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Considerations for the Use of Anesthetics in Neurotoxicity Studies

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Anesthetics are widely used in experiments investigating neurotoxicity and neuroprotection; however, these agents are known to interfere with the outcome of these experiments. The purpose of this overview is to review these effects and suggest methods for minimizing unintended consequences on experimental outcomes. Information on the neuroprotective and neurotoxic effects of isoflurane, dexmedetomidine, propofol, ketamine, barbiturates, halothane, xenon, carbon dioxide, and nitrous oxide is summarized. The pertinent cell signaling pathways of these agents are discussed. Methods of humane animal euthanasia without anesthetics are considered. Most anesthetics alter the processes of neuronal survival and death. When designing survival surgeries, sham controls subjected to anesthesia but not the surgical intervention should be compared with controls subjected to neither anesthesia nor surgery. Additional controls could include using an anesthetic with a different mechanism of action from the primary anesthetic used. Because the effects of anesthetics lessen with time after surgery, survival surgeries should include later time points until at least 7 d after the procedure. Humane methods of animal euthanasia that do not require anesthetics exist and should be used whenever appropriate.

Abbreviations: Bcl2, B cell lymphoma 2 protein; CA1, cornu ammonis 1 region; ERK, extracellular regulated kinase; $GABA_A$, γ-amino butyric acid A receptor; NMDAR, N-methyl D-aspartate receptor; NR, NMDAR subunit; OGD, oxygen–glucose deprivation.

Numerous molecules, including anesthetics, have been examined for their neuroprotective action, although some studies report neurotoxicity of certain anesthetic agents.[12,34,36,55] Anesthetic agents are widely and appropriately used in the laboratory setting for the humane treatment of animals. However, at times investigators fail to give due consideration to the possibility that these agents may alter the outcome of the experiment. Numerous studies show that anesthetics alter the processes being studied in neurotoxicity experiments.[1,2,8,10,15,21,25,28,30,32,33,35,61,62,64] In experimental models that use cell viability as an endpoint, anesthetics might interfere with the measurement of the outcome parameter. Anesthetics act through many of the same intracellular pathways involved in neurotoxicity and neuroprotection.[12] The purpose of this overview is to summarize the neuroprotective and neurotoxic effects of anesthetics and to suggest ways of managing their use in laboratory animal research.

For obvious humane reasons, research animals are treated with anesthetic agent(s) during common surgical methods of inducing ischemia. Depending on the method, anesthesia may be continued for part or all of the procedure. Clearly any effect of the anesthetic on the processes being studied is important, and sham surgical controls are used to account in part for these undesired effects. Another useful strategy is to allow the animal to serve as its own control by comparing the ipsilateral to contralateral hemisphere in animals exposed to unilateral ischemia. This approach allows the direct effect of the ischemia to be differentiated from nonspecific activation of stress responses or individual genetic variations. To truly test the effects of the anesthetic, these controls should be compared with animals not subjected to either anesthesia or surgery.

Often animals undergo surgical implantation of monitoring devices (such as arterial catheters or intracranial pressure monitors) a day or more before surgical ischemia. Although seemingly unrelated to the subsequent experimental paradigm, anesthesia used in these preparative procedures may produce an unintended effect known as preconditioning.[66] In preconditioning, a subthreshold or subtoxic treatment induces a cascade of neuroprotective mechanisms that lessen the effect of future neurotoxic insults; the preconditioning effect may last from 24 h to as long as 2 wk after the stimulus.[43] Obviously, a preconditioning stimulus may interfere with experimental outcomes and can be detrimental to animal studies involving neuronal death and survival. In any experiment in which surgical procedures must be done prior to the primary intervention, animals should be allowed to recover for 1 to 2 wk before proceeding.

Animals often are euthanized under anesthesia prior to analysis of the effects of neurotoxic insults or in the determination of neuroprotective properties of a test drug. Although present in the animal's system for only a short interval before death, an anesthetic may cause effects that persist in the tissue after circulation ceases, thereby potentially affecting cellular characteristics and processes. Appropriate and humane methods of euthanasia that do not require anesthesia, such as microwave irradiation, cervical dislocation or rapid decapitation,[3] are sometimes available and should be used whenever possible.

Clearly a thorough appreciation of the effects of anesthetics on neurotoxic or neuroprotective processes and information on how anesthesia may affect experimental outcome is essential for designing effective animal studies. Strategies to minimize

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unwanted influences of anesthetics are important. The anesthetic agents most commonly used in laboratory and clinical studies for which information on neuroprotective or neurotoxic effects exist are isoflurane, dexmedetomidine, propofol, ketamine, barbiturates, halothane, xenon, carbon dioxide (CO_2), and nitrous oxide (N_2O). Urethane, sevoflurane, fentanyl, chloral hydrate, and lidocaine are used less frequently and are less well-studied in regard to neurotoxicity and therefore are beyond the scope of this article. The following sections comprise a discussion of the neuroprotective or neurotoxic effects of each of the commonly used anesthetics.

Isoflurane. Isoflurane has been widely studied for its neuroprotective properties, and there is general agreement regarding its mechanisms of neuroprotection. Its anesthetic effect is thought to be mediated by antagonizing the N-methyl D-aspartate receptor (NMDAR) or by potentiating the γ-aminobutyric acid A receptor ($GABA_A$).[9] Activation of NMDAR requires coagonist binding of glycine at the NMDAR subunit (NR) 1 and glutamate binding at NR2.[16] Isoflurane inhibits the NR1 glycine binding site in NR1–NR2A and NR1–NR2B receptors transfected in HEK293T cells, explaining its NMDAR-mediated action.[16] Activation of $GABA_A$ or inhibition of NMDAR may provide a mechanism for potential neuroprotection by isoflurane.[9]

Isoflurane neuroprotection occurs through both preconditioning and effects caused at the time of surgery. Preconditioning effects have been studied primarily with in vitro model systems, such as primary culture and brain slices. In rat primary cortical neurons, isoflurane preconditioning (2.4% isoflurane for 1 h beginning 4 h before onset of insult) protected against subsequent isoflurane-mediated toxicity (2.4% isoflurane for 24 h).[64] Another study showed preconditioning in hippocampal slice cultures: 1.5% isoflurane for 2 h beginning 24 h before onset of oxygen glucose deprivation (OGD; an in vitro stroke model) reduced neuronal death in the cornu ammonis 1 (CA1) region for as long as 72 h after insult.[10] Clearly isoflurane can serve as a preconditioning agent.

In addition, isoflurane administered at the time of insult protects neurons. Although this effect has been observed in several different studies, discrepancy exists regarding the duration of protection. In a study using an organotypic hippocampal slice culture model of hypoxic injury, 1% isoflurane (administered during the insult) reduced neuronal injury after 60 min hypoxia in the CA1 and dentate gyrus regions of the hippocampus and induced phosphorylation of extracellular regulated kinase (ERK, an important player in the cell survival cascade).[21] In a model of rat focal cerebral ischemia, isoflurane was neuroprotective in samples collected until day 4 after ischemic injury, but this effect did not persist in samples assessed on day 7 after injury.[33] In another study of rat focal cerebral ischemia, a combination of isoflurane and a caspase inhibitor decreased cerebral injury and improved neurologic outcome measured on day 14 after injury.[28] Other experiments showed that isoflurane delays but does not prevent neuronal injury in rats subjected to focal ischemia.[35]

Several intracellular mechanisms for isoflurane neuroprotection have been proposed. Isoflurane's preconditioning effects may be mediated by inducible nitric oxide synthase,[65] hypoxia-inducible factor 1α, and ERK1/2[38] and by action on adenosine receptor A1.[39] When cultured rat hippocampal neurons were treated for 2 h with isoflurane and subjected to 2 h OGD, isoflurane decreased both neuronal injury and release of lactate dehydrogenase (a marker of cell death) in a dose-dependent manner.[38] Isoflurane preconditioning increased protein levels of hypoxia inducible factor 1α and subsequent inducible nitric oxide synthase mRNA.[38] In addition, isoflurane increased phosphoERK levels.[38]

When administered at the time of injury, isoflurane may protect by blocking NMDAR or potentiating GABA receptors. In rat hippocampal slices exposed to OGD or glutamate, isoflurane exhibited neuroprotective properties similar to those of an NMDAR antagonist.[49] A $GABA_A$ antagonist, bicuculline, lessened isoflurane-mediated neuroprotection in rat hippocampal slices subjected to OGD.[9] Isoflurane can increase cerebral blood volume and intracranial pressure, which might affect experimental results in neurotoxicity studies.[5,54] In summary, there is compelling evidence that isoflurane administered either before or at the time of neurotoxic insult affects neuronal viability. Isoflurane must be used with caution in experimental stroke models.

Dexmedetomidine. Dexmedetomidine is an α2-adrenergic receptor agonist that has been developed for human clinical use as an anesthetic and sedative. Its neuroprotective effects are thought to be related both to its agonism at α2 adrenergic receptors and to its binding at imidazoline 1 and 2 receptors.[41] Dexmedetomidine reduced lactate dehydrogenase release from mouse cortical neuronal cultures exposed to OGD.[41] In the same study, a combination of xenon and dexmedetomidine reduced infarct area and improved neurologic scores in rats subjected to focal ischemia.[41] Dexmedetomidine reduced isoflurane induced toxicity in neonatal rats as measured by caspase 3 immunostaining.[51] In that study, isoflurane impaired cognitive function in the experimental rats, whereas dexmedetomidine restored it. Dexmedetomidine also was found to reduce the neuronal injury induced by focal cerebral ischemia in rabbits.[42]

Several studies have focused on the mechanisms underlying dexmedetomidine neuroprotection. Dexmedetomidine increased B cell lymphoma 2 protein (Bcl2, an antiapoptotic protein) and reduced Bcl2-associated protein (a proapoptotic protein) in the hippocampus of adult, male Sprague–Dawley rats that underwent incomplete cerebral ischemia.[17] This anesthetic also decreased the levels of cleaved caspase 3 (an indicator of apoptosis) and reduced propidium iodide fluorescence (a measure of cell death) in hippocampal slices exposed to OGD.[14] Dexmedetomidine increased the basal levels of phosphorylated ERK1/2 by means of an α2-adrenergic receptor-independent mechanism, given that the α2-adrenergic antagonist yohimbine failed to prevent this increase in pERK1/2 after dexmedetomidine treatment. However, efaroxan (an α2-adrenergic receptor and imidazoline 1 receptor antagonist) blocked the dexmedetomidine-induced phosphorylation of ERK1/2. This result suggests that dexmedetomidine acts on the imidazoline 1 receptor to induce phosphorylated ERK1/2 and indicates that the neuroprotective actions of dexmedetomidine may require imidazoline 1 receptors.[13] In contrast, dexmedetomidine was shown to reduce cortical and white matter lesions induced by the excitotoxin ibotenate in α2C-adrenergic receptor knockout mice but not in α2A-adrenergic receptor knockout mice. This finding suggests that dexmedetomidine requires α2A-adrenergic receptors to be an effective neuroprotective agent.[48] Therefore, although data are somewhat contradictory, dexmedetomidine clearly affects neuronal viability and should be used with caution in light of its ability to affect the experimental outcome of studies involving neuroprotection.

Propofol. As with many anesthetics, the exact mechanism of action of propofol is not completely known. Several reports indicate that the anesthetic effects of propofol are mediated by potentiation of the $GABA_A$ receptor.[1] $GABA_A$ receptors allow chloride ion influx into the cell upon opening of the channel pore. This action leads to cell membrane hyperpolarization and prevention of further action potential propagation. In addition, propofol modulates NMDAR through phosphorylation of the NR1 subunit, which is associated with activation of NMDAR, in a dose-dependent manner.

Data relevant to neuroprotective effects of propofol are contradictory. Propofol lessened calcium-induced mitochondrial swelling (an indicator of apoptosis).[2] It also decreased propidium iodide staining in the CA1 region of hippocampal slices exposed to OGD[2] and reduced infarct size after middle cerebral artery occlusion in rats.[2] These data suggest that propofol is neuroprotective. In contrast, another study of rat hippocampal slices exposed to NMDA or glutamate showed that propofol was not neuroprotective in the CA1 region or dentate gyrus.[19] However, propofol did protect against OGD in the dentate gyrus but not the CA1 in this same study.[19]

Propofol affects many of the intracellular signaling pathways involved in cell death and survival, especially those related to NMDAR. NMDAR-mediated ERK phosphorylation and subsequent activation is important in synaptic plasticity and cell survival. Propofol at low doses (10 µM) inhibited long-term potentiation (a measure of synaptic plasticity) but at high doses (30 µM) completely blocked this parameter.[47] Propofol also alters intracellular mediators downstream of the NMDAR. Propofol increased Bcl2 protein (an antiapoptotic protein) and decreased Bcl2-associated protein (a proapoptotic protein) on day 3 after cerebral ischemia and reperfusion in the hippocampus.[18] Therefore, although some of the data are contradictory, it is clear that propofol modulates $GABA_A$ and NMDAR and affects downstream neuroprotective processes. For these reasons, the use of propofol as an anesthetic agent may alter the outcome of experiments involving the study of cell death and survival mechanisms.

Ketamine. Ketamine produces anesthesia by acting as an antagonist at NMDAR through blockage of the channel pore and by inhibiting the phencyclidine binding site.[25] In addition ketamine interacts with µ, κ, and δ opioid receptors.[25] At high doses, ketamine even blocks sodium channels.[25]

Several studies have shown that ketamine produces neurotoxicity. In rat and monkey cortical cultures, 10 µM ketamine increased DNA fragmentation (seen in apoptosis) but not release of lactate dehydrogenase (a well-established marker for necrotic cell death), suggesting that ketamine caused neuronal death specifically through apoptosis.[61,62] Other investigators demonstrated cell death after 100 µM ketamine treatment by using morphologic evidence of apoptosis as well as DNA fragmentation;[56] apoptosis in response to ketamine treatment was time-dependent. In contrast, other studies have shown neuroprotection with ketamine.[7,43,53] In acute striatal slices prepared from adult rats and exposed to OGD, 10 µM and 100 µM ketamine decreased lactate dehydrogenase release.[7] Primary cortical neuronal cultures from fetal rats showed dose-dependent protection by ketamine (5 to 50 µM) from NMDA treatment.[53] When given ketamine, gerbils subjected to global cerebral ischemia demonstrated dose-dependent improvements in motor function 24 h after ischemia. These ketamine-treated animals also displayed dose-dependent improvement in histopathologic scores of the hippocampal CA1 region 15 to 17 d after ischemia.[43] Several recent reviews have proposed that ketamine be investigated for neuroprotective potential in human clinical practice.[24,27]

As an NMDAR blocker, the potential neuroprotective effects of ketamine are associated with decreased calcium influx in the presence of excessive extracellular glutamate concentrations (that is, ischemia).[24] In contrast, a working mechanistic model of ketamine-induced neuronal death has been described as follows: ketamine blocks NMDAR, causing upregulation of NMDAR (as a compensatory mechanism). Therefore after exposure to ketamine, the release of normal amounts of endogenous glutamate results in increased NMDAR activation because of the increased numbers of receptors present, leading to pronounced calcium influx into the cell. Increased intracellular calcium results in neuronal death.[25] This model is supported by the observation that coadministration of NR1 antisense oligonucleotide blocked ketamine-induced neuronal death.[61,62] In another study, ketamine decreased activation of protein kinase B in a dose-dependent manner, which would increase the activity of glycogen synthase kinase 3 and lead to increased apoptosis.[56] Supporting this mechanism, insulin-like growth factor 1 (which increases protein kinase B activity) blocked ketamine-induced apoptosis.[56] In addition, glycogen synthase kinase 3β inhibitors prevented ketamine-induced apoptosis.[56] Therefore, although the role of ketamine as a neurotoxic or neuroprotective agent is controversial, a large body of evidence suggests that ketamine affects the NMDAR as well as neuronal fate after exposure to neurotoxic insults. Whenever possible, ketamine exposure in experimental stroke models should be avoided.

Barbiturates. Barbiturates are known to mediate their anesthetic action by acting as agonists at the $GABA_A$ receptor.[37] They have been shown to increase the open-channel time of $GABA_A$ and the probability of $GABA_A$ channel opening.[37] Although little work specifically addresses this issue, the effects on the $GABA_A$ channel are likely to be important in the mechanism behind the neuroprotective effects of barbiturates.

Pentobarbital (50 µg/mL) reduces cell death induced by serum-deprivation in PC12 cells.[46] A combination of 5 to 10 µM ketamine and 50 µM thiopental enhances the survival rate of cortical neurons cultured from embryonic day 16 Wistar rats.[53] In another similar study, cultured cortical neurons subjected to hypoxia for 24 h showed maximal survival after exposure to a combination of mild hypothermia (32 °C) and thiopental (at 40 and 400 µM).[59]

In vivo studies also have demonstrated neuroprotection with barbiturates. A study in gerbils reported that pentobarbital (50 mg/kg IP) given 30 min before global ischemia was neuroprotective, as measured by the preservation of CA1 hippocampal neuronal area on days 7 and 14 after ischemia.[29] Another study showed that pentobarbital reduced infarct volume in rats subjected to focal cerebral ischemia.[63] Thiopental protected against necrosis caused by the NMDAR antagonist MK801 in the posterior cingulate–retrosplenial cortex of rats.[31] Therefore, designing appropriate controls is essential when using barbiturates in experiments determining cell viability as the endpoint.

Halothane. Halothane has a long history of use as an anesthetic and currently is widely used in developing countries. Due to its hepatotoxic and cardiodepressant side effects, this anesthetic is used only rarely in developed countries. Halothane reduces nonNMDAR-mediated and NMDAR-mediated excitatory postsynaptic currents in pyramidal cells and interneurons of the CA1 hippocampal region. Its anesthetic action is thought to be via this

mechanism. Only a few studies have addressed halothane's neuroprotective actions, and we are unaware of any studies that have focused on the mechanisms through which halothane produces its neuroprotective effects.

Several studies suggest that halothane is neuroprotective. Halothane preconditioning (4% induction and 1.2% maintenance for 3 h) reduced cerebral infarct volume from middle cerebral artery occlusion measured 4 d after injury.[32] Short-term (less than 1 h) halothane treatment reduced cerebral infarct volume when compared with short-term propofol treatment after 2 h of middle cerebral artery occlusion.[8] One study found that electrical stimulation led to severe neuronal loss in the CA1 region of the hippocampus; this loss was prevented by administration of halothane (1% to 2%) during the stimulation. Halothane thus is established as a neuroprotective molecule, either when used as a preconditioning agent or when used during the neurotoxic insult. Halothane can increase cerebral blood volume and intracranial pressure, and these responses could also affect experimental results in neurotoxicity studies.[4,54] Caution must be applied when using halothane in any stroke experimental model.

Xenon. Although used in the 1940s as an anesthetic, xenon anesthesia currently is not available in the United States. Its attributes as a neuroprotective agent have begun to be appreciated, and several multicenter trials have favorably evaluated its clinical use in humans.[50,58] Xenon competitively inhibits the glycine site of NMDAR, thereby preventing activation of the receptor. This activity is most likely the mechanism by which xenon acts as an anesthetic.

Several studies have shown neuroprotective effects of xenon. Xenon preconditioning for 2 h prior to onset of OGD reduced both release of lactate dehydrogenase and propidium iodide staining in the CA1 and dentate gyrus regions of the hippocampus.[40] In a rat hypoxia ischemia model (right common carotid artery ligation), 2-h xenon preconditioning reduced infarct area and improved motor function scores.[40] The same researchers found that xenon improved gross pathophysiologic outcome 4 d after insult as well as neurologic scores 30 d after insult.[40] In another study, xenon improved neurobehavioral function and reduced infarct volume after middle cerebral artery occlusion.[26]

One study suggests a mechanism through which xenon might produce its neuroprotective effects. When given during OGD, 70% xenon upregulated Bcl2 protein and brain-derived neurotrophic factor, both well known prosurvival factors.[40] In conclusion, ample evidence indicates that xenon interferes in processes leading to cell death and its use may alter findings in animal stroke models.

Carbon dioxide. CO_2 is used frequently in small rodent anesthesia and euthanasia. Although not specifically studied as a neuroprotective or neurotoxic agent, CO_2 exerts noteworthy effects on these processes. One study demonstrated that CO_2 euthanasia increased brain glutamate levels postmortem.[20] In addition, anesthetic levels of CO_2 caused hemorrhage[23] and acidosis in the brain.[44] In some studies, anesthetic levels of CO_2 have been used for inducing stress[6] or as a pain stimulus,[57] suggesting that this agent alters brain metabolism associated with stress and pain, possibly through neurosteroids and cytokines. However, evidence to the contrary exists, which shows that CO_2 causes no inhumane distress in animals either at anesthetic or lethal doses.[22] Therefore, although no studies directly evaluate the neuroprotective or neurotoxic effects of CO_2, evidence suggests a potential for altering these processes. For these reasons, CO_2 should be avoided for use in experiments involving cell viability as the end-point parameter.

Nitrous oxide. N_2O has been used as an anesthetic in dental surgery since the 1800s and is commonly known as 'laughing gas.'[30] Like many anesthetics, N_2O is thought to work by antagonizing NMDAR as it inhibits NDMAR-mediated currents. N_2O likely mediates its anesthetic action by means of mixed competitive and noncompetitive inhibition of NMDAR. In one intriguing study, 3 h of 75% N_2O administered after 90 min of middle cerebral artery occlusion reduced infarct volume.[15] This protective effect of N_2O was restricted to cortical infarction but not striatal infarction. Conversely, N_2O actually can produce hypoxia if insufficient oxygen is given during its delivery (alveolar hypoxia) or during recovery from N_2O anesthesia (diffusion hypoxia).[11] Therefore, N_2O could potentially affect experimental results creating either neuroprotection or neurotoxicity and is best avoided.

Conclusion

As discussed, despite sometimes contradictory results, the commonly used anesthetic agents clearly all have substantial effects on neuronal survival and death. These agents modulate ion channels such as the NMDA and $GABA_A$ receptors and potentially produce effects on downstream signaling molecules, all of which are important in neurotoxicity and cell survival pathways.

How can these interferences be overcome so that laboratory research studies in the areas of neuroprotection and neurotoxicity are not complicated by the use of anesthetic agents? When planning a study involving in vivo cerebral ischemia, the choice of anesthetic must be considered carefully. Sham controls treated with anesthesia but no ischemia as well as control animals given neither anesthesia nor ischemia (and no experimental manipulations) are essential components of study design. The effects of most anesthetics tend to lessen over time as the animal recovers after ischemia, so measuring endpoints as far out as practical may also be helpful. Obviously if the animal is used in an acute-slice study in which there is minimal time for recovery after anesthesia, using no anesthetic prior to euthanasia is ideal.

Although not applicable to surgical procedures, one option for euthanizing animals is to forego anesthetics in favor of rapid nonpharmacologic (or physical) methods of euthanasia. The American Veterinary Medical Association has issued a statement on euthanasia in animals that provides guidance in this regard.[3] In addition to reviewing all anesthetics, the American Veterinary Medical Association also recommends physical means of animal euthanasia: "When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia."[2] Methods most applicable to laboratory research are cervical dislocation, decapitation, and microwave irradiation.

Cervical dislocation is very rapid and can be safely and humanely used in birds, mice, small (less than 200 g) rats, and small (less than 1 kg) rabbits.[3] The method becomes more difficult as the size of the animal increases, and proficiency with the technique should first be demonstrated with anesthetized animals. In a study in which animal stress was measured by brain acetylcholine levels, cervical dislocation induced more stress than decapitation in 6-mo-old adult rats; the authors attributed this difference to increased handling.[60] This result strengthens the idea that cervical dislocation is not a good technique for large rats because of the need for increased handling.

Decapitation can be used in mice, rats (including large rats), and small rabbits.[3] This method should be performed only with a sharp, well-maintained guillotine, and the animals usually need to be restrained in a plastic cone to reduce handling distress. Recent studies have confirmed that rats in the same room in which other rats are decapitated display only a low level of physiologic signs of stress (heart rate and blood pressure elevation), similar in intensity to responses to other innocuous stimuli, such as when investigators enter the room or other animals in the room have a cage change.[52] Therefore it is consistent with humane animal treatment to decapitate an animal in a room with other animals.

Microwave irradiation requires the use of special, commercially designed microwaves (*never* the common kitchen microwave oven).[3] Current instruments are designed only for mice and rats and cannot be used for larger animals or primates. The rodent is restrained in a cylinder with a removable plastic head cone attachment. A short pulse (1.5 to 2 s) of 10-kW (2450 Hz) focused irradiation is applied to the midpoint of the animal's skull. The animal then is decapitated, and the brain is isolated for further processing.[45] One study showed that high-energy microwave irradiation as a means of euthanasia produces high-integrity mRNA similar to the yield obtained by decapitation. In addition, rRNA was substantially degraded by the irradiation procedure, so that most of the RNA obtained was mRNA.[45] Drawbacks to this method are that appropriate microwave instruments are both expensive and unavailable for larger animals, thereby limiting widespread use.

Currently, all anesthetic agents available produce some downstream effect on cell viability, although the mechanisms by which they do this differ. Therefore, to design adequate controls, researchers using cell viability as an end point measurement must be aware of these effects and mechanisms. For example, if studying cellular pathways downstream of NMDAR, an anesthetic such as a barbiturate or α2 agonist, which acts on a receptor other than NMDAR, should be chosen. Another option is to compare 2 anesthetics with different mechanisms within the same study. In addition, nonsurgical controls should be used in addition to sham-operated animals. These nonsurgical controls should be euthanized by decapitation without anesthesia. Effects of anesthetics lessen with time so that by 1 to 2 wk after exposure, many of the differences between anesthetic exposed and nonexposed groups have dissipated. Therefore a late (beyond 2 wk) sample should be included even in studies of early mechanisms of neuroprotective action. Animals should be allowed to recover from any prestudy exposure to anesthesia (for example, implanting central catheters) for at least 1 to 2 wk prior to onset of study. Functional studies are important, even where the primary focus is mechanism of action of a potential neuroprotective drug, because these studies offer the most clinical relevance.

In conclusion, all anesthetics that have been studied have some effect on the cellular pathways. Therefore when studying neurotoxicity and neuroprotection in animal experiments, investigators must carefully choose the methods of anesthesia and euthanasia. In addition to being unacceptably inhumane, animal pain and suffering are likely to have negative effects on experimental pathways due to the effects of stress; therefore, for ethical and practical reasons, it is imperative that pain and suffering are minimized. Obviously, in vivo studies involving survival surgeries require the use of anesthetics. The anesthetic, as well as the dose, should be chosen carefully, and consideration of neuroprotective and neurotoxic effects must be included in the experimental design. However, for studies using in vitro models of ischemia such as primary culture or slice work, the acute effects of anesthesia are less difficult to overcome. For these types of studies, physical methods of animal euthanasia by cervical dislocation, decapitation, or microwave irradiation without anesthesia should be used whenever possible.

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