Investigation the convulsive effect of levosimendan in PTZ-induced seizure threshold in mice: possible involvement of KATP/NO

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Purpose

With a prevalence of 1-2%, epilepsies undoubtedly impose a major burden upon patients and societies. Despite major breakthroughs in the field of epilepsy research, anti-seizure medications do not provide sufficient seizure control in almost one-third of patients suffering from epilepsy. Thus, investigation on new evidence-based therapeutical strategies is mandatory, and neuropharmacology can be a promising field for this purpose. Levosimendan demonstrated neuroprotective effects and reduced mortality in conditions in which seizure can be an etiology of death; however, the underlying neuroprotective mechanisms of levosimendan still eludes us. In the light of evidence suggesting levosimendan can be a KATP channel opener and nitrergic pathway activator, levosimendan may exert anti-seizure effects through KATP channels and nitrergic pathway. This study aims to shed light on the effects of levosimendan on PTZ-induced seizure threshold in mice as a model of generalized clonic seizures (GCS) and then ascertain the nature of this effect by delineating the molecular pathways involved in the exertion of this effect.

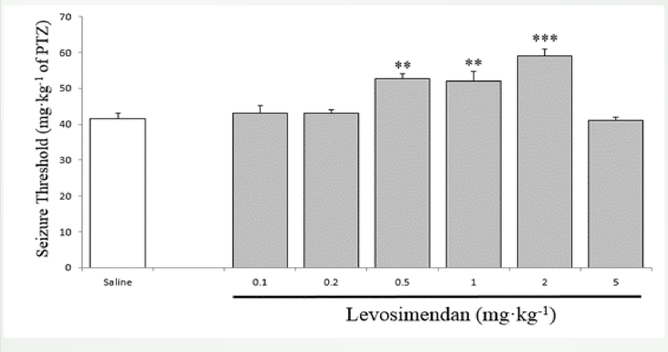
Methods

Clonic seizure threshold (CST) was determined by inserting a 30-gauge dental needle into the lateral tail vein of the mouse . The needle was then secured to the tail by a narrow piece of adhesive tape. With mouse moving freely, the PTZ solution (0.5%) was slowly infused into the tail vein at a constant rate of 1 ml/min using an infusion pump, which was connected to the dental needle by polyethylene tubing. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) was observed. The minimal dose of PTZ (mg/kg of mice weight) needed to induce general clonus was recorded as an index of clonic seizure threshold. As such, the seizure threshold is dependent on PTZ dose administered and time-related.

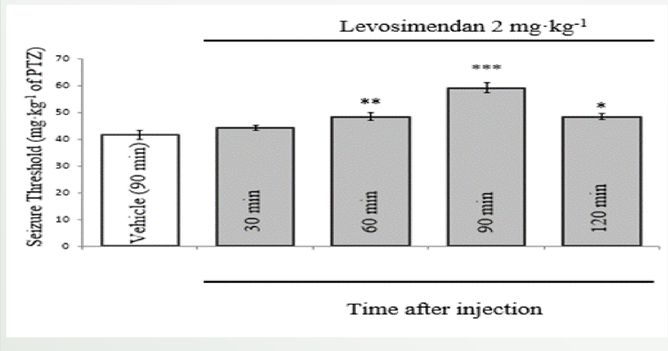
In order to measure brain's NO content, brain homogenates were centrifuged and aliquots of supernatants reacted with the same volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)- ethylenediamine dihydrochloride, 2.5% H3PO4. Nitrite concentrations were quantified spectrophotometrically at 540 nm using a standard curve plotted for known concentrations of sodium nitrite.

Results

Administration of a single effective dose of levosimendan significantly increased seizures threshold and the nitrite level in the hippocampus and temporal cortex.



Effects of different doses of levosimendan (0.1, 0.2, 0.5, 1, 2, and 5 mg.kg-1) on PTZ-induced seizure threshold in mice. Levosimendan was administered 90 min before determination of PTZ-induced seizure threshold. Data are expressed as the mean ± SEM of seizure threshold in each group. Each group consisted of 6-8 mice. **P<0.01, ***P < 0.001, compared with the saline-treated control group.

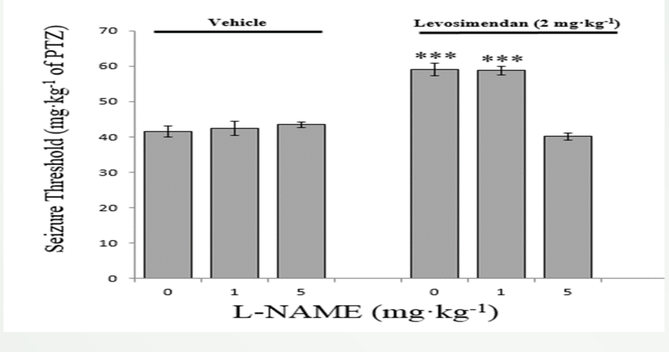


Time course effects of levosimendan (2 mg.kg^{-1}) on PTZ-induced seizure threshold in mice. Levosimendan was administered 30, 60, 90, or 120 min before determination of PTZ-induced seizure threshold, and its anticonvulsant effects were compared with the vehicle-treated control group (90 min before PTZ-induced seizure). Data are expressed as mean ± S.E.M. of seizure threshold in each group. Each group consisted of 6-8 mice. *P < 0.05, **P < 0.01, ***P < 0.001 in comparison to the vehicle-treated control group.

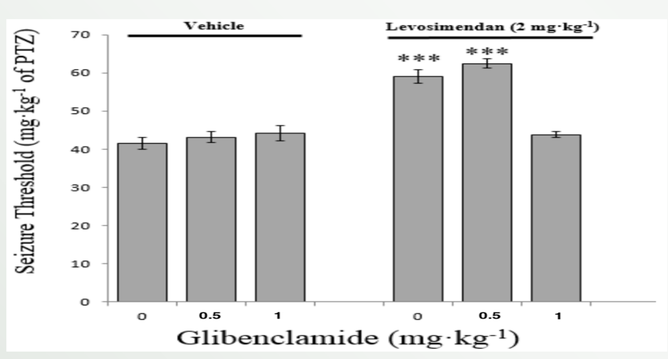
Table 1	Cerebral cortex				Hippocampus	Cerebellum	Brain homogenates
	Frontal	Temporal	Parietal	Occipital			
Saline	44.116 ± 1.993 (nM.mg^{-1})	75.018 ± 2.649 (nM.mg^{-1})	45.889 ± 2.386 (nM.mg^{-1})	68.339 ± 1.749 (nM.mg^{-1})	56.497 ± 1.694 (nM.mg^{-1})	33.229 ± 1.387 (nM.mg^{-1})	42.497 ± 2.486 (nM.mg^{-1})
Levosimendan (2 mg·kg^{-1})	43.489 ± 2.497 (nM.mg^{-1})	98.486 ± 1.296 (nM.mg^{-1}) AAA	41.396 ± 3.958 (nM.mg^{-1})	63.622 ± 2.795 (nM.mg^{-1})	73.497 ± 1.738 (nM.mg^{-1}) BBB	35.259 ± 3.628 (nM.mg^{-1})	54.397 ± 1.694 (nM.mg^{-1}) CC

Nitrite levels (nM.mg-1) in brain homogenates, frontal, temporal, parietal and occipital cortices, the hippocampus and the cerebellum of the mice treated with saline and levosimendan (2 mg·kg-1). Nitrite levels were significantly higher in the hippocampus and temporal cortex of the mice treated with levosimendan (2 mg·kg-1) in comparison to nitrite levels in the corresponding brain regions of the saline-treated control group. Each group consisted of 6-8 mice. AAA P < 0.001 in comparison to nitrite levels in the temporal cortex of the saline-treated control group, BBB P < 0.001 in comparison to nitrite levels in the hippocampus of the saline-treated control group and CC P < 0.01 in comparison to nitrite levels in brain homogenates of the saline-treated control group.

Pretreatment with noneffective doses of glibenclamide (a KATP channel blocker) and L-NAME (a non-selective NOS inhibitor) neutralize the anticonvulsant and nitrite elevating effects of levosimendan.



Graded doses of L-NAME (1 and 5 mg.kg-1; a non-selective NOS inhibitor), which independently did not alter PTZ-induced seizure threshold, decreased anticonvulsant effects of levosimendan (2 mg.kg-1) down to the saline-treated control group. L-NAME was administered 45 min before i.p. injection of levosimendan and 135 min before determination of PTZ-induced seizure threshold. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. ***P < 0.001, in comparison to the saline-treated control group.

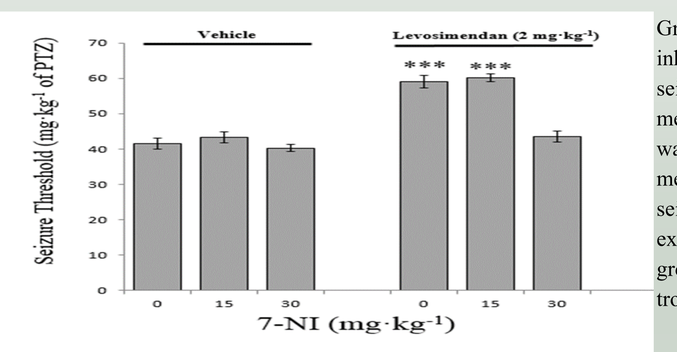


Graded doses of glibenclamide .0.5 and 1 mg.kg^{-1}; a KATP channel blocker), which independently did not alter PTZ-induced seizure threshold decreased anticonvulsant effects of levosimendan (2 mg.kg^{-1}) down to the saline-treated control group. Glibenclamide was administered 30 min before i.p. injection of levosimendan and 120 min before determination of PTZ-induced seizure threshold. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. ***P < 0.001, in comparison to the saline-treated control group.

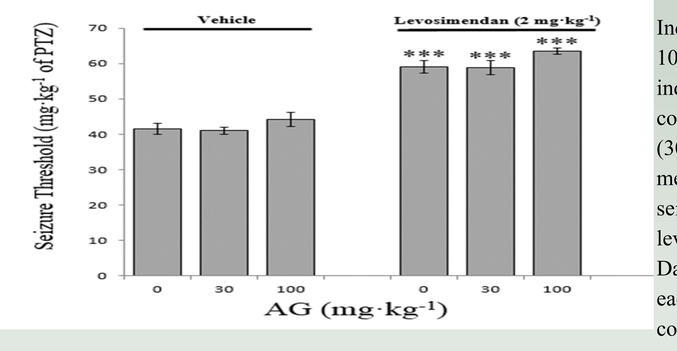
Table 2	Saline	Levosimendan (2 mg·kg^{-1})	L-NAME (5 mg·kg^{-1})	Glibenclamide (1 mg·kg^{-1})	L-NAME (5 mg·kg^{-1}) + levosimendan (2 mg·kg^{-1})	Glibenclamide (1 mg·kg^{-1}) + levosimendan (2 mg·kg^{-1})
Temporal cortex	75.018 ± 2.649 (nM.mg^{-1})	98.486 ± 1.296 (nM.mg^{-1}) ###	73.592 ± 1.285 (nM.mg^{-1})	71.385 ± 2.958 (nM.mg^{-1})	77.012 ± 0.947 (nM.mg^{-1})	78.379 ± 3.226 (nM.mg^{-1})
Hippocampus	56.497 ± 1.694 (nM.mg^{-1})	73.497 ± 1.738 (nM.mg^{-1}) ###	58.147 ± 3.519 (nM.mg^{-1})	56.594 ± 1.481 (nM.mg^{-1})	59.883 ± 0.439 (nM.mg^{-1})	52.297 ± 2.559 (nM.mg^{-1})

Nitrite levels (nM.mg^{-1}) in the temporal cortex and hippocampus of the mice treated with saline, levosimendan (2 mg·kg^{-1}), L-NAME (5 mg·kg^{-1}; a non-selective NO inhibitor), glibenclamide (1 mg·kg^{-1}; a KATP channel blocker), L-NAME (5 mg·kg^{-1}) and levosimendan (2 mg·kg^{-1}), glibenclamide (1 mg·kg^{-1}) and levosimendan (2 mg·kg^{-1}). Independent administration of L-NAME (5 mg·kg^{-1}) and glibenclamide (1 mg·kg^{-1}) did not change nitrite levels in the temporal cortex and hippocampus of the mice in comparison to nitrite levels in the corresponding brain regions of the saline-treated control group. However, pre-teatment with L-NAME (5 mg·kg^{-1}) 45 min before administration of levosimendan (2 mg·kg^{-1}) and also pre-teatment with glibenclamide (1 mg·kg^{-1}) 30 min before administration of levosimendan (2 mg·kg^{-1}) reversed nitrite levels of the temporal cortex and hippocampus of the mice down to the nitrite levels in the corresponding brain regions of the saline-treated control group. Each group consisted of 6-8 mice. ^{AAA}P < 0.001 in comparison to nitrite levels in the temporal cortex of the saline-treated control group, ^{BBB}P < 0.001 in comparison to nitrite levels in the hippocampus of the saline-treated control group and ^{CC}P < 0.01 in comparison to nitrite levels in brain homogenates of the saline-treated control group.

While 7-NI (a neural NOS inhibitor) blocked the anticonvulsant effect of levosimendan, Aminoguanidine (an inducible NOS inhibitor) failed to affect the anticonvulsant effects of levosimendan.

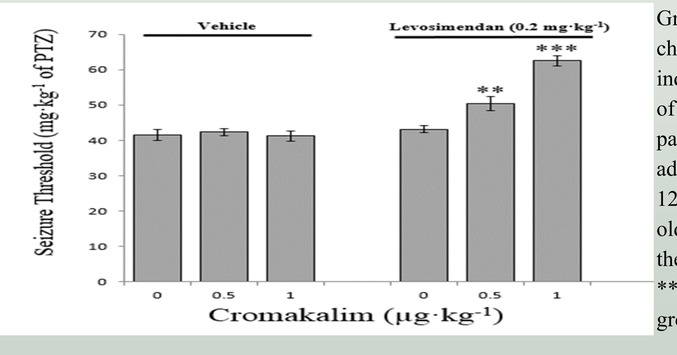


Graded doses of 7-NI (15 and 30 mg.kg-1; a selective nNOS inhibitor), which independently did not alter PTZ-induced seizure threshold, reversed anticonvulsant effects of levosimendan (2 mg.kg-1) to the saline-treated control group. 7-NI was administered 45 min before i.p. injection of levosimendan and 135 min before determination of PTZ-induced seizure threshold. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. ***P < 0.001, in comparison to the saline-treated control group.

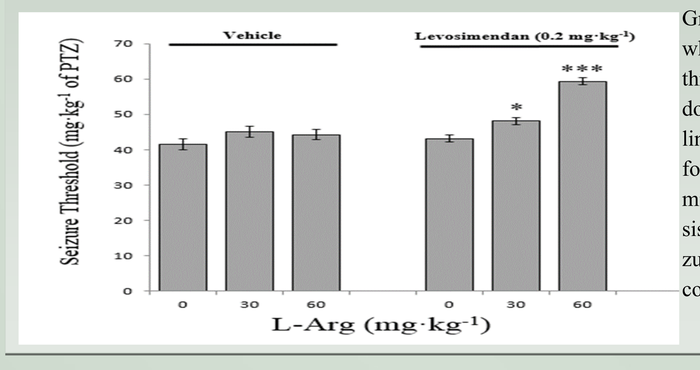


Independent administration of graded doses of AG (30 and 100 mg.kg^{-1}; a selective iNOS inhibitor) did not alter PTZ-induced seizure threshold in comparison to the saline-treated control group. Also, Pre-treatment with different doses of AG (30 and 100 mg.kg^{-1}) 45 min before i.p. injection of levosimendan and 135 min before determination of PTZ-induced seizure threshold could not alter anticonvulsant effects of levosimendan (2 mg.kg^{-1}). Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. ***P < 0.001, in comparison to the saline-treated control group.

Cromakalim (a KATP channel opener) or L-Arginine (an NO precursor) augmented the anticonvulsant effects of a subeffective dose of levosimendan.

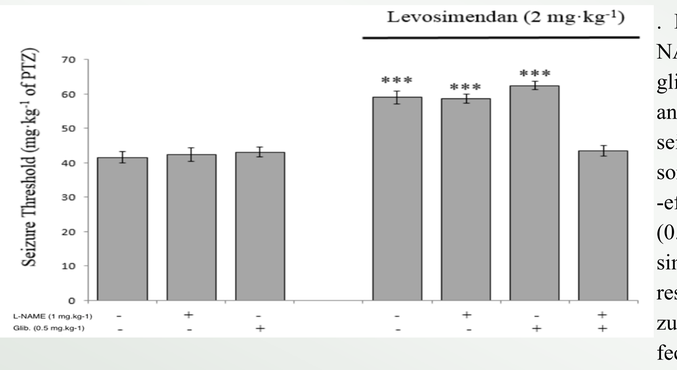


Graded doses of Cromakalim (0.5 and 1 µg·kg^{-1}; a KATP channel opener), which independently did not alter PTZ-induced seizure threshold, potentiated anticonvulsant effects of sub-effective dose of levosimendan (0.2 mg.kg^{-1}) in comparison to the saline-treated control group. Cromakalim was administered 30 min before i.p. injection of levosimendan and 120 min before determination of PTZ-induced seizure threshold. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. **P < 0.01, ***P < 0.001, in comparison to the saline-treated control group.



Graded doses of L-Arg (30 and 60 mg.kg^{-1}; NO precursor), which independently did not alter PTZ-induced seizure threshold, potentiated anticonvulsant effects of sub-effective dose of levosimendan (0.2 mg.kg^{-1}) in comparison to the saline-treated control group. L-Arg was administered 45 min before i.p. injection of levosimendan and 135 min before determination of PTZ-induced seizure threshold. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. *P < 0.05, ***P < 0.001, in comparison to the saline-treated control group.

Moreover, co-administration of noneffective doses of Glibenclamide and L-NAME demonstrated a synergistic effect in blocking the anticonvulsant effects of levosimendan.



. Independent administration of sub-effective doses of L-NAME (1 mg.kg-1; a non-selective NOS inhibitor) and glibenclamide (0.5 mg.kg-1; a KATP channel blocker) 45 min and 30 min respectively before determination of PTZ-induced seizure threshold did not alter seizure threshold in comparison to the saline-treated control group. Pre-treatment with sub -effective doses of L-NAME (1 mg.kg-1) or glibenclamide (0.5 mg.kg-1) 45 min and 30 min before i.p. injection of levosimendan (2 mg.kg-1) respectively and 135 min and 120 min respectively min before determination of PTZ-induced seizure threshold respectively did not alter anticonvulsant effects of levosimendan. However, Co-administration of sub-effective doses of L-NAME (1 mg.kg-1) and glibenclamide (0.5 mg.kg-1) 54 min and 30 min respectively before i.p. injection of levosimendan (2 mg.kg-1) and 135 min and 120 min respectively before determination of PTZ-induced seizure threshold reversed anticonvulsant effects of levosimendan down to the saline-treated control group. Each group consisted of 6-8 mice. Data are expressed as the mean ± SEM seizure threshold in each group. ***P < 0.001, in comparison to the saline-treated control group.

conclusion

Summing up, our findings for the first time elucidated the role of levosimendan in modulation of seizure threshold as a calcium sensitizer drug. We revealed the novel modulatory role of K_{ATP}/nNOS/NO activation in the hippocampus and temporal cortex in exertion of the anticonvulsant effects of levosimendan. Although the present findings need further investigation by experimental studies on other models of seizures and epilepsies, these findings add an important insight into the mechanism of neuroprotective effects of levosimendan and shall pave the way for innovative strategies in management of patients inflicted by acquired seizures as a result of heart failures induced hypoxia/ischemia or drug toxicities, such as CCBs. It also remains an open question, whether K_{ATP} channels openers and levosimendan in specific, are appropriate choices for treatment of epilepsy or can be an adjunctive therapy alongside other antiepileptic drugs.