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Dantrolene: mechanisms of neuroprotection and possible clinical applications in the neurointensive care unit

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Abstract

Background and aims—Calcium plays a central role in neuronal function and injury. Dantrolene, an inhibitor of the ryanodine receptor, inhibits intracellular calcium release from the sarcoplasmic reticulum and might serve as novel agent for neuroprotection and other applications in the Neurointensive Care Unit.

Methods—We reviewed the available data of dantrolene as a potential neuroprotective agent through literature searches on Ovid, Pubmed and Google Scholar.

Results—Dantrolene provides neuroprotection in multiple *in vitro* models and some *in vivo* models of neural injury. Its efficacy has an early and narrow time-window of protection. We briefly summarize its other pharmacologic effects that may have potential applications for patients in the neurointensive care unit. Areas with the need for continued research are identified.

Conclusion—Targeted use of dantrolene in selected ICU disease models of anticipated neural injury, such as impending ischemia from vasospastic syndromes, might provided neuroprotection.

INTRODUCTION

Many different forms of neuronal injury are managed in the neurointensive care unit (NICU). These include, but are not limited to, ischemic or hemorrhagic stroke, vasospasm after subarachnoid hemorrhage (SAH), elevated intracranial pressure (ICP), reperfusion injury, brain edema, and status epilepticus. Mechanisms of neuronal injury by these disease states include excitotoxicity, apoptosis, necrosis, disruption of membrane potentials and abnormal protein folding. Calcium (Ca^{2+}) plays an integral role in regulation or activation of these pathways.

Dantrolene, a drug that has been around since 1975, has a very narrow clinical use, i.e., malignant hyperthermia. It is rarely used in the NICU for other indications such as neuroleptic malignant syndrome, heat stroke, spasticity, shivering, serotonin syndrome and ecstasy overdose¹⁻⁶. Its unique mechanism of blocking intracellular Ca^{2+} release might make it an attractive drug in treating or preventing neuronal injury⁷.

PHARMACOLOGY OF DANTROLENE

A. MECHANISM OF ACTION AND RECEPTORS

Dantrolene inhibits ryanodine receptors (RYR)⁸. RYR are intracellular Ca^{2+} -release channels expressed on the surface of the sarcoplasmic reticulum⁹. RYR are regulated by the Ca^{2+} -dependent protein, calmodulin (CaM)¹⁰. Three different RYR isoforms have been identified in mammalian tissues. They have specific sites of expression: all three RYRs can be found in the brain, but RYR1 is found predominantly in skeletal muscle, RYR2 predominantly in cardiac muscle, and RYR3 is expressed at comparatively low levels in a variety of tissues, including the brain and smooth muscles cells¹¹. Dantrolene acts directly on the RYR1 and RYR3 to reduce channel activation by CaM, and thereby decreasing the Ca^{2+} -sensitivity of the channel activation¹². RYR2 is not inhibited by dantrolene¹³. This fortuitous selectivity explains why the drug has no negative inotropic effect on the heart. Binding of dantrolene to the RYRs in the brain may protect neurons from disruptions in Ca^{2+} -homeostasis. Dantrolene has recently been used in cell models and animal models to diminish cell death resulting from a multitude of neuronal injuries, including ischemia^{14, 15}, epileptic seizures^{16, 17}, and spinal cord injury¹⁸.

B. CHEMISTRY

Dantrolene sodium is a highly lipid soluble small molecule that is rather water insoluble, which led to the delay of the development of the intravenous (IV) formulation. Dantrolene was initially only available as an oral medication. One vial of dantrolene IV (60ml) contains 20 mg dantrolene sodium and 3000 mg mannitol and sodium hydroxide to yield a pH of 9.5, to assist with solubility. The resulting solution is irritating to veins, and should be injected preferably via a central venous catheter or large bore peripheral IV catheter.

C. PHARMACOKINETICS AND METABOLISM

Following dantrolene ingestion by mouth, about 70% of dantrolene is absorbed, with the peak plasma concentration reached in 6 hours¹⁹. However, especially in children, plasma concentrations can have great variations²⁰, although it is unclear whether this leads to a difference in clinical efficacy²¹. Flewellen et al.²² investigated the clinical response after IV administration of dantrolene in relation to plasma levels. In the conscious patient, the plateau maximal depression of muscle twitch response (75% depression) and grip strength (42% depression) was accomplished after administration of an accumulative dose of 2.4 mg/kg body weight. This resulted in a dantrolene plasma concentration of 4.2 $\mu\text{g/ml}$ (approximately 10 μM). Thereafter, the elimination half-life was 12 hours, although plasma concentration was maintained at a steady level within therapeutic range for about 5 hours. The residual dantrolene plasma concentration at 24 hours after such a dose was 1 $\mu\text{g/ml}$. Subjects reported a feeling of weakness at plasma levels of 1 $\mu\text{g/ml}$. This weakness persisted for up to 48 hours, by which time the residual plasma concentration of dantrolene had decreased to 0.3 $\mu\text{g/ml}$. Metabolism of dantrolene is achieved microsomally in the liver via oxidative and reductive pathways. Oxidation results in hydroxylation of the hydantoin ring to 5-hydroxydantrolene (5HD), while reduction leads to the formation of aminodantrolene, which is then acetylated to the reduced acetylated derivative (RAD) of dantrolene²⁰. The metabolites themselves can have some muscle relaxant properties²³. Excreted in both the urine and the bile, dantrolene is 79% excreted as 5HD, 17% as RAD, while 4% of the dose is cleared unchanged in the urine^{19, 20}.

D. SIDE EFFECTS

Most commonly reported side effects are dizziness, lightheadedness and drowsiness, but no other CNS effects with IV or oral administration have been reported^{19, 20}. Oral administration

can result in diarrhea. The most serious reported adverse effect, hepatotoxicity, has accompanied its chronic administration by mouth^{24, 25}. However, there have been several reports indicating the contrary, so that it remains unclear whether dantrolene is truly hepatotoxic. Flewellen et al.²² were unable to show any changes in liver function tests after prolonged oral exposure. No hepatotoxicity was achieved in mice exposed to dantrolene, even after enhancement of any hepatotoxic potential by the inhibition of acetylation, depletion of glutathione, induction of biotransformation and the promotion of reductive metabolism²⁶. In fact, dantrolene is hepato-protective in an *in vivo* rat model of liver injury²⁷. In a hepatocyte culture model, dantrolene did not produce any hepatotoxicity²⁸.

Other noteworthy adverse effects are rare cases of dantrolene-induced eosinophilic pleural and pericardial effusion^{29, 30}, which rapidly resolve after short steroid therapy³¹. Prolonged oral exposure to dantrolene has been associated with an acneiform rash¹⁹.

E. MAJOR DRUG INTERACTIONS

While dantrolene itself has no myocardial effects, marked myocardial depression was reported in swine and dogs after co-administration of verapamil³². It is important to note, however, that this observation cannot be extrapolated to other calcium-channel-blocking agents, which are widely used in the NICU and might raise concern regarding the use of dantrolene. No adverse cardiac effect was observed in swine treated with dantrolene and amlodipine³³, and our personal observations in over 30 patients have not witnessed bradycardia or hypotension in the presence of nimodipine or nicardipine.

MOLECULAR MECHANISMS OF NEUROPROTECTION

A. CALCIUM'S LINK TO THE PATHOPHYSIOLOGY OF NEURONAL INJURY

Fundamental for the survival of neurons is the fine balance between metabolic demand and energy supply. Any imbalance may lead to neuronal damage and potential cell demise. Calcium, in particular intracellular Ca^{2+} , plays a pivotal role in neuronal injury. The link between calcium and the following forms of neuronal injury will be introduced in order to understand the role of dantrolene later: excitotoxicity induced by excitatory amino acids (i.e. glutamate), apoptosis, membrane destabilization, protein misfolding and necrosis (Figure).

In case of metabolic failure, depolarization of the neuronal membrane ensues. This in turn results in elevated levels of glutamate, an excitotoxic amino acid released from the presynaptic vesicles³⁴, which binds to postsynaptic glutamate receptors³⁵. Many of the glutamate receptors are ligand-gated, ionotropic Ca^{2+} -channels (N-methyl-D-aspartic acid [NMDA], alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], and kainate [KA] receptors). The opening of glutamate-gated channels leads to Ca^{2+} -influx from the extracellular space into the cytosol, which in turn activates Ca^{2+} -efflux from intracellular stores⁷. The Ca^{2+} -overload in the cytosol activates various Ca^{2+} -dependent enzymes that can initiate irreparable cell injury and mitochondrial dysfunction leading to cell death³⁶. In neurons, the endoplasmic reticulum (ER) is the primary intracellular storage site of Ca^{2+} -ions^{37, 38}. As shown in animal models, metabolic failure results in massive Ca^{2+} -release from the ER³⁹. By inhibiting Ca^{2+} -discharge from the sarcoendoplasmic reticulum, dantrolene might have implications in neuroprotection.

Apoptosis is a genetically controlled, programmed and highly regulated cell death. It can be brought on by a loss of Ca^{2+} -homeostasis. It can trigger, as well as modulate, apoptosis by promoting mitochondria-mediated caspase activation⁴⁰.

Cell membranes, when disturbed by external toxins or other triggers, can lead to a rapid Ca^{2+} -influx, which in turn can activate cell death by mechanisms explained above.

The ER is not only the major intracellular Ca^{2+} -store, but it is also responsible for proper protein folding. Significant Ca^{2+} -efflux from the ER induces stress molecules and protein misfolding, which can trigger apoptosis^{41, 42}.

Necrosis on the other hand is characterized by cytoplasmic swelling, plasma membrane rupture and inflammation of the surrounding tissues⁴⁰. It is now known that specific pathways exist for necrosis⁴³. Glutamate is released, which results in excitatory cell death as described above.

B. DANTROLENE AND NEUROPROTECTION

1. CYTOTOXICITY BY EXCITATORY AMINO ACIDS—In an *in vitro* model of mouse cerebral cortical neurons, dantrolene reduced the glutamate-induced increases in intracellular Ca^{2+} -concentrations by 70% under physiologic conditions, and protected against glutamate-induced neurotoxicity⁷. Complete block of glutamate toxicity by dantrolene was seen in the absence of extracellular Ca^{2+} . This neuroprotective effect is dose-dependent⁴⁴. Dantrolene does not appear to affect the kinetics of glutamate binding to membranes or voltage-gated Ca^{2+} -channels⁴⁵, which indicates that release of Ca^{2+} from intracellular stores is essential for the propagation of glutamate-induced neuronal damage^{7, 45-47}. Dantrolene also protects against excitotoxicity induced by quisqualate (QA) and N-methyl-D-aspartate (NMDA), whereas no protection is observed on cell damage mediated by kainate (KA) or 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (AMPA) receptor stimulation⁴⁸. The discrepancy in glutamate receptor protection is likely explained by the fact that the increase in intracellular Ca^{2+} stimulated by glutamate, NMDA and QA is predominantly through release of Ca^{2+} from intracellular stores, whereas KA and AMPA increase intracellular Ca^{2+} largely from extracellular influx^{49, 50}. An *in vivo* ischemia model using microdialysis in rats showed several interesting findings: first, dantrolene and vehicle (DMSO) groups had the same level of extracellular glutamate, suggesting that dantrolene does not prevent the release of glutamate. Compared to vehicle and control, dantrolene had a significantly higher number of preserved hippocampal neurons when given 15 minutes prior to ischemia, suggesting that dantrolene is neuroprotective due to the downstream blockade in the cytoplasmic increase of Ca^{2+} ⁵¹.

2. APOPTOSIS—Several apoptosis models demonstrate dantrolene neuroprotection via different mechanisms. One mechanism is the protection from bioenergetic failure during DNA repair. Calmodulin-dependent nitric oxide (NO) synthase, an important Ca^{2+} -activated enzyme, forms NO from L-arginine³⁵. During ischemia and reperfusion, superoxide anion is produced, which can react with NO to form peroxynitrite. Both superoxide anion and peroxynitrite can cause DNA breaks. DNA cleavage activates the nuclear repair enzyme poly (ADP-ribose) polymerase (PARP). This enzyme utilizes NAD^+ to transfer ADP-ribose during DNA repair. Cell death may ensue due to consumption of NAD^+ , which may cause energy failure as ATP is depleted in the efforts to resynthesize NAD^+ ^{52, 53}. Dantrolene, when applied early (0-40 minutes), but not late (40-120 minutes) after ischemia ameliorated PARP-related bioenergetic failure and led to a significant 25% increase of high energy phosphates in an *in vitro* neonatal rat brain model⁵⁴. The exact mechanism of blocking PARP-related bioenergetic failure is unknown. Whether this is due to blocking activation of PARP via prevention of DNA fragmentation or blocking other downstream mechanism of PARP, such as activation of calpains (Ca^{2+} -dependent proteases) needs to be determined⁵⁵.

A different mechanism by which dantrolene protects against apoptosis was found in experiments using thapsigargin, an inducer of apoptosis. This chemical is a specific inhibitor of the sarcoendoplasmic reticulum Ca^{2+} -ATPase. In an *in vitro* neuronal cell model, dantrolene partially abolished apoptosis induced by thapsigargin⁵⁶. It is likely that the initial Ca^{2+} rise may be the signaling event in the apoptotic pathway, which is partially inhibited by dantrolene.

In an *in vitro* Alzheimer's disease model using cells expressing the proapoptotic presenilin-1 (PS-1) mutation, dantrolene significantly prevented amyloid- β induced apoptosis and resulted in lower cellular peroxide levels, thereby protecting against oxidative stress. This protection is likely due to an altered calcium release in these cells ⁵⁷.

Dantrolene has been reported to upregulate the anti-apoptotic protein Bcl-2. In two different cell lines pretreatment with dantrolene robustly suppressed 3-hydroxykynurenine-induced apoptosis ⁵⁸. Upregulation of the anti-apoptotic protein Bcl-2 was associated with this protection ^{58, 59}.

In an *in vitro* and *in vivo* apoptosis model with kainic acid-induced status epilepticus, dantrolene significantly reduced apoptosis in both models 24 hours after induction of status epilepticus ⁶⁰. Using a different *in vivo* apoptosis model with hypoxic-ischemic injury in neonatal rats, intraventricular dantrolene significantly reduced the lipid/creatine ratio as assessed by ¹H magnetic resonance spectroscopy in the alive rat pups, as well as the brain infarct area and morphologic appearance of the infarct ⁶¹.

The role of the mitochondria in apoptosis is well established ⁶². Elevated levels of intracellular Ca²⁺ released from the ER can rapidly be taken up by mitochondria ⁶³, causing collapse of the mitochondrial membrane potential and triggering the apoptotic cascade ⁶⁴. In a cell model of mitochondrial-induced apoptosis dantrolene has been shown to prevent cell death ⁶⁵.

3. MEMBRANE STABILIZATION—A single paper ⁶⁶ has investigated the properties of dantrolene as a possible plasma membrane stabilizer in a cell culture model of neuroblastoma cells. The authors exposed the cells to compound 48/80 (C48/80), a membrane stimulator, leading to rapid, dose-dependent Ca²⁺-influx. Dantrolene inhibited the induced increase in Ca²⁺-permeability of plasma membrane in a concentration-dependent manner. In addition, dantrolene (10 μ M) itself did not affect membrane fluidity, but it significantly reduced the C48/80-induced increase in membrane fluidity. These results suggest that dantrolene is a direct neuronal plasma membrane stabilizer, possibly through the inhibition of phospholipase A2 ⁶⁷.

4. PROTEIN FOLDING—In an *in vivo* ischemia model of 90-minute middle cerebral artery (MCA) occlusion, Li et al. studied the effect of dantrolene on ER stress-mediated apoptotic signal pathway activation ⁶⁸. Dantrolene significantly decreased infarct volume and DNA fragmentation assessed 24 hours after transient ischemia. At the same time, apoptotic markers were significantly reduced in the ischemic penumbra, but not the ischemic core. With dantrolene treatment, various markers of ER stress were significantly downregulated within the penumbra. These results suggest that dantrolene has its greatest effect within the penumbra and may decrease infarct volume by preventing protein misfolding.

5. NECROSIS—In a *C. elegans* model of necrosis, ryanodine receptors and Ca²⁺-release from the ER were demonstrated to play a critical role ⁶⁹. In this *in vivo* model, dantrolene also rescued neuronal cell death. Likewise, in an *in vivo* mouse model of Alzheimer's disease where presenilin-1 mutation predisposes neurons to excitotoxic necrosis, dantrolene pretreatment also protected 80% of neurons from cell death measured by maintenance of plasma membrane integrity ^{70, 71}. Dantrolene has been shown to protect other tissues from necrosis, such as heart, liver, and pancreas ⁷²⁻⁷⁵.

CHALLENGES IN THE TRANSLATION FROM BENCH TO BEDSIDE

A. EXPERIMENTAL MODELS: DANTROLENE DOES NOT ALWAYS WORK

The neuroprotective effect of dantrolene appears rather consistent across multiple cell and animal models of neurological injury that include excitotoxicity, oxygen glucose deprivation (OGD), global ischemia, forebrain ischemia, focal ischemia, seizure, and traumatic injury (see Table). Three reports showed no effect on neuronal injury⁷⁶⁻⁷⁸. In the first study, a neurological score without histology was performed. The neurological score on a small number of animals might have been too insensitive to find differences⁷⁶. In the second study, only a low dose of dantrolene (10 μM) was used, which suggests that neuroprotection may be dose-dependent⁷⁸. In the third negative study, no change in neuronal viability was found for unclear reasons⁷⁷. This study stands in contrast to other studies with evidence for neuroprotection by dantrolene using the same *in vitro* hippocampal slice model^{16, 17, 54, 79}.

B. TIMING AND DOSING: WHEN AND HOW MUCH?

The neuroprotection provided by dantrolene has a narrow temporal therapeutic window. As in most other neuroprotective agents, timing is one of the major limitations in its application in the human. For *in vitro* neonatal rat brain or neuronal cell models, pretreatment, intra-injury treatment and post-treatment out to 40 minutes have shown consistent protection. However, experiments with analysis at later time points (either 40 minutes or 16 hours after the injury) did not show any neuroprotection^{54, 80}. Using *in vivo* brain ischemia or epilepsy models, dantrolene pre- and post-treatment extending out to 30 minutes appears neuroprotective, whereas delayed treatment at 120-140 minutes provides little or no protection^{81, 82} (see Table).

The dose of dantrolene that achieves neuroprotection appears to be consistent among studies (see Table). Doses of 10-100 μM in cell culture or tissues are effective. *In vitro* and *in vivo* doses between 1-10 μM may or may not be effective^{44, 46, 78, 81, 83}. Doses exceeding 120 μM may be toxic to cells in culture⁵⁸, but *in vivo* doses as high as 1mM and 2mM were well tolerated in a brain ischemia and epilepsy model^{61, 84}. In another study of dose toxicity the dose at which caused 50% death (LD_{50}) for dantrolene in rats was 500mg/kg (2mM) intraperitoneally⁸⁵.

C. ROUTE OF ADMINISTRATION

The ideal route of dantrolene administration - as with all neuroprotective drugs - remains an unsolved problem. Despite dantrolene's high lipid solubility, low molecular weight, and pK_a of 7.7 there are suggestions that dantrolene has poor permeability of the blood brain barrier (BBB) based on an older oral dose C_{14} -dantrolene experiments with monkeys⁸⁶. However, more recent evidence based on microdialysis and HPLC suggests that dantrolene penetrates the BBB with a tissue/plasma concentration ratio K_p of 6.4 at steady state⁸⁷. This partition coefficient across the BBB is similar to oxycodone⁸⁸. This new evidence is consistent with the finding that drowsiness is a common side-effect of dantrolene and is noted when blood drug levels are 0.64 $\mu\text{g}/\text{ml}$ ⁸⁹ and dantrolene's ability to upregulate dopamine metabolism as measured by metabolites in human CSF⁹⁰. Most experiments that reported successful neuroprotection used intracerebroventricular delivery, with doses as low as 2.5 μM ⁸¹, whereas two negative studies used intravenous delivery at low doses of either 8 or 16 μM ^{76, 78} (see Table). However, during neuronal injury the BBB frequently is not intact. Whether or not dantrolene has improved BBB penetration with brain injury requires further study. In fact, in an *in vivo* model of global cerebral ischemia dantrolene administered intravenously at doses 40-80 μM immediately following a five minute global cerebral ischemia was effective in protecting CA1 neurons, suggesting that not only the mode of delivery but also the injury state of the brain might play a role¹⁵. Furthermore, other *in vivo* non-ischemic models of

neuroprotection have used intraperitoneal delivery successfully at doses as low as 40 μ M (see Table).

POSSIBLE CLINICAL APPLICATIONS IN THE NICU

Neuroprotection continues to be challenging in clinical practice. As with the recent negative results of the Phase III trial of NXY-059 in ischemic stroke⁹¹, taking promising therapeutic agents from the animal lab to the bedside can be disappointing when done prematurely. However, the field of neurocritical care encounters many different forms of neurological injury, and therefore neuroprotection is particularly interesting in the diseases seen in the NICU.

However, not all cell or animal models described in our article apply to clinical practice. Pre-injury administration might only be feasible pre-operatively prior to high-risk procedures during which brain injury might ensue (i.e. carotid endarterectomy, cerebral angiography, brain surgery). For all other brain injuries that cannot be foreseen, administration just minutes after the injury is unrealistic. Given that only 5% of all acute stroke patients receive the FDA approved tissue plasminogen activator within 3 hours⁹², it is unlikely that a neuroprotective agent can be given sooner. As discussed above, the mode of administration remains an important unsolved problem and must be investigated. Intravenous administration is certainly the easiest way; however further study on BBB permeability comparing intravenous and intracerebroventricular administration is required in injured and uninjured brains of animals and possibly humans.

In addition to being potentially neuroprotective, dantrolene has several other pharmacologic effects that might be of additional benefit to patients with neurologic injuries. It inhibited stretch-induced smooth muscle contraction in rabbits⁹³. Recently, in an *ex vivo* rat basilar artery vasoconstriction model, dantrolene has been shown to inhibit serotonin induced vasoconstriction⁹⁴. Synergistic effects were seen with the combination of nimodipine and dantrolene. Other animal studies have confirmed synergistic neuroprotective effects of nimodipine and dantrolene^{44, 83}. We have observed dantrolene-mediated vasorelaxation in three of our own NICU patients [submitted to the Neurocritical Care Journal]. Whether dantrolene might be a potential treatment for cerebral vasoconstriction syndromes such as vasospasm after SAH, trauma or “Call-Fleming Syndrome” will require prospective human trials^{95, 96}.

Dantrolene has been shown to significantly reduce the gain of shivering and the shivering threshold in healthy adults, thereby reducing shivering thermogenesis by as much as 30 kcal/h². Induced normothermia or hypothermia has become a treatment modality to reduce fever burden in neurological injury⁹⁷⁻¹⁰². The use of therapeutic fever reduction is compromised by shivering as a thermoregulatory defense, which causes an increase in oxygen consumption and doubling of the metabolic rate¹⁰³⁻¹⁰⁵. Drugs that are used to inhibit shivering may have side effects that restrict their use. Propofol, fentanyl, benzodiazepines or neuromuscular blocking agents might work well in limiting shivering, but are often avoided in order to preserve the neurologic exam. Meperidine may also alter the mental status, but most importantly can lower seizure threshold¹⁰⁶ as well as blood pressure, as can benzodiazepines and central alpha-2 agonists^{107, 108}. Although occasionally used by us as an add-on antishivering drug, dantrolene usually causes less sedation and muscle relaxation than the medications commonly used to treat shivering, and at the same time may have a dual pharmacodynamic benefit of neuroprotection as discussed above.

CONCLUSION

This review provides scientific evidence in animal studies for the neuroprotective potential of dantrolene. Further animal investigations are needed for timing, dose and route of

administration. Prospective studies are necessary in order to determine whether dantrolene is effective in humans. Dantrolene has few side effects, and is generally well tolerated. In addition, dantrolene reduces shivering, and inhibits vasoconstriction mediated by serotonin with synergism in the presence of nimodipine. Dantrolene or other novel ryanodine receptor blockers might provide a promising new line of direction for neuroprotection with multiple application possibilities in the NICU.

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Abbreviations

ADP, adenosine diphosphate
 AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
 ATP, adenosine triphosphate
 CaM, calmodulin
 CICR, calcium-induced calcium release
 CytC, Cytochrome C
 DNA, Deoxyribonucleic acid
 KA, kainic acid
 MtPTP, mitochondrial permeability transition pore
 NAD, Nicotinamide adenine dinucleotide
 NMDA, N-methyl-D-aspartic acid
 NO, nitric oxide
 NOS, nitric oxide synthase
 PARP, Poly (ADP-ribose) polymerase
 RyR, ryanodine receptor
 SERCA, Sarcoendoplasmic Reticulum Ca²⁺-ATPase
 UPR, unfolded protein response
 VGCC, voltage gated calcium channel

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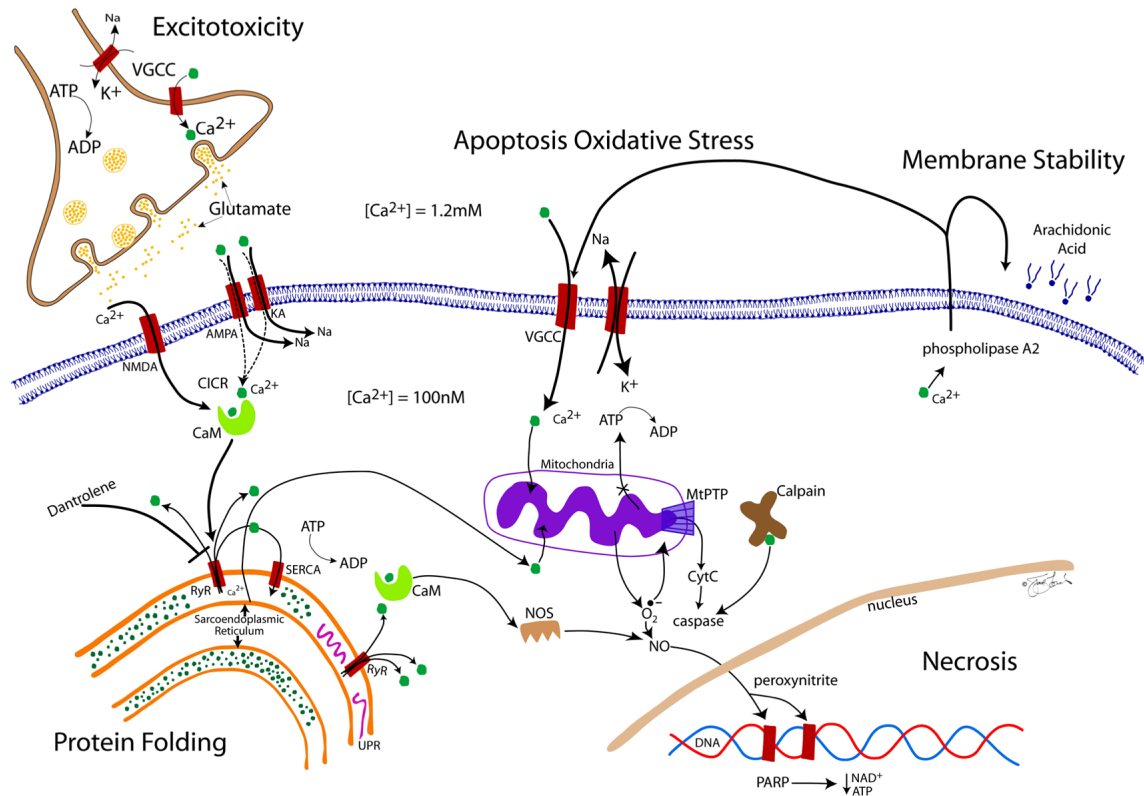


FIGURE. The role of calcium in cellular injury

Under normal conditions, there is a 10,000-fold Ca^{2+} -gradient between the intra- and extracellular space. When cellular oxygen levels reach below-threshold levels, the Na^+ - K^+ -pump, which consumes 70% of neuronal ATP, fails, leading to depolarization of the membrane and influx of Ca^{2+} through VGCC and glutamate release into the synaptic cleft. Glutamate binds to NMDA, AMPA and KA receptors, leading to direct and indirect influx of Ca^{2+} into the cell. Elevated levels of intracellular Ca^{2+} lead to further calcium-induced calcium release (CICR) via binding of Ca^{2+} to CaM. CICR is mediated by RyR binding and calcium release from the sarcoendoplasmic reticulum, the largest store of intracellular Ca^{2+} . Increases in intracellular Ca^{2+} cannot be compensated for by the energy-dependent SERCA pump, located in the membrane of the sarcoendoplasmic reticulum. A reduction in endoplasmic Ca^{2+} results in misfolding of proteins, leading to UFR as a cellular stress response. Influx of Ca^{2+} into mitochondria leads to formation of oxygen radicals and energy failure through decreased production of ATP. Binding of Ca^{2+} to CaM also leads to activation of NOS and formation of NO. Oxygen radicals react with NO to form peroxynitrite, a highly reactive molecule that damages DNA and proteins. DNA cleavage activates the DNA-repair enzyme PARP, which requires ATP for its action. PARP-related energy depletion adds further to cellular stress causing necrosis. The increased (toxic) levels of intracellular Ca^{2+} lead to increased mitochondrial permeability via MtPTP. This causes mitochondria to become permeable to molecules smaller than 1.5 kDa, which increases the osmolar load inside the mitochondria, causing mitochondrial swelling and outer membrane rupture, releasing CyC. CyC activates pro-apoptotic factors (caspases) by reacting with Ca^{2+} -activated calpain. Elevated intracellular Ca^{2+} activates phospholipase A2 which cleaves cell membrane phospholipids, releasing arachidonic acid, which in turn can be further activated to proinflammatory molecules, such as prostaglandins, leukotrienes, and thromboxanes (not shown). Dantrolene is neuroprotective by blocking ryanodine receptors at the sarcoendoplasmic reticulum, and thereby inhibiting further increase in intracellular Ca^{2+} -levels.

Studies testing the potential neuroprotective effect of dantrolene in animal or cell models are listed by injury model, animal model, timing of dantrolene administration in relation to the injury, dantrolene dose, findings, and the citation.

TABLE

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
Excitotoxicity (NMDA)	Cortical neuron culture	at the time of injury	30 μ M	iCa^{2+} reduced by 30% in presence of extracellular Ca^{2+} and 100% in absence of extracellular Ca^{2+} ; toxic effect of glutamate entirely prevented	7
Excitotoxicity (Glutamate, NMDA, QA, KA, AMPA)	Cortical neuron culture	at the time of injury	30 μ M	Dantrolene blocks LDH and calcium release by NMDA, QA and glutamate, no effect on KA or AMPA	48
Excitotoxicity (NMDA)	Cerebellar granule neuron culture	45 min prior	1-100 μ M alone and in combination with nimodipine and ruthenium red (RuR)	dose-dependent neuroprotection at 5 and 15 min, alone and in combination with RuR and nimodipine	44
Excitotoxicity (NMDA, KA, QA)	Cerebellar granule neuron culture	Simultaneous	50 μ M	Dantrolene inhibited glutamate induced Ca^{2+} release	45
Excitotoxicity (NMDA)	Cerebellar granule neuron culture	at the time of injury	5-30 μ M	decrease in iCa^{2+} in response to NMDA and K^+ , proving that NMDA/ K^+ released Ca^{2+} from intracellular stores	46
Excitotoxicity (NMDA)	Mixed cortical and retinal cell cultures	at the time of injury	30 μ M	iCa^{2+} decreased by 56%; cell toxicity reduced by 50%	49
Excitotoxicity (NMDA)	Hippocampal neuron culture	10-20 min prior	10 μ M	reduced NMDA-induced increased iCa^{2+}	50
Neurotoxicity by 3-HK	Cerebellar granule neuron culture, PC12 cells, and GT1-7 hypothalamic neurosecretory cells	30 min prior	120 μ M	3-HK induced cell death of PC12 and GT1-7 cells inhibited; marked increase in protein level Bcl-2	58
HIV-1 infection (cytotoxicity by HIV coat protein gp 120)	Cortical synaptosomes	Immediately prior and 10 min during experiment	10 μ M	decreased Gp120-induced iCa^{2+} rise	109
Status epilepticus	Kainic acid 8 mg/kg, rat	30-60 min after	10 mg/kg i.p. (approx 40 μ M)	CA3 protected and CA1 preserved at 4 days	110
Status epilepticus (140min)	Electrogenic limbic status epilepticus, rat	Simultaneous, 30 or 140 min after onset	10 mg/kg i.p. (approx 40 μ M)	early administration reduced injury in all areas of hippocampus, late	82

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
Epilepsy	Rat	15, 30, or 60 min prior	62.5 mg/kg, 125 mg/kg, 250mg/kg, 500 m/kg, all i.p. (approx 250-2000 μ M)	administration reduced injury in CA3 region dose dependent reduction in limbic seizures	84
Epilepsy	EL mouse (mutant susceptible to convulsive seizures)	60 min prior	20, 40 and 80 mg/kg i.p (approx 80-320 μ M)	Seizure suppression; no influence on NO levels at any dose	111
Epilepsy	Hippocampal slice, rat	n/a	10-100 μ M	Dose-dependent suppression of pilocarpine- and DHPG-induced ictal activity	17
Epilepsy	Entorhinal cortex and hippocampus slice, rat	30 min prior and during	30 μ M	complete cell death prevention after 1, 3, or 12-14hrs; no effect on synaptic transmission or epileptic discharges	16
OGD(0-15min)	intracellular recordings in cortical slice, rat	15 min prior	10-30 μ M	no effect on membrane depolarization or intracellular calcium increase	112
OGD (40min)	Hippocampal slice, rat	120 min prior or simultaneously or 120 min after	10 μ M	No effect	77
OGD(0-10min)	Hippocampal slice, gerbil	60 min prior and during	50 μ M	Dantrolene reduced iCa^{2+} increase from ER	79
OGD (10-16 hours)	Neuroblastoma cell line	Simultaneous or 16 hours or 48 hours after	40 and 80 μ M	viability doubled at 48 hours; treatment after OGD achieves same effect as during OGD	80
OGD (40min)	Neonatal cortical slice	0-40 or 40-120 min after	20 μ M	early but not late energy failure reduced	54
OGD (45 min)	Retinal cell culture, rat	30 min prior and during	100 μ M	ganglion cell death prevented; 54% reduction of dead cells in the retinal layer	47
OGD (48 hrs)	Cortical neuron cell culture with presenilin-1 expression	60 min prior	10 μ M	80% of neurons rescued	71
Forebrain ischemia (20 min)	4 vessel occlusion, rat	15 min prior	20 μ M topical	Reduction of free fatty acids from ischemia and reperfusion	113
Forebrain ischemia (3 min)	Gerbil	30 or 120 min after	6 μ l of 1.6 mM intracerebroventricular (approx 10 μ M in the brain); 0.4mM	3 and 4.5 fold neuronal protection for 0.4 and 1.6mM. No effect at 120 min.	81

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
Forebrain ischemia (5 min)	4 vessel occlusion, rat	15 min after followed by infusion for 3 days	solution (approx 2.5 μ M in brain) 33 μ g intracerebroventricular (approx 50 μ M) followed by 40 μ g/day (approx 60 μ M)	50% CA1 cell loss reduction at 4 days; reduced TUNEL staining in the CA1 region.	114
Forebrain ischemia (10 min)	4 vessel occlusion, rat	15 min prior	33 μ g intracerebroventricular (approx 50 μ M)	40% more viable neurons in CA1 at 7 days.	51
Focal ischemia (90 min)	Middle cerebral artery occlusion, rat	Immediately after	20 μ g intracerebroventricular (approx 30 μ M)	65% infarct volume reduction at 24 hours; reduced ER stress markers and TUNEL staining in the penumbra	68
Focal ischemia (120 min)	Middle cerebral artery occlusion, rat	30 min prior	2 mg/kg (approx 8 μ M)	No effect	78
Global cerebral ischemia (11 min)	Dog	Immediately prior	4mg/kg intravenous (approx 16 μ M)	No effect	76
Apoptosis (<i>in vitro</i>); global ischemia (<i>in vivo</i>)	GT1-7 hypothalamic neurosecretory cells (<i>in vitro</i>), gerbil (<i>in vivo</i>)	30min prior (<i>in vitro</i>), Immediately after (<i>in vivo</i>)	120 and 240 μ M (<i>in vitro</i>); 10, 25, and 30 mg/kg intravenous (approx 40-200 μ M)	Dose-dependent reduction of iCa^{2+} , DNA-fragmentation and cell death (<i>in vitro</i>), dose-dependent increase of CA1 cells at 7 days (<i>in vivo</i>); 50mg/kg was toxic.	15
Apoptosis	PC12 cells	120 min prior	1 μ M	Suppression of peroxide accumulation and protection against oxidative stress	57
Apoptosis (<i>in vitro</i>) / status epilepticus (<i>in vivo</i>)	Cerebellar granule neuron culture (<i>in vitro</i>) and rat (<i>in vivo</i>)	Simultaneous (<i>in vitro</i>), 60 min prior (<i>in vivo</i>)	10 μ M (<i>in vitro</i>); 10 mg/kg i.p. (approx 40 μ M, <i>in vivo</i>)	cell death and apoptosis decreased at 3h and 24h (<i>in vitro</i>), CA1 region and cerebral cortex protected against apoptosis at 24hrs (<i>in vivo</i>)	60
Apoptosis (<i>in vivo</i>)	Hypoxic-ischemic neonatal rat brain	Immediately after	1 mM intracerebroventricular	Lip/Cr ratio decreased (1H MR spectroscopy) at 24hrs; no effect on NAA/Cr ratio; infarct area decreased at 14d; no effect on TUNEL stain and survival rate at 14d.	61
Membrane fluidity	Neuroblastoma cell line	Immediately after	10 μ M	Dantrolene decreased Ca^{2+} mobilization by 40%, reduced increase in membrane fluidity and stabilized neuronal plasma cell membranes	66

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
Spinal cord injury (mechanical compression)	Ex-vivo spinal cord, rat	15 min prior until 20min after	10 μ M	Electrophysiological recovery improved 1h after injury	18
Trauma	Hippocampal neuron culture	Simultaneously	30 μ M and 100 μ M	Dose-dependent reduction in cell death	115

Abbreviations: NMDA, N-methyl-D-aspartate; QA, quisqualate; KA, kainate; AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid; 3-HK, 3-hydroxykynurenine; HIV-1, human immunodeficiency virus type-1; gp120, glycoprotein; mGluR, metabotropic glutamate receptor; 1S,3R-ACPD, (1S,3R)-1-aminocyclopentane-1-3-dicarboxylic acid; OGD, oxygen glucose deprivation; PC, pheochromocytoma cells; RuR, ruthenium red; ip., intraperitoneal; Ca^{2+} , intracellular calcium; LDH, lactate dehydrogenase; NO, nitric oxide; DHPG, dihydroxyphenylglycine; ER, endoplasmic reticulum; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling; TTC, tetrazolium chloride; Lip/Cr, lipid/creatine; NAA/Cr, N-acetyl aspartate/creatine.