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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 sarcoendoplasmic 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 overdose [1-6]. Its unique mechanism of blocking intracellular Ca^{2+} release might make it an attractive drug in treating or preventing neuronal injury [7].

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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 sarcoendoplasmic reticulum [9]. 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 cells [11]. Dantrolene acts directly on the RYR1 and RYR3 to reduce channel activation by CaM, and thereby decreasing the Ca^{2+}-sensitivity of the channel activation [12]. RYR2 is not inhibited by dantrolene [13]. 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 injury [18].

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 hours [19]. However, especially in children, plasma concentrations can have great variations [20], although it is unclear whether this leads to a difference in clinical efficacy [21]. 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 μg/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 μg/ml. Subjects reported a feeling of weakness at plasma levels of 1 μg/ml. This weakness persisted for up to 48 hours, by which time the residual plasma concentration of dantrolene had decreased to 0.3 μg/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 dantrolene [20]. The metabolites themselves can have some muscle relaxant properties [23]. 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 metabolism [26]. In fact, dantrolene is hepato-protective in an *in vivo* rat model of liver injury [27]. In a hepatocyte culture model, dantrolene did not produce any hepatotoxicity [28].

Other noteworthy adverse effects are rare cases of dantrolene-induced eosinophilic pleural and pericardial effusion [29, 30], which rapidly resolve after short steroid therapy [31]. Prolonged oral exposure to dantrolene has been associated with an acneiform rash [19].

E. MAJOR DRUG INTERACTIONS

While dantrolene itself has no myocardial effects, marked myocardial depression was reported in swine and dogs after co-administration of verapamil [32]. 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 amlodipine [33], 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 vesicles [34], which binds to postsynaptic glutamate receptors [35]. 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 stores [7]. 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 death [36]. In neurons, the endoplasmatic 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 ER [39]. 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 activation [40].

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 cytoplasmatic swelling, plasma membrane rupture and inflammation of the surrounding tissues [40]. It is now known that specific pathways exist for necrosis [43]. 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 neurotoxicity [7]. Complete block of glutamate toxicity by dantrolene was seen in the absence of extracellular Ca^{2+}. This neuroprotective effect is dose-dependent [44]. Dantrolene does not appear to affect the kinetics of glutamate binding to membranes or voltage-gated Ca^{2+}-channels [45], 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 stimulation [48]. 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-arginine [35]. 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 model [54]. 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 determined [55].

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 thapsigargin [56]. 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-hydroxykynurenineinduced 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 ^{1}H 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 Ca^{2+} 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 Ca^{2+}-influx. Dantrolene inhibited the induced increase in Ca^{2+}-permeability of plasma membrane in a concentration-dependent manner. In addition, dantrolene (10μM) 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 Ca^{2+}-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 injury [76-78]. 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 differences [76]. In the second study, only a low dose of dantrolene (10 μM) was used, which suggests that neuroprotection may be dose-dependent [78]. In the third negative study, no change in neuronal viability was found for unclear reasons [77]. 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 culture [58], 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) intraperitoneally [85].

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 monkeys [86]. However, more recent evidence based on microdialysis and HPLC suggests that dantrolene penetrates the BBB with at tissue/plasma concentration ratio K_p of 6.4 at steady state[87]. This partion coefficient across the BBB is similar to oxycodone[88]. 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 μg/ml[89] and dantrolene's ability to upregulate dopamine metabolism as measured by metabolites in human CSF[90]. 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 role [15]. Furthermore, other *in vivo* non-ischemic models of

neuroprotection have used intraperitoneal delivery successfully at doses as low as 40μM (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 stroke [91], 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 hours [92], 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 rabbits [93]. Recently, in an *ex vivo* rat basilar artery vasoconstriction model, dantrolene has been shown to inhibit serotonin induced vasoconstriction [94]. 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/h [2]. Induced normothermia or hypothermia has become a treatment modality to reduce fever burden in neurological injury [97-102]. 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 rate [103-105]. 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 threshold [106] 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 Ca^{2+}-ATPase
UPR, unfolded protein response
VGCC, voltage gated calcium channel

REFERENCES

1. Harrison GG. Control of the malignant hyperpyrexic syndrome in MHS swine by dantrolene sodium. British journal of anaesthesia 1975;47(1):62–5. [PubMed: 1148076]
2. Lin CM, Neeru S, Doufas AG, et al. Dantrolene reduces the threshold and gain for shivering. Anesth Analg 2004;98(5):1318–24. [PubMed: 15105208]table of contents
3. Lydiatt JS, Hill GE. Treatment of heat stroke with dantrolene. JAMA 1981;246(1):41–2. [PubMed: 7241727]
4. Hadad E, Cohen-Sivan Y, Heled Y, Epstein Y. Clinical review: Treatment of heat stroke: should dantrolene be considered? Critical care (London, England) 2005;9(1):86–91.
5. Hall AP, Henry JA. Acute toxic effects of `Ecstasy' (MDMA) and related compounds: overview of pathophysiology and clinical management. British journal of anaesthesia 2006;96(6):678–85. [PubMed: 16595612]
6. Graber MA, Hoehns TB, Perry PJ. Sertraline-phenelzine drug interaction: a serotonin syndrome reaction. The Annals of pharmacotherapy 1994;28(6):732–5. [PubMed: 7919561]
7. Frandsen A, Schousboe A. Dantrolene prevents glutamate cytotoxicity and Ca2+ release from intracellular stores in cultured cerebral cortical neurons. Journal of neurochemistry 1991;56(3):1075–8. [PubMed: 1671584]
8. MacLennan DH, Duff C, Zorzato F, et al. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 1990;343(6258):559–61. [PubMed: 1967823]

9. Marks AR, Tempst P, Hwang KS, et al. Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America 1989;86(22):8683–7. [PubMed: 2813419]
10. Balshaw DM, Yamaguchi N, Meissner G. Modulation of intracellular calcium-release channels by calmodulin. The Journal of membrane biology 2002;185(1):1–8. [PubMed: 11891559]
11. Giannini G, Conti A, Mammarella S, Scrobogna M, Sorrentino V. The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. The Journal of cell biology 1995;128(5):893–904. [PubMed: 7876312]
12. Fruen BR, Mickelson JR, Louis CF. Dantrolene inhibition of sarcoplasmic reticulum Ca2+ release by direct and specific action at skeletal muscle ryanodine receptors. The Journal of biological chemistry 1997;272(43):26965–71. [PubMed: 9341133]
13. Zhao F, Li P, Chen SR, Louis CF, Fruen BR. Dantrolene inhibition of ryanodine receptor Ca2+ release channels. Molecular mechanism and isoform selectivity. The Journal of biological chemistry 2001;276(17):13810–6. [PubMed: 11278295]
14. Mody I, MacDonald JF. NMDA receptor-dependent excitotoxicity: the role of intracellular Ca2+ release. Trends in pharmacological sciences 1995;16(10):356–9. [PubMed: 7491714]
15. Wei H, Perry DC. Dantrolene is cytoprotective in two models of neuronal cell death. Journal of neurochemistry 1996;67(6):2390–8. [PubMed: 8931471]
16. Pelletier MR, Wadia JS, Mills LR, Carlen PL. Seizure-induced cell death produced by repeated tetanic stimulation in vitro: possible role of endoplasmic reticulum calcium stores. Journal of neurophysiology 1999;81(6):3054–64. [PubMed: 10368420]
17. Rutecki PA, Sayin U, Yang Y, Hadar E. Determinants of ictal epileptiform patterns in the hippocampal slice. Epilepsia 2002;43(Suppl 5):179–83. [PubMed: 12121317]
18. Thorell WE, Leibrock LG, Agrawal SK. Role of RyRs and IP3 receptors after traumatic injury to spinal cord white matter. Journal of neurotrauma 2002;19(3):335–42. [PubMed: 11939501]
19. Dykes MH. Evaluation of a muscle relaxant: dantrolene sodium (Dantrium). Jama 1975;231(8):862–4. [PubMed: 1089167]
20. Lietman PS, Haslam RH, Walcher JR. Pharmacology of dantrolene sodium in children. Archives of physical medicine and rehabilitation 1974;55(8):388–92. [PubMed: 4853611]
21. Meyler WJ, Mols-Thurkow HW, Wesseling H. Relationship between plasma concentration and effect of dantrolene sodium in man. European journal of clinical pharmacology 1979;16(3):203–9. [PubMed: 499321]
22. Flewellen EH, Nelson TE, Jones WP, Arens JF, Wagner DL. Dantrolene dose response in awake man: implications for management of malignant hyperthermia. Anesthesiology 1983;59(4):275–80. [PubMed: 6614536]
23. Ellis KO, Wessels FL. Muscle relaxant properties of the identified metabolites of dantrolene. Naunyn-Schmiedeberg's archives of pharmacology 1978;301(3):237–40.
24. Faling LJ, Petusevsky ML, Snider GL. Nitrofurantoin and dantrolene; liver and lung. Annals of internal medicine 1980;93(1):151. [PubMed: 7396303]
25. Chan CH. Dantrolene sodium and hepatic injury. Neurology 1990;40(9):1427–32. [PubMed: 2392230]
26. Durham JA, Gandolfi AJ, Bentley JB. Hepatotoxicological evaluation of dantrolene sodium. Drug and chemical toxicology 1984;7(1):23–40. [PubMed: 6723545]
27. Yamagishi F, Komoda T, Ohnishi K, Itoh S. Protective effect of dantrolene sodium on carbon tetrachloride induced liver injury in the rat. Research communications in chemical pathology and pharmacology 1993;82(2):237–40. [PubMed: 8303092]
28. Sorensen EM, Acosta D. Comparison of dantrolene sodium with erythromycin estolate using primary cultures of rat hepatocytes. Drug and chemical toxicology 1985;8(4):219–37. [PubMed: 4075997]
29. Petusevsky ML, Faling LJ, Rocklin RE, et al. Pleuropericardial reaction to treatment with dantrolene. Jama 1979;242(25):2772–4. [PubMed: 501885]
30. Mahoney JM, Bachtel MD. Pleural effusion associated with chronic dantrolene administration. The Annals of pharmacotherapy 1994;28(5):587–9. [PubMed: 8068995]

31. Felz MW, Haviland-Foley DJ. Eosinophilic pleural effusion due to dantrolene: resolution with steroid therapy. Southern medical journal 2001;94(5):502–4. [PubMed: 11372801]
32. Saltzman LS, Kates RA, Corke BC, Norfleet EA, Heath KR. Hyperkalemia and cardiovascular collapse after verapamil and dantrolene administration in swine. Anesth Analg 1984;63(5):473–8. [PubMed: 6711841]
33. Freysz M, Timour Q, Bernaud C, Bertrix L, Faucon G. Cardiac implications of amlodipine-dantrolene combinations. Canadian journal of anaesthesia = Journal canadien d'anesthesie 1996;43(1):50–5.
34. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. Journal of neurochemistry 1984;43(5):1369–74. [PubMed: 6149259]
35. Small DL, Buchan AM. Mechanisms of cerebral ischemia: intracellular cascades and therapeutic interventions. Journal of cardiothoracic and vascular anesthesia 1996;10(1):139–46. [PubMed: 8634379]
36. Kristian T, Siesjo BK. Calcium in ischemic cell death. Stroke 1998;29(3):705–18. [PubMed: 9506616]
37. Somlyo AP, Bond M, Somlyo AV. Calcium content of mitochondria and endoplasmic reticulum in liver frozen rapidly in vivo. Nature 1985;314(6012):622–5. [PubMed: 3990795]
38. Carafoli E. Intracellular calcium homeostasis. Annual review of biochemistry 1987;56:395–433.
39. Kohno K, Higuchi T, Ohta S, Kohno K, Kumon Y, Sakaki S. Neuroprotective nitric oxide synthase inhibitor reduces intracellular calcium accumulation following transient global ischemia in the gerbil. Neuroscience letters 1997;224(1):17–20. [PubMed: 9132680]
40. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nature reviews 2003;4(7):552–65.
41. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes & development 1999;13(10):1211–33. [PubMed: 10346810]
42. Welihinda AA, Tirasophon W, Kaufman RJ. The cellular response to protein misfolding in the endoplasmic reticulum. Gene expression 1999;7(46):293–300. [PubMed: 10440230]
43. Syntichaki P, Tavernarakis N. The biochemistry of neuronal necrosis: rogue biology? Nature reviews 2003;4(8):672–84.
44. Duzenli S, Bakuridze K, Gepdiremen A. The effects of ruthenium red, dantrolene and nimodipine, alone or in combination, in NMDA induced neurotoxicity of cerebellar granular cell culture of rats. Toxicol In Vitro 2005;19(5):589–94. [PubMed: 15896552]
45. Bouchelouche P, Belhage B, Frandsen A, Drejer J, Schousboe A. Glutamate receptor activation in cultured cerebellar granule cells increases cytosolic free Ca2+ by mobilization of cellular Ca2+ and activation of Ca2+ influx. Experimental brain research Experimentelle Hirnforschung 1989;76(2): 281–91.
46. Simpson PB, Challiss RA, Nahorski SR. Involvement of intracellular stores in the Ca2+ responses to N-Methyl-D-aspartate and depolarization in cerebellar granule cells. Journal of neurochemistry 1993;61(2):760–3. [PubMed: 8336152]
47. Massote PD, Pinheiro AC, Fonseca CG, et al. Protective Effect of Retinal Ischemia by Blockers of Voltage-dependent Calcium Channels and Intracellular Calcium Stores. Cell Mol Neurobiol. 2008
48. Frandsen A, Schousboe A. Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate and kainate in cultured cerebral cortical neurons. Proceedings of the National Academy of Sciences of the United States of America 1992;89(7):2590–4. [PubMed: 1372982]
49. Lei SZ, Zhang D, Abele AE, Lipton SA. Blockade of NMDA receptor-mediated mobilization of intracellular Ca2+ prevents neurotoxicity. Brain research 1992;598(12):196–202. [PubMed: 1486480]
50. Segal M, Manor D. Confocal microscopic imaging of [Ca2+]i in cultured rat hippocampal neurons following exposure to N-methyl-D-aspartate. The Journal of physiology 1992;448:655–76. [PubMed: 1534370]

51. Nakayama R, Yano T, Ushijima K, Abe E, Terasaki H. Effects of dantrolene on extracellular glutamate concentration and neuronal death in the rat hippocampal CA1 region subjected to transient ischemia. Anesthesiology 2002;96(3):705–10. [PubMed: 11873048]
52. Eliasson MJ, Sampei K, Mandir AS, et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nature medicine 1997;3(10):1089–95.
53. Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. Science 1994;263(5147):687–9. [PubMed: 8080500]
54. Tasker RC, Sahota SK, Cotter FE, Williams SR. Early postischemic dantrolene-induced amelioration of poly(ADP-ribose) polymerase-related bioenergetic failure in neonatal rat brain slices. J Cereb Blood Flow Metab 1998;18(12):1346–56. [PubMed: 9850147]
55. Moubarak RS, Yuste VJ, Artus C, et al. Sequential Activation of Poly(ADP-Ribose) Polymerase 1, Calpains, and Bax Is Essential in Apoptosis-Inducing Factor-Mediated Programmed Necrosis. Molecular and cellular biology 2007;27(13):4844–62. [PubMed: 17470554]
56. Nath R, Raser KJ, Hajimohammadreza I, Wang KK. Thapsigargin induces apoptosis in SH-SY5Y neuroblastoma cells and cerebrocortical cultures. Biochemistry and molecular biology international 1997;43(1):197–205. [PubMed: 9315298]
57. Guo Q, Sopher BL, Furukawa K, et al. Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid beta-peptide: involvement of calcium and oxyradicals. J Neurosci 1997;17(11):4212–22. [PubMed: 9151738]
58. Wei H, Leeds P, Chen RW, et al. Neuronal apoptosis induced by pharmacological concentrations of 3-hydroxykynurenine: characterization and protection by dantrolene and Bcl-2 overexpression. Journal of neurochemistry 2000;75(1):81–90. [PubMed: 10854250]
59. Kim BC, Kim HT, Mamura M, Ambudkar IS, Choi KS, Kim SJ. Tumor necrosis factor induces apoptosis in hepatoma cells by increasing Ca(2+) release from the endoplasmic reticulum and suppressing Bcl-2 expression. The Journal of biological chemistry 2002;277(35):31381–9. [PubMed: 12077131]
60. Popescu BO, Oprica M, Sajin M, et al. Dantrolene protects neurons against kainic acid induced apoptosis in vitro and in vivo. Journal of cellular and molecular medicine 2002;6(4):555–69. [PubMed: 12611640]
61. Gwak M, Park P, Kim K, et al. The effects of dantrolene on hypoxic-ischemic injury in the neonatal rat brain. Anesth Analg 2008;106(1):227–33. [PubMed: 18165582]table of contents
62. Gupta S. Molecular signaling in death receptor and mitochondrial pathways of apoptosis (Review). International journal of oncology 2003;22(1):15–20. [PubMed: 12469180]
63. Bucholz J, Tsai H, Duckles SP. Intracellular calcium release induced by caffeine is buffered by mitochondria in superior cervical ganglion cells. FASEB J 1996;10:A 142.
64. Hermes-Lima M. How do Ca2+ and 5-aminolevulinic acid-derived oxyradicals promote injury to isolated mitochondria? Free radical biology & medicine 1995;19(3):381–90. [PubMed: 7557553]
65. Saunders R, Szymczyk KH, Shapiro IM, Adams CS. Matrix regulation of skeletal cell apoptosis III: Mechanism of ion pair-induced apoptosis. J Cell Biochem. 2006
66. Hayashi T, Kagaya A, Motohashi N, Yamawaki S. Possible mechanism of dantrolene stabilization of cultured neuroblastoma cell plasma membranes. Journal of neurochemistry 1994;63(5):1849–54. [PubMed: 7931341]
67. Bronner C, Wiggins C, Monte D, et al. Compound 48/80 is a potent inhibitor of phospholipase C and a dual modulator of phospholipase A2 from human platelet. Biochimica et biophysica acta 1987;920 (3):301–5. [PubMed: 3607084]
68. Li F, Hayashi T, Jin G, et al. The protective effect of dantrolene on ischemic neuronal cell death is associated with reduced expression of endoplasmic reticulum stress markers. Brain research 2005;1048(12):59–68. [PubMed: 15921666]
69. Xu K, Tavernarakis N, Driscoll M. Necrotic cell death in C. elegans requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. Neuron 2001;31(6): 957–71. [PubMed: 11580896]
70. Guo Q, Fu W, Sopher BL, et al. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nature medicine 1999;5(1):101–6.

71. Mattson MP, Zhu H, Yu J, Kindy MS. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. J Neurosci 2000;20(4):1358–64. [PubMed: 10662826]
72. Zucchi R, Yu G, Ghelardoni S, Ronca F, Ronca-Testoni S. A3 adenosine receptor stimulation modulates sarcoplasmic reticulum Ca(2+) release in rat heart. Cardiovascular research 2001;50(1): 56–64. [PubMed: 11282078]
73. Yu G, Zucchi R, Ronca-Testoni S, Ronca G. Protection of ischemic rat heart by dantrolene, an antagonist of the sarcoplasmic reticulum calcium release channel. Basic research in cardiology 2000;95(2):137–43. [PubMed: 10826506]
74. Lopez-Neblina F, Toledo-Pereyra LH, Toledo AH, Walsh J. Ryanodine receptor antagonism protects the ischemic liver and modulates TNF-alpha and IL-10. The Journal of surgical research 2007;140 (1):121–8. [PubMed: 17359999]
75. Husain SZ, Prasad P, Grant WM, Kolodecik TR, Nathanson MH, Gorelick FS. The ryanodine receptor mediates early zymogen activation in pancreatitis. Proceedings of the National Academy of Sciences of the United States of America 2005;102(40):14386–91. [PubMed: 16186498]
76. Kross J, Fleischer JE, Milde JH, Gronert GA. No dantrolene protection in a dog model of complete cerebral ischaemia. Neurological research 1993;15(1):37–40. [PubMed: 8098851]
77. Martinez-Sanchez M, Striggow F, Schroder UH, Kahlert S, Reymann KG, Reiser G. Na(+) and Ca (2+) homeostasis pathways, cell death and protection after oxygen-glucose-deprivation in organotypic hippocampal slice cultures. Neuroscience 2004;128(4):729–40. [PubMed: 15464281]
78. Kim JH, Kim SH, Min KTM, Kim MH, Song SK, Lee BH. Comparison of the Cerebral Protective Effects of Thiopental, Propofol and Dantrolene on Focal Cerebral Ischemia Induced by Temporary Middle Cerebral Artery Occlusion in the Rat Under the Monitoring of Compressed Spectral Array. J Korean Neurosurg Soc 2002;32:239–45.
79. Mitani A, Yanase H, Sakai K, Wake Y, Kataoka K. Origin of intracellular Ca2+ elevation induced by in vitro ischemia-like condition in hippocampal slices. Brain research 1993;601(12):103–10. [PubMed: 8431758]
80. Wang C, Nguyen HN, Maguire JL, Perry DC. Role of intracellular calcium stores in cell death from oxygen-glucose deprivation in a neuronal cell line. J Cereb Blood Flow Metab 2002;22(2):206–14. [PubMed: 11823718]
81. Zhang L, Andou Y, Masuda S, Mitani A, Kataoka K. Dantrolene protects against ischemic, delayed neuronal death in gerbil brain. Neuroscience letters 1993;158(1):105–8. [PubMed: 8233063]
82. Niebauer M, Gruenthal M. Neuroprotective effects of early vs. late administration of dantrolene in experimental status epilepticus. Neuropharmacology 1999;38(9):1343–8. [PubMed: 10471088]
83. GepdIremen A, Duzenl IS, Hacimuftuoglu A, Suleyman H, Oztas S. The effects of dantrolene alone or in combination with nimodipine in glutamate-induced neurotoxicity in cerebellar granular cell cultures of rat pups. Pharmacol Res 2001;43(3):241–4. [PubMed: 11401415]
84. Tizzano JP, Griffey KI, Johnson JA, Fix AS, Helton DR, Schoepp DD. Intracerebral 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) produces limbic seizures that are not blocked by ionotropic glutamate receptor antagonists. Neuroscience letters 1993;162(12):12–6. [PubMed: 8121613]
85. Borowicz KK, Gasior M, Kleinrok Z, Czuczwar SJ. Influence of isradipine, niguldipine and dantrolene on the anticonvulsive action of conventional antiepileptics in mice. European journal of pharmacology 1997;323(1):45–51. [PubMed: 9105875]
86. Wuis EW, Rijntjes NV, Van der Kleijn E. Whole-body autoradiography of 14C-dantrolene in the marmoset monkey. Pharmacology & toxicology 1989;64(1):156–8. [PubMed: 2502774]
87. Enokizono J, Kusuhara H, Ose A, Schinkel AH, Sugiyama Y. Quantitative investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) in limiting brain and testis penetration of xenobiotic compounds. Drug metabolism and disposition: the biological fate of chemicals 2008;36(6):995–1002. [PubMed: 18322075]
88. Bostrom E, Hammarlund-Udenaes M, Simonsson US. Blood-brain barrier transport helps to explain discrepancies in in vivo potency between oxycodone and morphine. Anesthesiology 2008;108(3): 495–505. [PubMed: 18292687]

89. Meyler WJ, Bakker H, Kok JJ, Agoston S, Wesseling H. The effect of dantrolene sodium in relation to blood levels in spastic patients after prolonged administration. Journal of neurology, neurosurgery, and psychiatry 1981;44(4):334–9.

90. Nisijima K, Ishiguro T. Does dantrolene influence central dopamine and serotonin metabolism in the neuroleptic malignant syndrome? A retrospective study. Biological psychiatry 1993;33(1):45–8. [PubMed: 7678377]

91. Shuaib A, Lees KR, Lyden P, et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007;357(6):562–71. [PubMed: 17687131]

92. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117(4):e25–146. [PubMed: 18086926]

93. Nakayama K, Suzuki S, Sugi H. Physiological and ultrastructural studies on the mechanism of stretch-induced contractile activation in rabbit cerebral artery smooth muscle. The Japanese journal of physiology 1986;36(4):745–60. [PubMed: 3784149]

94. Sims JR, Salomone S. Dantrolene inhibits serotonin and endothelin-1 vasoconstriction in the rat basilar artery. Neurocritical care 2007;6(3):267–A107.

95. Call GK, Fleming MC, Sealfon S, Levine H, Kistler JP, Fisher CM. Reversible cerebral segmental vasoconstriction. Stroke 1988;19(9):1159–70. [PubMed: 3046073]

96. Calabrese LH, Dodick DW, Schwedt TJ, Singhal AB. Narrative review: reversible cerebral vasoconstriction syndromes. Annals of internal medicine 2007;146(1):34–44. [PubMed: 17200220]

97. Dietrich WD. The importance of brain temperature in cerebral injury. Journal of neurotrauma 1992;9 (Suppl 2):S475–85. [PubMed: 1613807]

98. Azzimondi G, Bassein L, Nonino F, et al. Fever in acute stroke worsens prognosis. A prospective study. Stroke 1995;26(11):2040–3. [PubMed: 7482646]

99. Reith J, Jorgensen HS, Pedersen PM, et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. Lancet 1996;347(8999):422–5. [PubMed: 8618482]

100. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. Stroke 1998;29(2):529–34. [PubMed: 9472901]

101. Fernandez A, Schmidt JM, Claassen J, et al. Fever after subarachnoid hemorrhage. Risk factors and impact on outcome. Neurology. 2007

102. Wartenberg KE, Schmidt JM, Claassen J, et al. Impact of medical complications on outcome after subarachnoid hemorrhage. Crit Care Med 2006;34(3):617–23. [PubMed: 16521258]quiz 24

103. Lopez M, Sessler DI, Walter K, Emerick T, Ozaki M. Rate and gender dependence of the sweating, vasoconstriction, and shivering thresholds in humans. Anesthesiology 1994;80(4):780–8. [PubMed: 8024131]

104. Ralley FE, Wynands JE, Ramsay JG, Carli F, MacSullivan R. The effects of shivering on oxygen consumption and carbon dioxide production in patients rewarming from hypothermic cardiopulmonary bypass. Canadian journal of anaesthesia = Journal canadien d'anesthesie 1988;35 (4):332–7.

105. Horvath SM, Spurr GB, Hutt BK, Hamilton LH. Metabolic cost of shivering. Journal of applied physiology 1956;8(6):595–602. [PubMed: 13331842]

106. Modica PA, Tempelhoff R, White PF. Pro- and anticonvulsant effects of anesthetics (Part II). Anesth Analg 1990;70(4):433–44. [PubMed: 2180345]

107. Buggy D, Higgins P, Moran C, O'Donovan F, McCarroll M. Clonidine at induction reduces shivering after general anaesthesia. Canadian journal of anaesthesia = Journal canadien d'anesthesie 1997;44 (3):263–7.

108. Bicer C, Esmaoglu A, Akin A, Boyaci A. Dexmedetomidine and meperidine prevent postanaesthetic shivering. Eur J Anaesthesiol 2006;23(2):149–53. [PubMed: 16426470]

109. Nath A, Padua RA, Geiger JD. HIV-1 coat protein gp120-induced increases in levels of intrasynaptosomal calcium. Brain research 1995;678(12):200–6. [PubMed: 7620888]

110. Berg M, Bruhn T, Frandsen A, Schousboe A, Diemer NH. Kainic acid-induced seizures and brain damage in the rat: role of calcium homeostasis. Journal of neuroscience research 1995;40(5):641–6. [PubMed: 7602615]

111. Nagatomo I, Hashiguchi W, Tominaga M, Akasaki Y, Uchida M, Takigawa M. Effects of MK-801, dantrolene, and FK506 on convulsive seizures and brain nitric oxide production in seizure-susceptible EL mice. Brain research 2001;888(2):306–10. [PubMed: 11150489]

112. Pisani A, Calabresi P, Tozzi A, D'Angelo V, Bernardi G. L-type Ca2+ channel blockers attenuate electrical changes and Ca2+ rise induced by oxygen/glucose deprivation in cortical neurons. Stroke 1998;29(1):196–201. [PubMed: 9445351]discussion 2

113. Phillis JW, Diaz FG, O'Regan MH, Pilitsis JG. Effects of immunosuppressants, calcineurin inhibition, and blockade of endoplasmic reticulum calcium channels on free fatty acid efflux from the ischemic/reperfused rat cerebral cortex. Brain research 2002;957(1):12–24. [PubMed: 12443975]

114. Yano T, Nakayama R, Imaizumi T, Terasaki H, Ushijima K. Dantrolene ameliorates delayed cell death and concomitant DNA fragmentation in the rat hippocampal CA1 neurons subjected to mild ischemia. Resuscitation 2001;50(1):117–25. [PubMed: 11719138]

115. Yoon KW, Mitchell HL, Broder LD, Brooker RW, Delisle RK. Trauma-induced neurotoxicity in rat hippocampal neurons. Stroke 1996;27(1):122–6. [PubMed: 8553387]

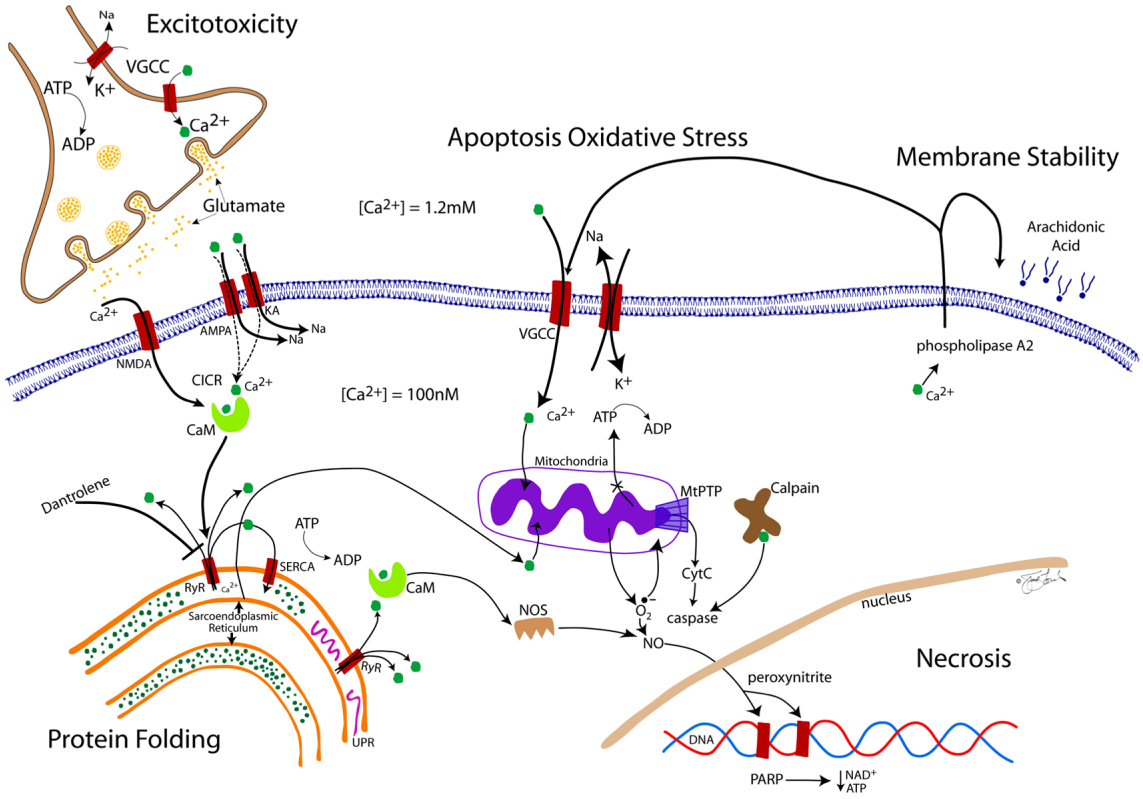

FIGURE. The role of calcium in cellular injury

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 sarcoendoplasmatic 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 sarcoendoplasmatic 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 peroxinitrite, 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 sarcoendoplasmatic reticulum, and thereby inhibiting further increase in intracellular Ca^{2+}-levels.

TABLE

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.

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
Excitotoxicity (NMDA)	Cortical neuron culture	at the time of injury	30 μM	iCa2+ reduced by 30% in presence of extracellular Ca2+ and 100% in absence of extracellular Ca2+; toxic effect of glutamate entirely prevented	7
Excitotoxicity (Glutamate, NMDA, QA, KA, AMPA)	Cortical neuron culture	at the time of injury	30 μM	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 μM 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 μM	Dantrolene inhibited glutamate induced Ca^{2+}-release	45
Excitotoxicity (NMDA)	Cerebellar granule neuron culture	at the time of injury	5-30 μM	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 μM	iCa^{2+} decreased by 56%; cell toxicity reduced by 50%	49
Excitotoxicity (NMDA)	Hippocampal neuron culture	10-20 min prior	10 μM	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 μM	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 μM	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 μM)	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 μM)	early administration reduced injury in all areas of hippocampus, late	82

Injury	Model	Timing of Dantrolene Relative to Injury	Dose of Dantrolene	Findings	Citation
				administration reduced injury in CA3 region	
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 μM)	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 μM)	Seizure suppression; no influence on NO levels at any dose	111
Epilepsy	Hippocampal slice, rat	n/a	10-100 μM	Dose-dependent suppression of pilocarpine- and DHPG-induced ictal activity	17
Epilepsy	Entorhinal cortex and hippocampus slice, rat	30 min prior and during	30 μM	complete cell death prevention after 1, 3, or 12-14hrs; no effect on synaptic transmission or epileptic discharges	l6
OGD(0-15min)	intracellular recordings in cortical slice, rat	15 min prior	10-30 μM	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 μM	No effect	77
OGD(0-10min)	Hippocampal slice, gerbil	60 min prior and during	50 μM	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 μM	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 μM	early but not late energy failure reduced	54
OGD (45 min)	Retinal cell culture, rat	30 min prior and during	100 μM	ganglion cell death prevented; 54% reduction of dead cells in the retinal layer	47
OGD (48 hrs)	Cortical neuron cell culture with presenlin-1 expression	60 min prior	10 μM	80% of neurons rescued	71
Forebrain ischemia (20 min)	4 vessel occlusion, rat	15 min prior	20 μM topical	Reduction of free fatty acids from ischemia and reperfusion	113
Forebrain ischemia (3 min)	Gerbil	30 or 120 min after	6μ1 of 1.6 mM intracerebroventricular (approx 10 μM 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
			solution (approx 2.5 μM in brain)		
Forebrain ischemia (5 min)	4 vessel occlusion, rat	15 min after followed by infusion for 3 days	33 μg intracerebroventricular (approx 50 μM) followed by 40 μg/day (approx 60 μM)	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 μg intracerebroventricular (approx 50 μM)	40% more viable neurons in CA1 at 7 days.	51
Focal ischemia (90 min)	Middle cerebral artery occlusion, rat	Immediately after	20 μg intracerebroventricular (approx 30 μM)	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 μM)	No effect	78
Global cerebral ischemia (11 min)	Dog	Immediately prior	4mg/kg intravenous (approx 16 μM)	No effect	76
Apoptosis (*in vitro*); global ischemia (*in vivo*)	GT1-7 hypothalamic neurosecretory cells (*in vitro*), gerbil (*in vivo*)	30min prior (*in vitro*), Immediately after (*in vivo*)	120 and 240 μM (*in vitro*); 10, 25, and 50 mg/kg intravenous (approx 40-200 μM)	Dose-dependent reduction of iCa^{2+}, DNA-fragmentation and cell death (*in vitro*). dose-dependent increase of CA1 cells at 7 days (*in vivo*); 50mg/kg was toxic.	15
Apoptosis	PC12 cells	120 min prior	1 μM	Suppression of peroxide accumulation and protection against oxidative stress	57
Apoptosis (*in vitro*) / status epilepticus (*in vivo*)	Cerebellar granule neuron culture (*in vitro*) and rat (*in vivo*)	Simultaneous (*in vitro*), 60 min prior (*in vivo*)	10 μM (*in vitro*); 10 mg/kg i.p. (approx 40 μM, *in vivo*)	cell death and apoptosis decreased at 3h and 24h (*in vitro*). CA1 region and cerebral cortex protected against apoptosis at 24hrs (*in vivo*)	60
Apoptosis (*in vivo*)	Hypoxic-ischemic neonatal rat brain	Immediately after	1 mM intracerebroventricular	Lip/Cr ratio decreased (^{1}H 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 μM	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 μM	Electrophysiological recovery improved 1h after injury	18
Trauma	Hippocampal neuron culture	Simultaneously	30 μM and 100 μM	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; $iCa2^{+}$, 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.