23
120	39.8 ± 0.09	38.8 ± 0.16	38.8 ± 0.10	38.5 ± 0.30
180	39.1 ± 0.14	38.9 ± 0.13	38.5 ± 0.15	37.9 ± 0.30

Table data are presented as mean temperatures ± SEM of at least 10 determinations.
Statistical analysis was performed using two-way repeated measurements ANOVA followed by Bonferroni post-hoc test. Control – vehicle-treated animals; LY – LY 300164; PB – phenobarbital.

***Table 2*. Effects of LY300164, valproate and its combination on body temperature in mice**

Time (min)	Temperature (°C)			
	Control	LY [2.0]	VPA [165]	VPA[165]+LY[2.0]
0	39.1 ± 0.15	39.5 ± 0.10	38.2 ± 0.23	37.9 ± 0.28
15	39.2 ± 0.11	38.2 ± 0.16	38.3 ± 0.19	37.7 ± 0.37
30	39.8 ± 0.05	38.7 ± 0.25	38.9 ± 0.18	37.0 ± 0.32
45	39.7 ± 0.07	38.9 ± 0.23	38.4 ± 0.19	37.2 ± 0.44
60	39.6 ± 0.11	39.1 ± 0.17	37.8 ± 0.18	36.9 ± 0.58
90	39.7 ± 0.11	38.5 ± 0.16	37.8 ± 0.24	35.7 ± 0.59***
120	39.8 ± 0.09	38.8 ± 0.16	37.5 ± 0.18	35.1 ± 0.74***, a
180	39.1 ± 0.14	38.9 ± 0.13	37.1 ± 0.41	34.5 ± 0.93***, b, ‡

Table data are presented as mean temperatures ± SEM of at least 10 determinations. Statistical analysis was performed using two-way repeated measurements ANOVA followed by Bonferroni post-test. ***$P<0.001$ vs control group; [a]$P<0.01$ vs LY-treated animals; [b]$P<0.001$ vs LY-treated animals; [‡]$P<0.05$ vs VPA group. Control – vehicle-treated animals; LY – LY 300164; VPA – valproate

In experimental studies, several conventional AEDs have evoked also the hypothermic effects, especially if the drugs were administered at higher doses. For instance, a substantial reduction in body temperature has been noted following i.p. administration of diazepam (2-5 mg/kg), carbamazepine (20-50 mg/kg) and valproate (100-300 mg) [18]. On the contrary, lamotrigine, phenobarbital, diphenylhydantoin, felbamate, and gabapentin have not affected the temperature of experimental animals. Hence, our findings are generally consistent with those presented by Gareri et al. [18], confirming that valproate reduced the temperature, whereas phenobarbital had no impact on this parameter in experimental mice.

Since some NMDA and AMPA/kainate receptor antagonists have exerted strong hypothermic effects [19] and possessed the neuroprotective activity in both focal and global ischemic models [20], LY 300164 might be considered as a good candidate drug to share these two properties. Although, the agent per se did not produce the reduction in animal body temperature, but it considerably enhanced the hypothermic effect, when combined with VPA.

In conclusion, the reduction of body temperature by the mixture of LY 300164 with valproate may occur neuroprotective in both, cerebral ischemia and seizure models. Nevertheless, more advanced investigations are required to fully understand the real mechanisms to be responsible for the coexistence of seizures, neuronal loss and regulation of temperature in living organisms.

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