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Influence of LY 300164 alone or in combination with carbamazepine or diphenylhydantoin on the body temperature in mice

Excessive activation of glutamate receptors is implicated in neuronal degeneration and loss of neurons caused by acute brain pathology, such as cerebral ischemia/hypoxia, stroke, cerebral trauma or epilepsy (7), as well as, certain neurodegenerative diseases including Huntington's chorea, Parkinson's disease, Alzheimer's disease and AIDS neuropathology (11). Diverse pathophysiological processes may lead to overstimulation of glutamate receptors, which usually develop as a result of specific brain pathology. Of these pathophysiological processes, an increased release of excitatory amino acid neurotransmitters, high levels of excitotoxins in cerebral parenchyma, impaired neurotransmitter reuptake and/or metabolism, and imbalance between excitatory and inhibitory neurotransmitters, are the most important (2). No doubts that prolonged glutamate receptor activation is responsible for serious disturbances in the cellular signalling of the central nervous system (16). These main effects of excitatory amino acids to be involved in molecular mechanisms underlying the seizure induction, propagation and amplification had been documented several years ago (4). Moreover, in numerous *in vivo* and *in vitro* models, a neuroprotective effect of some *N*-methyl-*D*-aspartate (NMDA)- and α-amino-3-hydroxy-5,7-methylisoxazole-4-propionic acid (AMPA)-receptor antagonists has been demonstrated and some AMPA/kainate receptor antagonists occurred more advantageous than NMDA receptor antagonists, since produced less pronounced acute neurotoxic side effects (12).

In experimental animal models, hypothermia provides a marked protective effect against brain damage following cerebral ischemia and/or trauma brain injury, whereas hyperthermia evidently aggravates brain pathology (6). It was documented that intracerebroventricular (i.c.v.) administration of NMDA increased a brain temperature in experimental rats (9), and this effect was reversed by NMDA receptor antagonists, such as MK-801 (dizocilpine) and (±)-2-amino-5-phosphonopentanoic acid (10). Also, a low dose of AMPA (1 μg), injected i.c.v., produced a great hyperthermic response in rats, while this agent at high doses (up to 2.5 μg), similarly to kainic acid (0.1 μg) produced a biphasic effect: short-lasting hypothermia followed by hyperthermia (15). Thus, it seems likely that glutamate receptors are involved in the development of hyperthermia after brain injuries. In such cases, a suppression of this phenomenon by an appropriate drug administration might be useful in the treatment of patients with brain damage. Therefore, it was of some scientific importance to explore some mechanisms to be responsible for the occurrence of changes in body temperature evoked by a modulation of glutamate receptors.

It was previously found that LY 300164 {7-acethyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo-[4,5-h][2,3]-benzodiazepine), an antagonist of AMPA/kainate receptors, potentiated

the anticonvulsant activity of some antiepileptic drugs (AEDs) in various seizure models: maximal electroshock- (5) and aminophylline-induced seizures in mice (14). In contrast, LY 300164 was much more resistant to a convulsant activity of aminophylline or strychnine than conventional or novel AEDs such as valproate, phenobarbital, diphenylhydantoin (13) and lamotrigine (1). Therefore, in the present study, the effects of LY 300164 alone or in combination with carbamazepine or diphenylhydantoin on body temperature of experimental animals were examined. The doses of the AEDs and LY 300164, as well as, the time to the temperature monitoring were based upon the study by Czuczwar et al. (5).

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 8–12 animals were randomly collected. The procedure of temperature monitoring was carried out between 10:00 a.m. and 2:00 p.m. and each mouse was used only once. All experiments were approved by the Local Ethics Committee of the Medical University of Lublin.

Drugs. The following AEDs were used in this study: carbamazepine (Amizepin) and diphenylhydantoin (Phenytoinum), both from Polfa (Warsaw, Poland). Carbamazepine and diphenylhydantoin were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). LY 300164 {7-acethyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo-[4,5-h][2,3]-benzodiazepine; kindly supplied by Eli-Lilly, Indianapolis, USA} was dissolved in sterile saline. All drugs were injected intraperitoneally (i.p.), in a volume of 10 ml/kg, carbamazepine – 30 min, diphenylhydantoin – 120 min, and LY 300164 – 15 min before the test.

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

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

RESULTS

Influence of LY 300164 given alone on body temperature in mice. In a carbamazepine study group, LY 300164 (at 2 mg/kg) significantly decreased the body temperature of mice after the period of 60–90 min, at $^*p < 0.05$ (Fig. 1A). Likewise, in a diphenylhydantoin study group, LY 300164 reduced the temperature of animals tested in the time periods ranged between 15–30 and 60–90 min, at $^*p < 0.05$ (Fig. 2A).

Influence of carbamazepine and its combination with LY 300164 on body temperature in mice. Carbamazepine at the dose of 15.8 mg/kg significantly decreased the body temperature of the examined animals between 60–90 min, at *$p < 0.05$ (Fig. 1B). On the other hand, carbamazepine (5 mg/kg) given alone or concomitantly with LY 300164 (2 mg/kg) did not affect this parameter and no significant changes, with respect to any hypothermic effects, were observed (Fig. 1C, 1D).

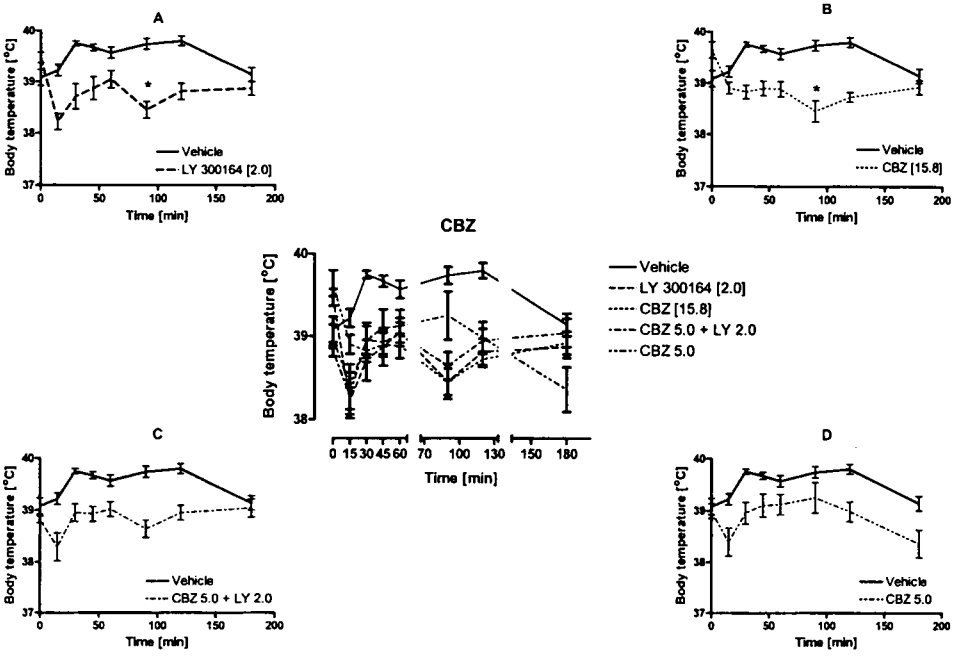

Fig. 1. Influence of LY 300164 and carbamazepine on body temperature in mice

Temperature of animals following i.p. injection of LY 300164 alone or in combination with carbamazepine (CBZ) was measured by rectal probe thermistor thermometer, and presented on the graphs in a time-dependent manner. Results are presented as mean temperature ± SEM (as the error bars) of 10 determinants. In the central position of this figure, one collective graph represents all obtained data for the combination of LY 300164 with CBZ. Additionally, each compound of this combination is presented on a separate graph in order of examination (as 1A – for LY 300164 alone; 1B – for CBZ at 15.8 mg/kg alone; 1C – for the mixture of LY 300164 with CBZ at 5 mg/kg; 1D – for CBZ at 5 mg/kg alone). CBZ – carbamazepine; * - significant difference at $p < 0.05$ vs. the respective vehicle-treated animals. Statistical evaluation of data was performed with two-way ANOVA followed by Bonferroni *post-hoc* test.

Influence of diphenylhydantoin alone or combined with LY 300164 on body temperature in mice. Diphenylhydantoin (at 11.8 mg/kg) significantly reduced the temperature of animals when compared to vehicle-treated group in the time period ranging between 0–15 min at **$p < 0.01$ (Fig. 2B). Also, the combination of diphenylhydantoin (at 3.6 mg/kg) with LY 300164 (2 mg/kg) considerably decreased the temperature of mice between 0–15 and 15–30 min, at ***$p < 0.001$ and *$p < 0.05$, respectively (Fig. 2C). No significant changes as to the hypothermic effects were observed when diphenylhydantoin (3.6 mg/kg) was administered alone (Fig. 2D).

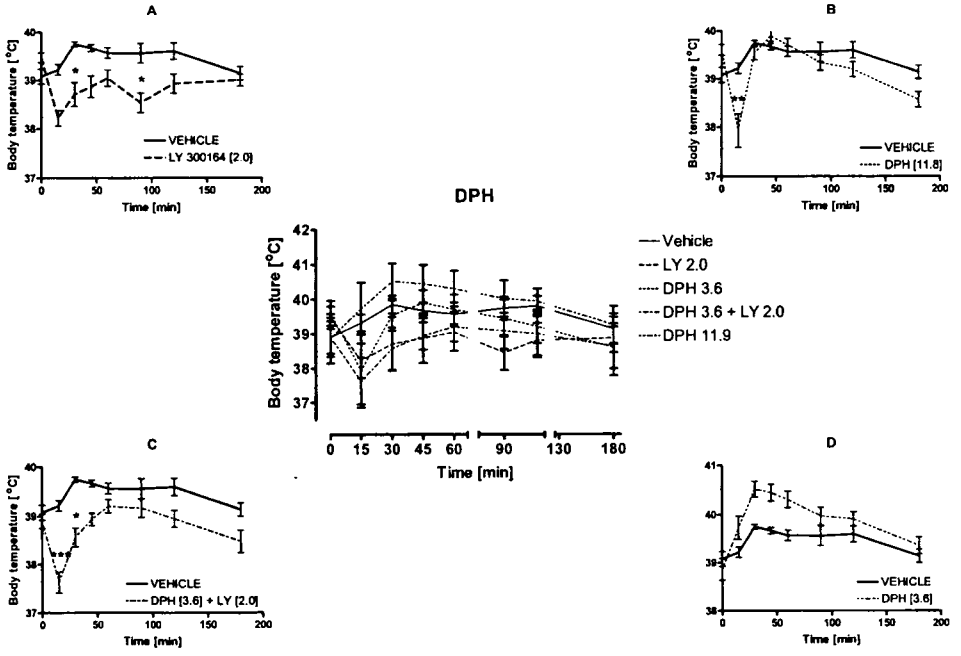

Fig. 2. Influence of LY 300164 and diphenylhydantoin on body temperature in mice

Temperature of animals following i.p. injection of LY 300164 alone or in combination with diphenylhydantoin (DPH) was measured by rectal probe thermistor thermometer, and presented on the graphs in a time-dependent manner. Results are presented as mean temperature ± SEM (as the error bars) of 10 determinants. In the central position of this figure, one collective graph represents all obtained data for the combination of LY 300164 with DPH. Additionally each compound of this combination is presented on a separate graph in order of examination (as 1A – for LY 300164 alone; 1B – for DPH at 11.8 mg/kg alone; 1C – for the mixture of LY 300164 with DPH at 3.6 mg/kg; and 1D – for DPH at 3.6 mg/kg alone). DPH – diphenylhydantoin; *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$ vs. the respective vehicle-treated animals. Statistical evaluation of data was performed with two-way ANOVA followed by Bonferroni *post-hoc* test.

DISCUSSION

Results clearly demonstrated that LY 300164, an AMPA/kainate receptor antagonist, significantly decreased the temperature of the experimental mice. However, in comparison to the anticonvulsant properties of this drug, being established at 15 min, hypothermic potential of LY 300164 was strongly pronounced after 30 and 90 min of injection of the drug. Also, carbamazepine (15.8 mg/kg) and diphenylhydantoin (11.8 mg/kg) – two sodium channel blockers, applied at their anticonvulsant doses, substantially reduced the temperature in mice. Moreover, a significant potentiation of hypothermic effects was observed in mice receiving the mixture of diphenylhydantoin with LY 300164.

As it was previously shown, a hypothermic effect of some AEDs was observed after the administration of high doses of carbamazepine (20–50 mg/kg), diazepam (3–5 mg/kg) or valproate (100–300 mg/kg). Surprisingly, no significant differences in body temperature among animals treated with lamotrigine, phenobarbital diphenylhydantoin, felbamate, gabapentin, or low doses of carbamazepine, diazepam and valproate were detected (8).

It is widely accepted that AMPA antagonists provide neuroprotection in several experimental models of cerebral ischemia (3). In such cases, the reduction of lesion size in ischemic brain seems to be secondary to drug-induced hypothermia, since decrease of body temperature in rodents has ameliorated neuronal damage in both, focal and global (3) ischemic models. Typically, in experimental studies dealing with the evaluation of neuroprotective effects of AMPA receptor antagonists, a body temperature in animals is monitored at times to the peak of maximum effects of administered substances or surgical procedure. The fact that temperature of animals could be decreased by AMPA/kainate receptor antagonists for a long time, after this period of examination, is widely neglected. For the first time, it was demonstrated herein that LY 300164 combined with diphenylhydantoin lowered the body temperature of examined animals up to 2°C (after 0–15 min-interval).

Bearing in mind that glutamate (NMDA and AMPA/kainate) receptor antagonists exerted hypothermic effects (15), it seems likely that both, hypothermia evoked by LY 300164 and the anticonvulsant activity of the drug, contribute to the reduction of seizures (5). Thus, a protection against seizures may be enhanced by hypothermic effects produced by LY 300164.

Finally, LY 300164 significantly potentiated a hypothermic effect produced by diphenylhydantoin. Moreover, both sodium channel blockers, i.e., diphenylhydantoin and carbamazepine when applied at high doses, significantly decreased the body temperature in the tested mice.

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REFERENCES

1. Borowicz K. K. et al.: Influence of several convulsants on the protective activity of a non-competitive AMPA/kainate antagonist, LY 300164, and lamotrigine against maximal electroshock in mice. J. Physiol. Pharmacol., 53, 859, 2002.
2. Choi D. W., Rothman S. M.: The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu. Rev. Neurosci., 13, 171, 1990.
3. Coimbra C. et al.: Long-lasting neuroprotective effect of postischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug. Evidence for chronic encephalopathic processes following ischemia. Stroke, 27, 1578, 1996.
4. Czuczwar S. J., Meldrum B.: Protection against chemically induced seizures by 2-amino-7-phosphonoheptanoic acid. Eur. J. Pharmacol., 83, 335, 1982.
5. Czuczwar S. J. et al.: LY 300164, a novel antagonist of AMPA/kainate receptors, potentiates the anticonvulsive activity of antiepileptic drugs. Eur. J. Pharmacol., 359, 103, 1998.
6. Dietrich W.D.: The importance of brain temperature in cerebral injury. J. Neurotrauma, 9 Suppl. 2, S475, 1992.
7. Dingledine R. et al.: Excitatory amino acid receptors in epilepsy. Trends Pharmacol. Sci., 11, 334, 1990.
8. Gareri P. et al.: Influence of carbenoxolone on the anticonvulsant efficacy of conventional antiepileptic drugs against audiogenic seizures in DBA/2 mice. Eur. J. Pharmacol., 484, 49, 2004.
9. Hara S. et al.: Local changes in oxygen tension and blood flow in the brain under hyperthermia induced by intracerebroventricular NMDA in rats. Brain Res., 737, 339, 1996.
10. Hara S. et al.: Distinct effects of MK-801 and (+/-)-2-amino-5-phosphonopentanoic acid on N--methyl-D-aspartate-induced rise of brain temperature in rats. Life Sci., 61, L, 1997.
11. Lipton S.A.: Models of neuronal injury in AIDS: another role for the NMDA receptor? Trends Neurosci., 15, 75, 1992.

12. Loscher W. et al.: Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. Eur. J. Pharmacol., 152, 9, 1988.
13. Pilip S. et al.: Anticonvulsant action of chlormethiazole is prevented by subconvulsive amounts of strychnine and aminophylline but not by bicuculline and picrotoxin. Pol. J. Pharmacol., 52, 267, 2000.
14. Świąder M. et al.: Influence of LY 300164, an AMPA/kainate receptor antagonist upon the anticonvulsant action of antiepileptic drugs against aminophylline-induced seizures in mice. Pol. J. Pharmacol., 55, 103, 2003.
15. Turski W. et al.: (RS)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid: wet dog shakes, catalepsy and body temperature changes in rats. Pharmacol. Biochem. Behav., 15, 545, 1981.
16. Wong B.Y. et al.: Dextrorphan and dextromethorphan, common antitussives, are antiepileptic and antagonize N-methyl-D-aspartate in brain slices. Neurosci. Lett., 85, 261, 1988.
17. Xue D. et al.: Immediate or delayed mild hypothermia prevents focal cerebral infarction. Brain Res., 587, 66, 1992.

SUMMARY

This study was aimed at evaluating the body temperature of mice following the injection of LY 300164, an AMPA/kainate receptor antagonist, alone or in combination with carbamazepine or diphenylhydantoin. LY 300164, injected alone at the dose of 2 mg/kg, produced a potent hypothermic effect between 15 and 30 min, or 60 and 90 min, after the drug administration. The combined treatment of LY 300164 (2 mg/kg) with diphenylhydantoin (3.6 mg/kg) resulted in a significant decrease of body temperature at the time period between 0 and 30 min, whilst LY 300164 (2 mg/kg) co-administered with carbamazepine (5 mg/kg) did not affect the animal temperature. Moreover, either diphenylhydantoin (11.8 mg/kg) or carbamazepine (15.8 mg/kg) injected alone exerted the hypothermic effects elicited at times ranging between 0 and 15 min, or 60 and 90 min, after the respective drug dose administration. In conclusion, hypothermia induced by LY 300164 along with its neuroprotective effects, may be useful in various brain conditions related with neuronal loss in which hypothermia offers some profitable effects, prolonging a survival rate of neurons in the central nervous system.

Wpływ LY 300164 podawanego osobno lub w kombinacji z karbamazepiną lub difenylohydantoiną na temperaturę ciała u myszy

Celem pracy było oznaczenie temperatury ciała myszy po podaniu LY 300164, antagonisty receptorów AMPA/kainianowych, zarówno osobno jak i w kombinacji z karbamazepiną lub difenylohydantoiną. LY 300164 w dawce 2 mg/kg wywoływał wyraźny efekt hipotermiczny w 15–30 min i 60–90 min po szczycie maksymalnego działania przeciwdrgawkowego substancji. Łączne zastosowanie LY 300164 (2 mg/kg) z diphenylohydantoiną (w dawce 3,6 mg/kg) prowadziło do istotnego obniżenia temperatury ciała zwierząt w czasie 0–30 min, podczas gdy LY 300164 (2 mg/kg) podawany łącznie z karbamazepiną (5 mg/kg) nie wpływał na temperaturę ciała zwierząt badanych. Difenylohydantoina (11,8 mg/kg) oraz karbamazepina (15,8 mg/kg) podawane osobno wykazywały silne efekty hipotermiczne w 0–15 min oraz 60–90 min po szczycie maksymalnego działania przeciwdrgawkowego odpowiednich leków. Podsumowując, hipotermiczne działanie wywoływane przez LY 300164 może być korzystne w różnych schorzeniach mózgu związanych z utratą neuronów, w których hipotermia może zwiększyć przeżycie neuronów w ośrodkowym układzie nerwowym.