Lang F (ed): Mechanisms and Significance of Cell Volume Regulation.
Contrib Nephrol. Basel, Karger, 2006, vol 152, pp 221–240

Volume Changes in Neurons: Hyperexcitability and Neuronal Death

Herminia Pasantes-Morales, Karina Tuz

Department of Biophysics, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico

Abstract

Hyponatremia propitiates and increases susceptibility to seizure episodes. In vitro, hyposmolarity induces hyperexcitability and epileptiform activity and increases the amplitude of excitatory postsynaptic potentials. Synaptic (increased glutamate vesicular release) and non-synaptic (swelling-induced extracellular space shrinkage and ephaptic interactions) might be responsible for the hyposmolarity effects on brain excitability. Neuronal volume constancy in hyponatremia is preserved by the isovolumetric regulation, relying importantly on organic osmolytes. Changes in cell volume are closely linked to neuronal death: swelling characterizes necrotic death as in acute ischemic episodes or brain trauma, whereas volume decrease is typical of apoptotic death. Swelling in necrotic death results from the intracellular Na^+ increase followed by Cl^- and water influx. Na^+ accumulation is due initially to the Na^+/K^+ ATPase dysfunction and subsequently from the Na^+ influx through the overactivated ionotropic glutamate receptors. A second wave of swelling generates by excitotoxic derived formation of reactive oxygen species, membrane lipoperoxidation and further ion overload. Excessive swelling contributes to membrane rupture and release of cell debris, propagating the damage to adjacent cells. Apoptotic death is characterized by cell volume decrease termed apoptotic volume decrease, which in neurons seems to occur by mechanisms remarkably similar to those operating in the hyposmotic swelling-activated volume regulatory decrease, i.e. channel-mediated efflux of K^+ and Cl^-. A variety of K^+ channels and the volume-regulated anion channel participate in apoptotic volume decrease. K^+ has a protagonic role as an early element in neuronal apoptosis since a delayed rectifier K^+ current IK_{DR} is enhanced by apoptosis prior to the caspase activation, increased extracellular K^+ and IK_{DR} blockers attenuate apoptosis and intracellular K^+ loss through ionophores induces apoptosis. Volume-regulated anion channel participates as well in the Cl^- efflux although its role and hierarchy in the apoptotic program are not well defined. Efflux of organic osmolytes, such as taurine participate as well in apoptotic volume decrease.

Swelling in hyposmotic or isosmotic conditions is closely linked to ion movements. Regulatory volume decrease (RVD) activated by hyposmotic swelling relies on the outflow of K^+, Cl^- and small organic osmolytes. Swelling in isosmotic conditions occurs by ion redistribution, modifying the ionic homeostasis in the cell. All these changes are of crucial importance in brain, because the extracellular/intracellular ionic equilibrium determines the resting potential and the discharge pattern of neurons, as excitatory and inhibitory synaptic events are driven by ionic gradients. Besides, some of the organic osmolytes released in connection with volume recovery play in the brain a prominent role as synaptic transmitters. Moreover, changes in cell volume, swelling or shrinkage, may be critical signals in directing the cell death type to necrosis or apoptosis.

Brain Cell Volume and Hyperexcitability

The influence of changes in cell volume on brain excitability was suggested by the seizure occurrence during acute hyponatremia, and the increased susceptibility to seizures in chronic hyponatremia or psychotic polydipsia. That the hyperexcitable condition was due to cell swelling and not to changes in the Na^+ concentration in plasma, was demonstrated by seizure attenuation after infusion of hypertonic solutions or by water restriction. At the cellular level, also hyposmolarity induces hyperexcitability and increases evoked epileptiform activity as shown in CA3 neurons of hippocampal slices and in neocortical pyramidal neurons [Rosen and Andrew, 1990; Saly and Andrew, 1993]. Hyposmolarity also affects excitatory synaptic transmission, increasing the amplitude of excitatory postsynaptic potentials. These effects may result from events connected with cell swelling and volume recovery, occurring in both neurons and astrocytes. Cell swelling may increase brain excitability by one or both of these factors: (i) swelling-induced release of excitatory neurotransmitters, notably glutamate, (ii) reduction in the size of the extracellular space, propitiating ephaptic interactions and restraining the diffusion of neurotransmitters and depolarizing agents [Schwartzkroin et al., 1998].

The first possibility, i.e. a swelling-evoked release of excitatory neurotransmitters is documented in a variety of brain preparations [de la Paz et al., 2002; Franco et al., 2001; Kimelberg et al., 1990; Saransaari and Oja, 1999]. Interestingly, the glutamate efflux in hippocampal slices has features which deviate from the release of typical organic osmolytes represented by taurine. The efflux of taurine inactivates slowly, is sensitive to the Cl^- channel blockers NPPB and niflumic acid and is markedly reduced by tyrosine kinase blockers. Glutamate efflux, in contrast, is rapidly inactivated and is insensitive to all the agents which affected taurine, being only decreased by DIDS [Franco et al., 2001].

Hyposmotic efflux of taurine and glutamate is also observed in rat cortical isolated nerve endings (synaptosomes) [Tuz et al., 2004] (fig. 1a, b). In the case of glutamate, the release results from a chain of events triggered by hyposmolarity and ultimately leading to an increase in synaptic vesicle discharge. The initial event is a Na^+-dependent depolarization, sensitive to La^{3+}, Gd^{3+} and ruthenium red, mediated possibly by TRP channels. A subtype of this family of channels, the TRPV4, is almost exclusively present in the nervous tissue, it is osmotically- and mechano-sensitive and is blocked by Gd^{3+}, La^{3+} and ruthenium red [Gunthorpe et al., 2002]. Depolarization is followed by a Na^+-dependent, La^{3+} sensitive, PKC-modulated $[Ca^{2+}]_i$ rise, originated mostly from internal stores. The mitochondrial Ca^{2+} pool released by activation of the mitochondrial Na^+/Ca^{2+} exchanger, as result of the increase in cytosolic Na^+ has an important contribution. $[Ca^{2+}]_i$ rise evoked by hyposmolarity has been reported in cerebellar granule neurons and in hippocampal pyramidal neurons, with contributions of extracellular Ca^{2+} as well as of Ca^{2+} released from internal sources [Borgdorff et al., 2000; Pasantes-Morales and Morales-Mulia, 2000]. Depolarization and $[Ca^{2+}]_i$ rise evoked by hyposmolarity leads to enhanced exocytosis in the nerve endings, which is Ca^{2+}-dependent and prevented by tetanus toxin (TeTX) (fig. 1). This vesicular release is the mechanism responsible for a fraction of the glutamate release from nerve endings, which accordingly, is La^{3+}- and Ca^{2+}-dependent, PKC-modulated and blocked by TeTX. Another fraction of this release occurs via the reversal operation of the carrier and is consequently suppressed by the transporter blockers [Tuz et al., 2004]. Hyposmolarity also increases the efflux of taurine and at a lesser extent, that of GABA (fig. 1b). Noteworthy, the hyposmotic release of taurine does not occur via the Ca^{2+}-dependent vesicular release but it has the features of the volume-activated diffusion pathway, characteristic of the organic osmolyte outflow found in most cell types, i.e. reduced by Cl^- channel blockers and modulated by tyrosine kinase phosphorylation [Tuz et al., 2004]. The hyposmolarity-induced vesicular synaptic release of glutamate from nerve endings may explain in part, the increase in amplitude of spontaneous or evoked excitatory postsynaptic potentials found in CA3 cells in the hippocampus and in neocortical pyramidal neurons. Since hyposmolarity has no effect on the neuronal intrinsic properties such as resting membrane potential, cell input resistance, action potential threshold and duration, its effect on the excitatory postsynaptic potentials must reflect a synaptic phenomenon [Baraban and Schwartzkroin, 1998; Rosen and Andrew, 1990; Saly and Andrew, 1993].

The effects evoked by hyposmolarity resulting ultimately in enhanced exocytosis predict a more general effect of this condition on neurotransmitter release, even when the transmitter is unrelated to an osmolyte function. This was confirmed by data showing a hyposmolarity-evoked norepinephrine

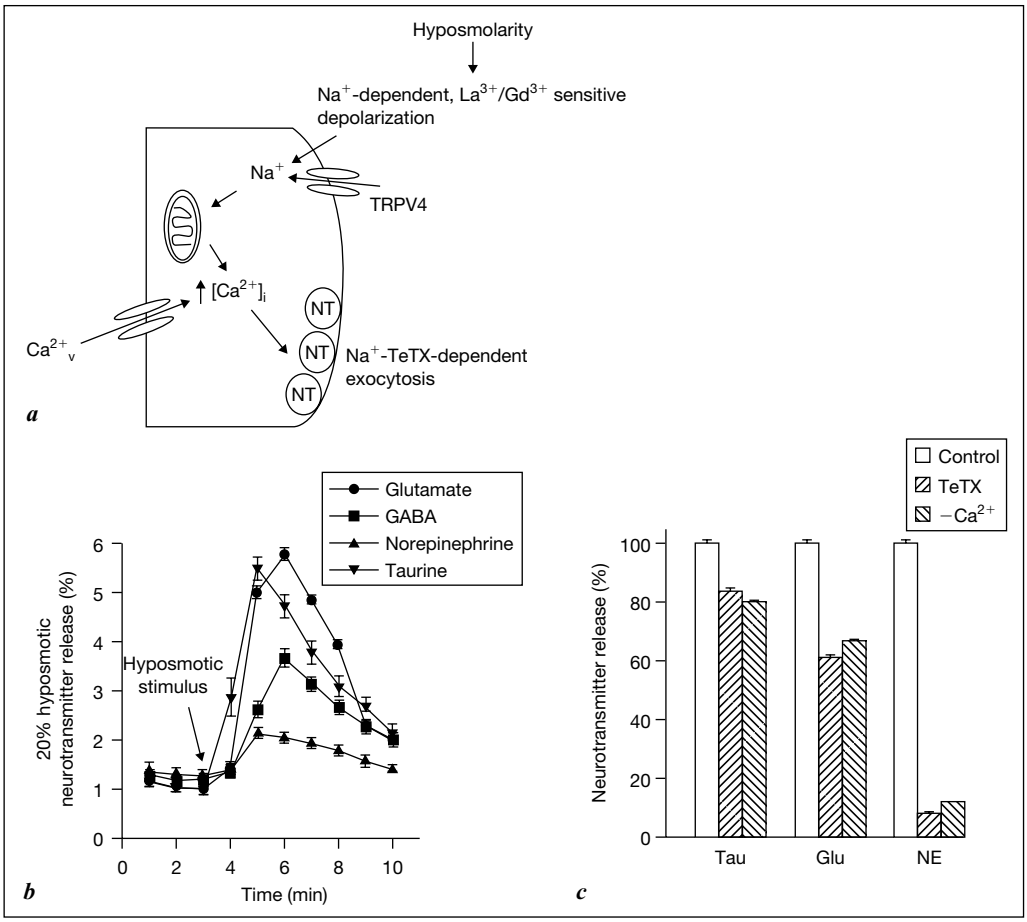

Fig. 1. Hyposmolarity and neurotransmitter release. ***a*** Events sequence of the hyposmolarity-induced release of neurotransmitters from isolated nerve endings (synaptosomes). The initial event is a Na^+-dependent, La^{3+}/Gd^{3+} sensitive depolarization, followed by $[Ca^{2+}]_i$ increase from external and internal sources and activation of a PKC-dependent exocytotic release of neurotransmitters. ***b*** Time course of neurotransmitter release evoked by hyposmolarity from isolated nerve endings. Synaptosomes were prepared and loaded with ^{3}H-glutamate (●), ^{3}H-GABA (■), ^{3}H-norepinephrine (▲) or ^{3}H-taurine (▼), washed and superfused (1 ml/min) during 3 min with isosmotic medium to obtain a constant basal efflux. At the arrow, the medium was replaced by 20% hyposmotic medium, and superfusion continued for 7 min. Results (radioactivity released per min) are expressed as percentage of the total radioactivity incorporated. SE is represented as vertical bars when they exceed the size of symbols. ***c*** Effect of EGTA-AM and TeTX on hyposmotic neurotransmitter release from synaptosomes obtained and treated as in (***b***). Bars represent the radioactivity released (%) at the peak release fractions (5–9). Empty bars represent net release (hyposmotic minus isosmotic release) as 100%. Dashed bars correspond to percentage decrease by EGTA-AM or TeTX.

release from isolated nerve endings, which is fully dependent on depolarization, $[Ca^{2+}]_i$ rise and exocytosis [Tuz and Pasantes-Morales, 2005] (fig. 1c). In comparison with the norepinephrine release evoked by depolarizing concentration of K^+, the hyposmotic norepinephrine efflux is more dependent on intracellular than on extracellular sources of $[Ca^{2+}]_i$ rise and is insensitive to blockers of the voltage-dependent L-type Ca^{2+} channels (fig. 1). Altogether these results confirm that despite the differences in the mechanism to induce depolarization and the source of $[Ca^{2+}]_i$ increase, the synaptic events elicited by hyposmolarity result at the end, on a TeTX-sensitive mechanism not different from the vesicular exocytosis characteristic of the classical release of neurotransmitters. This opens the intriguing possibility of a modulatory effect of changes in cell volume in synaptic transmission.

Swelling may affect neuronal excitability also by non-synaptic mechanisms. Swelling of neurons, but particularly of astrocytes, leads to narrowing of the extracellular space. Astrocyte swelling occurs either in hyponatremia or during clearance of the high extracellular K^+ resulting from intense neuronal activity. As cells expand, there is a reduction in the size of the extracellular space, enhancing ephaptic interactions. This is likely in the origin of the non-synaptic mechanisms of the hypersynchronous behavior of cortical neurons typical of seizures and of the synchronization of epileptiform activity. The influence of cell swelling and the mirrored extracellular space decrease may be of particular importance in brain regions such as the hippocampus where the tight packing of the cell somata restricts the size of the extracellular space. In line with this notion, hyperosmotic solutions block the high K^+-induced epileptiform activity [Dudek et al., 1998] and furosemide suppresses the neuronal synchronized activity generated by episodes of electrically evoked afterdischarges by reducing astrocyte swelling and extracellular space shrinkage [Hochman et al., 1995].

Neuronal Protection in Hyponatremia

Exposure of cultured neurons, astrocytes or brain slices to hyposmotic solutions has been a current experimental device to simulate hyponatremia in vivo. The typical response characterized by rapid swelling followed by return towards the original volume, i.e. the RVD, observed when cells are exposed to

Synaptosomes were 15 min preincubated with 50 μM EGTA-AM in Ca^{2+} free medium containing 0.1 mM EGTA + 10 mM $MgCl_2$, or during 90 min in isosmotic medium in the presence of 50 nM TeTX. Other experimental details in Tuz et al. [2004, 2005]. Glu = Glutamate, NE = norepinephrine, tau = taurine.

sudden and large osmolarity reductions, has been useful to magnify the process and facilitate the identification of signals and mechanisms responsible for this adaptive cell response. However, changes of this magnitude probably never occur in brain under physiological conditions nor even in pathological situations. When the external osmolarity is gradually reduced (−2.2 mOsm/l) some cells do not swell and others swell less than after an abrupt decrease in osmolarity. This is what was named isovolumetric regulation by Lohr and Grantham [1986] who first showed this phenomenon in renal proximal tubule cells. Exposed to gradual changes in osmolarity, renal cells were able to maintain a constant volume within a broad range of osmolarities. The term 'isovolumetric regulation' reflects the active nature of this process, as the unchanged volume is not due to the absence of swelling, but to a continuous volume adjustment accomplished by the extrusion of intracellular osmolytes. This paradigm has been applied to other cell types and marked differences have been found with respect to the efficiency of the process. Interestingly, these differences appear to be related to the contribution of amino acids.

Cerebellar granule neurons, as renal cells, respond to the gradual decrease in external osmolarity by a constancy in cell volume even if osmolarity reductions reached up to 50% [Tuz et al., 2001] (fig. 2a). Glioma C6 cells and cultured astrocytes exhibit some swelling, but significantly lower that if an osmotic stimulus of the same magnitude is suddenly imposed [Ordaz et al., 2004a; 2004b] (fig. 2a). Finally, trout erythrocytes respond with similar swelling to gradual or sudden exposure to hyposmotic solutions [Godart et al., 1999].

The osmolytes involved in volume corrective mechanisms during isovolumetric regulation are the same as in RVD, i.e. K^+, Cl^- and organic molecules. The activation threshold of the osmolyte fluxes appears related to the efficiency of the different cell types to counteract the changes in external osmolarity. A correlation is observed between the efflux threshold of taurine and glutamate and the extent of swelling during gradual osmolarity changes. In cerebellar granule neurons, which show the typical isovolumetric regulation, the efflux of taurine and glutamate activates very early after the hyposmolarity reduction, as early as 2% for taurine [Tuz et al., 2001]. In astrocytes and C6 cells the efflux is delayed up to 15 and 39% for taurine and glutamate, respectively [Ordaz et al., 2004a; 2004b], and in trout erythrocytes which do not exhibit isovolumetric regulation, the efflux of taurine occurs in low amounts and is very delayed [Godart et al., 1999]. The higher ability of neurons as compared to astrocytes to resist to changes in external osmolarity, which seems based primarily on the contribution of organic osmolytes, may represent a protective mechanism to spare neurons from the deleterious consequences of swelling. In line with this notion is the interesting observation by Nagelhus et al. [1993] in the cerebellum

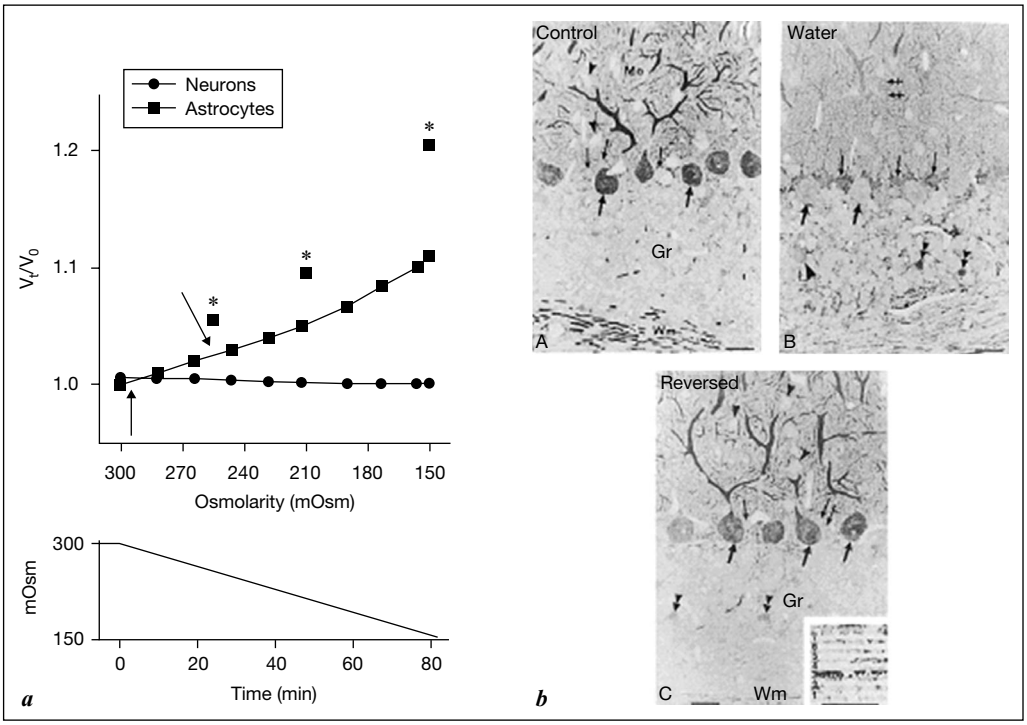

Fig. 2. ***a*** Cell volume constancy i.e. isovolumetric regulation, in cultured cerebellar granule neurons (●) exposed to small and gradual changes in osmolarity (−1.8 mOsm/min). The same treatment in astrocytes (■) increases cell volume but not at the extent observed when the osmotic change is suddenly imposed (■*). For experimental details see Tuz et al. [2001] and Ordaz et al. [2004a, b]. ***b*** Redistribution of taurine-like immunoreactivity in rat cerebellar cortex following water loading. Details of the experiment are in Nagelhus et al. [1993]. In control, isosmotic conditions, taurine is found accumulated in Purkinje cells (large arrows). Upon water loading taurine is transferred to the adjacent glial elements (arrows) which contain very low taurine in isosmotic conditions. As consequence of this redistribution, astrocytes swell while neurons are spared. The original distribution is restored after hyposmolarity correction. Reproduced from Naghelhus et al. [1993], with permission.

of water loaded rats. In the isosmotic condition, the cerebellar Purkinje cells contain high concentrations of taurine, while the nearby astrocytes contain essentially no taurine. In response to the osmolarity reduction there is a remarkable change in the location of taurine: all taurine is lost from the Purkinje cells and is then accumulated in astrocytes (fig. 2b). As consequence of this redistribution, astrocyte swell and neurons are spared. This notable protective action of astrocytes suggests the importance that for neuronal function may have the

maintenance of cell volume, not only in the soma but also in dendrites, axons and nerve endings. Special mechanisms developed for preserving intact the cytoarchitecture of neurons appear to involve importantly organic osmolytes.

Swelling and Necrotic Neuronal Death

Brain cell swelling occurs during pathological conditions such as ischemia, epilepsies, trauma and hepatic encephalopathy. Vasogenic as well as cellular edema are found coincidently in these pathologies. Cellular swelling occurs also in pathologies associated with hyponatremia. Hyposmotic swelling results from a decrease in the external osmolarity and the subsequent water flow tending to establish a new osmotic equilibrium. Isosmotic (cytotoxic) swelling is generated by redistribution of ions or molecules responding to phenomena inherent to the pathology, such as the energetic failure and dissipation of Na^+ gradients during hypoxia/ischemia, the increase of extracellular K^+ during ischemia and epilepsies, or the ammonium accumulation during hepatic encephalopathy. Hyposmotic swelling, even if drastic, rarely results in cell death, while cytotoxic swelling commonly ends in excitotoxicity and necrotic death.

It is generally accepted that cytotoxic swelling occurs mainly in astrocytes whereas neurons are less affected. This is in line with the known essential role of astrocytes in protecting neurons from the disturbing effects of changes in the composition of the extracellular space, or from excessive concentration of potentially toxic molecules. Typical examples of this role of astrocytes are the K^+ clearance from the extracellular space by spatial buffering or the glutamate uptake persisting in astrocytes longer than in neurons due to their ability of facing the energy failure by generating ATP via the glycolitic pathway [rev. in Pasantes-Morales and Franco, 2005]. When the pathological conditions are too severe or prolonged, the protective mechanisms in astrocytes are exceeded and neurons could then be affected in several ways, including swelling occurrence.

The mechanisms of swelling-induced necrotic neuronal death will be discussed essentially for the ischemic condition. During trauma, vasogenic is the predominant type of edema, while cellular edema is consequent to the ischemic conditions. Swelling in hepatic encephalopathy affects essentially astrocytes, since ammonia detoxification, which is the primary swelling inductor, occurs only in astrocytes [Butterworth, 2002].

The chain of events responsible for cytotoxic swelling and necrotic cell death in ischemia is initiated by the energy failure and the consequent Na^+/K^+ ATPase dysfunction. The resultant elevation of intracellular Na^+ and extracellular K^+ is followed by depolarization and glutamate release. High extracellular

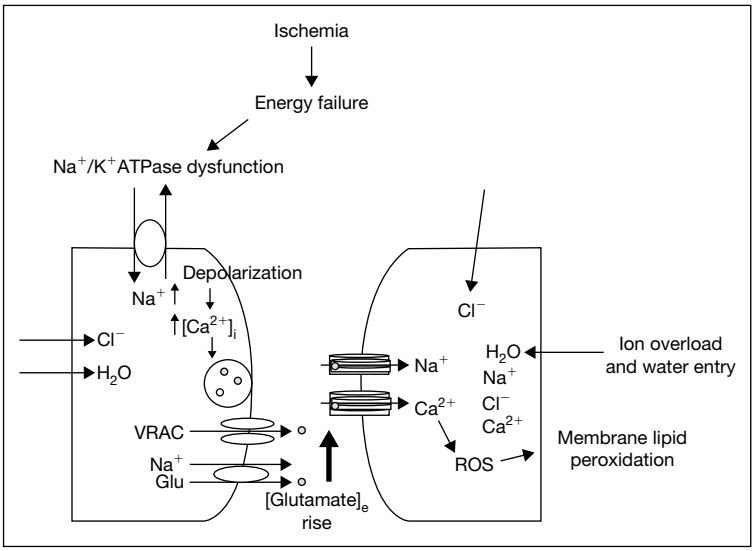

Fig. 3. Swelling inductors in ischemic necrotic neuronal cell death. The energy failure produced by ischemia provokes Na^+/K^+ ATPase dysfunction, intracellular Na^+ increase, and depolarization. This is followed by Ca^{2+}-dependent glutamate release, both from exocytosis, reversal of the transporter and activation of the volume-sensitive Cl^- channel. The overfunction of ionotropic glutamate receptors further increases intracellular Na^+ followed again by Cl and water. The massive Ca^{2+} influx entry promotes production of ROS, membrane lipid peroxidation and a new wave of swelling due to ion overload through the injured membranes. Excessive swelling contributes to membrane rupture and release of cell debris, propagating the damage to adjacent cells.

glutamate levels resulting from depolarization cannot be removed by the Na^+-driven glutamate transporters since the Na^+ gradient is dissipated. The transporters may even operate in a reverse mode, further increasing extracellular glutamate, and overfunction of ionotropic glutamate receptors leads to massive Na^+ influx. Then, Cl^- and water influx driven by the intracellular high concentration of Na^+ generates a first wave of cytotoxic swelling. Further glutamate release via the swelling-activated glutamate efflux pathway also contributes to the increase and persistence of glutamate in the extracellular space (fig. 3). A second wave of swelling occurs as consequence of Ca^{2+} influx through the ionotropic glutamate receptors. This $[Ca^{2+}]_i$ rise produces reactive oxygen species (ROS) through the activation of prooxidant mechanisms, such as phospholipases, xantine oxidase and nitric oxid synthase. The free fatty acid release and the generation of several free radical species affects membrane integrity and favors ion overload and a further wave of Na^+ and Ca^{2+} increase (fig. 3). There is almost general agreement on that elevation of $[Ca^{2+}]_i$ is the primary

cause of excitotoxic neuronal death, mediated by proteases of the family of calpains and cathepsins. At this state, swelling contributes to necrotic neuronal death propagation propitiating plasma membrane rupture and cell lysis. The release of cellular content and necrotic debris to the extracellular space precipitates damage to cells in the vicinity, via the chain of events involving ROS, membrane lipid peroxidation and Na^+ and Ca^{2+} overload through the affected membranes.

Lactacidosis generated in ischemia and trauma is another factor of swelling and neuronal death. Lactate is formed during the operation of the glycolitic pathway in astrocytes, which provides an alternate source of energy as far as there is residual glucose. Cytotoxic swelling by lactacidosis occurs preferentially in astrocytes but there is evidence of neuronal swelling in vivo as well in cultured neurons exposed to extracellular lactate levels as those present in ischemia [Alojado et al., 1996; Staub et al., 1993]. Swelling is a function of the severity of acidosis and duration of the exposure, although not all neurons appear to be equally susceptible [Cronberg et al., 2005].

How neurons respond to cytotoxic swelling has not been examined in detail. Neurons exhibit a complex morphology, with specialized functions in soma, dendrites, axon or nerve terminals. Then, swelling may not be homogeneous and its functional consequences will depend on the affected area. There is no clear evidence of active volume recovery in neurons after cytotoxic swelling, but a low efficiency or inability is predictable, in view of the nature of the swelling inductors above discussed. Cell volume regulation relies on the expulsion of intracellular osmolytes, which occurs in general, via diffusion pathways or channels through which the osmolytes move following the gradient direction. In ischemia and epilepsies, the excessive extracellular K^+ concentration resulting from the Na^+/K^+ ATPase dysfunction and neuronal overexcitability will limit the operation of the volume-activated K^+ channels. The ATP drop impairs also the volume-sensitive Cl^- channel which characteristically requires non-hydrolytic ATP binding. Organic osmolytes also have this ATP requirement [Okada et al., 2006]. In addition, the volume-sensitive Cl^- channel seems impaired by lactacidosis [Mori et al., 2002]. Altogether, these conditions accentuate the difficulty of brain cells, including neurons, to accomplish the set of reactions necessary to restore the normal cell volume in conditions of ischemic cytotoxic swelling.

Cell Volume and Apoptosis in Neurons

Apoptosis is a highly regulated process of cell deletion directed to eliminate a definite group of cells at a precise time, for preserving the optimal

operation of tissues and organs. In the developing brain, apoptosis is an essential process to accomplish the numerical match of neuronal populations and their targets, for organizing the regional cytoarchitecture, and to establish functional synapses and building of neural circuitry. In the adult brain, apoptosis has some contribution to neuronal loss in acute neuropathologies, in some neurodegenerative disorders and in brain aging. Apoptosis occurs according to a strictly ordered set of biochemical events, one of which is a characteristic reduction in cell volume, termed apoptotic volume decrease (AVD).

When the temporary resolution of apoptosis was established, it was clear that AVD is an early event in the program, occurring before the surge of other characteristic traits such as caspase 3 cleavage, cytochrome c release and translocation, endonuclease activation and DNA fragmentation. Moreover, it is now considered that AVD may be part of the causal signals and not only consequence of apoptosis [Bortner and Cidlowski, 2002, 2004]. Noteworthy, AVD is not followed by any compensatory mechanism directed to counteract the cell volume loss. There is no evidence of regulatory volume increase, which is apparently inhibited or overridden by an as yet unknown signal [Bortner and Cidlowsly, 2004]. Although AVD occurs in isosmotic conditions, it relies on the same mechanisms of RVD, i.e. the active translocation of intracellular osmolytes and osmotically obligated water flow. K^+ and Cl^- are the main intracellular osmotically active solutes and are natural candidates to accomplish AVD. Some organic molecules also participate.

K^+ Efflux and AVD in Neurons, K^+ Channels Involved

The importance of K^+ efflux and AVD as part of the apoptotic signaling chain is now well established, and there is reasonable agreement on that K^+ outflow occurs via K^+ channels [Bortner and Cidlowski, 2004; Burg et al., 2006; Yu, 2003]. Studies in mice cultured cortical neurons undergoing apoptosis by treatment with staurosporin or ceramide have shown AVD, K^+ loss and the selective and early enhancement of a voltage-dependent delayed rectifier current (IK_{DR}) in the apoptotic neurons. Decreasing cell K^+ by the ionophores valinomycin and beauvericin induced apoptosis and accordingly, high external K^+ levels suppressed apoptosis. The antiapoptotic effect of increasing external K^+ was Ca^{2+}- and caspase-independent [Yu et al., 1997, 1999]. IK_{DR} blockade by tetraethyl ammonium or clofilium also attenuates apoptosis. These results suggest an early location of K^+ efflux and AVD in the apoptotic signaling chain in mice cortical neurons. However, a study in rat cortical neurons, showed that staurosporine-induced apoptosis is not reduced by tetraethyl ammonium, clofilium or high external K^+ but it is prevented by SITS [Small et al., 2002]. These differences may be due to the higher concentration of staurosporine used in this study, which may induce both apoptosis and necrosis.

Cerebellar granule neurons require for survival a depolarizing environment provided by 25 mM K^+ or NMDA receptor agonists. Cell death prevention in this case depends on the Ca^{2+} influx through voltage-gated channels [Alavez et al., 2003]. In conditions of low K^+, cells die by apoptosis which occurs concomitant to the increase of an outwardly rectifying K^+ current named standing outward K^+ current (IK_{so}) [Lauritzen et al., 2003].

Apoptosis is induced by staurosporine in neuronal progenitor cells generated from mice striatal stem cells [Hribar et al., 2004]. Similar to mice cultured cortical neurons, a delayed rectifier K^+ current already expressed in the neuronal precursors, is enhanced by staurosporine since the first days of differentiation. IK_{DR} enhancement precedes the activation of caspase 3 and increasing external K^+ reduced IK_{DR} and attenuates apoptosis. These results are similar to those found in cortical neurons but in the neuronal progenitor cells the amplitude of IK_{DR} is reduced by caspase blockers.

Apoptotic cell death concurs with necrotic cell death in some neurodegenerative disorders as well as in ischemia and trauma. Necrotic neuronal death predominates at the ischemic focus while apoptotic cell death prevails in the perifocal area. Apoptosis is triggered and sustained by multiple factors concurrent with the ischemic condition: acidosis, increased expression of death receptors or proapoptotic molecules, activation of MAP kinases and generation of nitric oxid and peroxynitrite. Activation of apoptosis directly by nitric oxide in mice cortical neurons led to IK_{DR} enhancement, K^+ outflow and cell K^+ loss, all resistant to the general caspase blocker zVAD [Bossy-Wetzel, 2004]. The same set of reactions occurs in cortical neurons in a thiol-oxidant model of apoptosis [Aizenman et al., 2000]. The β-amyloid peptide linked to Alzheimer disease, enhances an outward K^+ current in cortical neurons in conjunction with apoptosis [Yu et al., 1998]. In cerebellar Purkinje cells, apoptosis associated to lurcher gene occurs after activation of a voltage-gated channel, suggesting the involvement of K^+ in the apoptotic program in this neurodegenerative condition [Norman et al., 1995].

The molecular identity of the ADV-linked K^+ channels is now extensively investigated and from the diversity of cell types examined, including neurons, an important point emerged: there is apparently not a specific channel, devoted exclusively to permeate K^+ for reducing cell volume during apoptosis. This function seems accomplished by K^+ channels present in the non-apoptotic cell, and performing a variety of tasks in the physiological condition. The type of apoptosis-activated K^+ channel may be cell specific and could be different according to the apoptotic inductor. K^+ channels linked to AVD and apoptosis include: Kv channels (isoforms Kv1.1, Kv1.3, Kv1.5, Kv2.1), K2P channels (TASK-1 and TASK-3), HERG and BK channels. The K_{ATP} channels are involved in the intrinsic mid-and late phase of apoptosis [Yu, 2003]. Kv channels

are those linked to AVD in cortical neurons. These channels fit an important requirement for the K^+ exit route i.e. large K^+ conductance and slow inactivation during depolarization. The Kv2.1 isoform carries a significant fraction of the enhanced IK_{DR} in apoptotic cortical neurons. This is concluded by the effect of two dominant negative mutant forms of Kv2.1 decreasing IK_{DR}, and complementary, the apoptosis attenuation in neurons deficient in functional Kv2.1 [Pal et al., 2003]. In cultured cerebellar granule neurons, the standing outward K^+ current (IK_{so}) which increases with cell maturation in the culture is suggested as the apoptotic K^+ exit route. IK_{so} has biophysical, pharmacological and regulation properties characteristic of the TASK-1 and TASK-3 2P-domain K^+ channels (K2P) [Lauritzen et al., 2003]. The following evidence connects IK_{so} and K2P channels to apoptosis: (i) young cerebellar neurons lacking IK_{so} are resistant to low K^+-induced cell death, (ii) conditions or agents decreasing K2P prevent apoposis in mature neurons, (iii) apoptosis occurs in hippocampal neurons lacking IK_{so} after viral-induced expression of TASK-1 or TASK-3 channels (iiii) inactivation of endogenous TASK channels by expression of the dominant-negative loss of function TASK mutants, protects neurons from the low-K^+ induced apoptosis [Lauritzen et al., 2003]. In some conditions, glutamate induces apoptosis and, similar to other apoptogenic models, K^+ efflux is linked to apoptotic death. Kv channels, increased K^+ permeability through the activated receptors or activation of high conductance Ca^{2+}-activated K^+ channels are all proposed routes for K^+ exit [Isaacson and Murphy, 2001; Yu, 2003]. Most studies on AVD in neurons have addressed to the early phase of the apoptotic program and the K^+ efflux driven by plasmalemmal K^+ currents. However, mitochondrial K^+ channels may also be involved in the mid- and late phases characterized by mitochondrial depolarization and cytochrome c release. K_{ATP} channels are those commonly associated with these phenomena.

AVD and Cl^- Fluxes

K^+ efflux is a characteristic trait of apoptosis in neurons as in many other cell types, but in order to effectively contribute to water outflow and AVD, it has to occur in conjunction with Cl^- exit. Activation of Cl^- currents in CD95-induced apoptosis was first shown by Lang and coworkers in Jurkat cells [Lang et al., 2005; Szabo et al., 1998]. This was confirmed thereafter in other cell types in apoptosis driven by staurosporine, FAS ligand, TNFα, ceramide or doxorubicin [Maeno et al., 2000; Okada et al., 2006]. The apoptosis-linked anion currents have properties in all similar to those carried by the volume-sensitive anion channel which plays a prominent role in RVD, but noteworthy in apoptosis this activation occurs in isosmotic conditions or even in shrinking, not swelling cells. The mechanism(s) or signals for the volume-sensitive Cl^- channel

activation are as yet unknown, neither in RVD nor in AVD. Cl^- channel blockers, DIDS, NPPB or phloretin prevent staurosporine-induced apoptosis in neural PC12 and in neuroblastoma NG108–15 cells [Maeno et al., 2000], and SITS is effective in hippocampal neurons undergoing apoptosis by ischemia/reperfusion [Inoue et al., 2005]. However, a study in cortical neurons shows that DIDS, NPPB or phloretin provide only limited protection against apoptosis induced by staurosporine, ceramide or serum deprivation, while K^+ channel blockers supply complete protection [Wei et al., 2004]. This failure of Cl^- channel blockers has been attributed to the low concentration of the blockers used in the cortical neurons preparation [Okada et al., 2006].

In addition to the volume-sensitive Cl^- channel, the voltage-dependent anion channel (VDAC) is proposed to participate in AVD. VDAC is a large conductance anion channel (300–400 pS), located at the outer mitochondrial membrane. In staurosporine-induced apoptosis in the hippocampal cell line HT-22 and in the human neuroblastoma cell line SK-N-MC, VDAC was found functionally expressed in the cell membrane in 48% of apoptotic cells and its blockade by functional antibodies or by high concentrations of sucrose reduce the number of apoptotic cells [Elinder et al., 2005]. In physiological conditions, membranal VDAC may function as an NADH-reductase involved in transmembrane redox regulation. The Cl^-/HCO_3^- exchanger is also suggested as mechanism for Cl^- outflow in apoptosis, based on the strong effect of the typical anion exchanger blocker DIDS, in suppressing the apoptotic program. This has been challenged by experiments showing no change in the antiapoptotic effect of DIDS in the absence of bicarbonate, a condition reducing the exchanger operation [Okada et al., 2006].

Signals for Activation of Ion Fluxes in Apoptosis

The signals in the apoptotic process activating Cl^- and K^+ fluxes for AVD remain largely unknown. Since various types of K^+ channel are involved, a common signal may not operate. The stress-reacting MAPK p38 is suggested as a signal [McLaughlin et al., 2001] under the upstream influence of another MAPK, the MAPKKK also called ASK1 (apoptosis signal-regulating kinase). Inactive ASK-1 or expression of a dominant-negative form of ASK-1 suppresses IK_{DR} and prevents apoptosis in cortical neurons [Aras and Aizenman, 2005]. Tyrosine kinases may also participate in the AVD-linked K^+ fluxes in cortical neurons, since the tyrosine kinase general blockers herbimycin or lavendustin reduce IK_{DR} in these cells [Yu et al., 1999]. Tyrosine kinases may be directly or indirectly associated with p38. Another proposed mechanism for apoptotic IK_{DR} upregulation is the membrane insertion of preformed, endogenous channels as shown for Kv2.1 channels in cortical neurons [Pal et al., 2006]. This translocation requires t-SNARE proteins, the same involved in the

exocytotic mechanism of neurotransmitter release. Disruption of SNARE proteins by botulin neurotoxin suppresses the apoptotic enhanced IK_{DR} [Pal et al., 2006].

Cl^- outflow seems to occurs via the same anion channel in RVD and AVD, in spite of the different, even opposite volume of cells in the two conditions, suggesting a change in the volume set point for the channel gating. The mechanism for this adjustment in the set point is unknown. Reactive oxygen species or tyrosine kinases such as the Src kinase p56Lck might be involved [Lambert, 2004; Leppe-Wienhues et al., 1998]. A role for the cytosolic concentration of ATP has also been considered [Okada et al., 2006].

Volume Decrease or Ionic Homeostasis: Which Is the Apoptotic Signal?

Cell volume decrease has been the key morphological trait to distinguish between necrotic and apoptotic death, but it is only in the last decade that insertion of AVD in the signaling chain of the apoptotic process has been demonstrated. Clearly AVD results essentially from K^+ and Cl^- outflow, but the question remains of whether the relevant point in terms of apoptotic program, is the cell volume reduction or the decrease in the intracellular ion concentrations. If K^+ or Cl^- at physiological levels could have an inhibitory influence on factors or reactions of the apoptotic chain, the activation of an efflux pathway and the consequent decrease in cell levels would relieve this inhibition. AVD would then be just the consequence of the ion extrusion. This hypothesis is supported by two types of results: (i) preventing K^+ loss by increasing extracellular K^+ interrupts apoptosis even when the K^+ exit route is fully active and (ii) decreasing cell K^+ concentrations with no activation of the apoptogen-induced K^+ currents is sufficient to set in motion the apoptotic machinery. Caspases and nucleases may be the sites of K^+ influence as suggested by the inhibitory effect of physiological K^+ concentrations on the activity of caspase 3. The K^+ threshold concentration for activating the apoptotic reactions has not been precisely defined [Bortner and Cidlowski, 2004]. A possible influence of Cl^- levels on the apoptotic steps has not being examined in detail. It is also unclear if K^+ and Cl^- have equal importance as a relevant signal, or if only one of them is actively expelled as part of the apoptotic signaling chain, and the other one is just passively carried to support the persistence and magnitude of the active ion outflow. That Cl^- may play this passive role is suggested by a study in staurosporine-induced apoptosis in cortical neurons showing that DIDS prevents AVD but not caspase-3 nor DNA fragmentation, whereas K^+ channel blockers have a full inhibitory action on all the apoptotic events [Wei et al., 2004]. The temporal sequence of apoptosis-linked Cl^- and K^+ fluxes has not been examined in detail. In RVD the order of K^+ and Cl^- efflux activation is dependent on the cell type. Thus, Cl^- channels activate prior to K^+ channels in

most epithelial cells and the opposite is found in non-epithelial cells [Pasantes-Morales and Morales-Mulia, 2000].

A role for Na^+ in AVD and apoptosis has recently raised attention. An early and transient increase in intracellular Na^+ has been detected in apoptotic Jurkat cells [Bortner and Cidlowki, 2003] which if suppressed, prevents K^+ loss, cell shrinkage and DNA degradation. Na^+ influx is a hallmark of necrotic death and therefore, if Na^+ entry is also crucial for apoptosis, the influx mechanisms and their activation and inactivation signals must be critical for deciding the ultimate direction of the cell death type.

AVD and Taurine Efflux

Organic osmolytes contribute to RVD and they could also participate in AVD. Taurine efflux concurrent with apoptosis has been shown in a variety of cell types including cerebellar granule neurons. The mechanism of the apoptotic taurine release is not well characterized. In cerebellar granule neurons as well as in Jurkat cells, the Cl^- channel blockers which are very efficient in preventing taurine efflux in RVD do not decrease but increase the AVD-linked taurine efflux [Lang et al., 2000; Morán et al., 2000]. Also, the tyrosine kinase influence observed in taurine efflux in RVD is not found in AVD. Raising external taurine up to 20 mM, a condition likely preventing taurine efflux or/and cell taurine loss, attenuates apoptosis in cortical neurons [Huang et al., 2006]. Thus, the arguments raised about the key role of K^+ cell loss and not AVD, are also valid for taurine. A consistent observation is that in cells treated with high external taurine, the apoptotic step corresponding to the assembly of apoptosomes and the further activation of caspase 9 is prevented [Takatani et al., 2004], suggesting an inactivating role of physiological concentrations of taurine in certain apoptotic signals. In line with this interpretation is the apoptotic death of photoreceptors in taurine transporter knockout mice [Heller-Stilb et al., 2002].

Final Comments

Excitability, hyperexcitability, hypersynchrony, survival and death, all have a link with one of the most ancient and preserved traits of the cell biology: the cell volume control. Even in the highly specialized neuron, cell volume influences its life cycle, from rescue to death, from proliferation to extinction, from rest to excitation. More question than answers still characterize our knowledge of this essential biological function. Among the most intriguing are the crossing points between RVD and AVD, while regulatory volume increase becomes protagonist by its apparently programmed repression. The nature of a volume

sensor finely tuned to detect in RVD, the imperative of activating osmolyte outflow routes for rescue, and in AVD, activating apparently similar routes for death. The set point adjustment allowing the activation of the same channels in swollen or shrunken cells, the similarities or dissimilarities between the signaling cascades in RVD and AVD, connecting the same sensors and the same effectors, the sequence, consequence and interdependence of K^+ and Cl^- fluxes leading cells to normality or to death, are still unsolved questions in the seminal topic of neuronal death and survival in pathologies related to changes in cell volume.

Acknowledegement

The work of the authors was supported by grants No.IN206403 from DGAPA UNAM and 46465 from CONACYT.

References

Aizenman E, Stout AK, Hartnett KA, Dineley KE, McLaughlin B, Reynolds IJ: Induction of neuronal apoptosis by thiol oxidation: putative role of intracellular zinc release. J Neurochem 2000;75: 1878–1888.

Alavez S, Pedroza D, Morán J: Mechanisms of cell death by deprivation of depolarizing conditions during cerebellar granule neurons maturation. Neurochem Int 2003;43:581–590.

Alojado ME, Morimoto Y, Morimoto Y, Kemmotsu O: Mechanism of cellular swelling induced by extracellular lactic acidosis in neuroblastoma-glioma hybrid (NG108–15) cells. Anesth Analg 1996;83:1002–1008.

Aras MA, Aizenman E: Obligatory role of ASK1 in the apoptotic surge of K^+ currents. Neurosci Lett 2005;387:136–140.

Baraban SC, Schwartzkroin PA: Effects of hyposmolar solutions on membrane currents of hippocampal interneurons and mossy cells in vitro. J Neurophysiol 1998;79:1108–1112.

Borgdorff AJ, Somjen GG, Wadman JW: Two mechanisms that raise free intracellular calcium in rat hippocampal neurons during hypoosmotic and low NaCl treatment. J Neurophysiol 2000;83:81–89.

Bortner CD, Cidlowski JA: Apoptotic volume decrease and the incredible shrinking cell. Cell Death Differ 2002;9:1307–1310.

Bortner CD, Cidlowski JA: Uncoupling cell shrinkage from apoptosis reveals that Na^+ influx is required for volume loss during programmed cell death. J Biol Chem 2003;278:39176–39184.

Bortner CD, Cidlowski JA: The role of apoptotic volume decrease and ionic homeostasis in the activation and repression of apoptosis. Pflugers Arch 2004;448:313–318.

Bossy-Wetzel E, Talantova MV, Lee WD, Schölzke MN, Harrop A, Mathews E, Götz T, Han J, Ellisman MH, Perkins GA, Lipton SA: Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p-38-activated K^+ channels. Neuron 2004;41: 351–365.

Burg ED, Remillard CV, Yuan JX-J: K^+ channels in apoptosis. J Membr Biol 2006;209:1–18.

Butterworth MF: Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab Brain Dis 2002;17:221–227.

Cronberg T, Jensen K, Rytter A, Wieloch T: Selective sparing of hippocampal CA3 cells following in vitro ischemia is due to selective inhibition by acidosis. Eur J Neurosci 2005;22:310–316.

de La Paz LD, Lezama R, Torres-Marquez ME, Pasantes-Morales H: Tyrosine kinases and amino acid efflux under hyposmotic and ischaemic conditions in the chicken retina. Pflugers Arch 2002;445:89–96.
Dudek FE, Yasumura T, Rash JE: 'Non-synaptic' mechanisms in seizures and epileptogenesis. Cell Biol Int 1998;22:793–805.
Elinder F, Akanda N, Tofighi R, Shimizu S, Tsujimoto Y, Orrenius S, Ceccatelli S: Opening of plasma membrane voltage-dependent anion channels (VDAC) precedes caspase activation in neuronal apoptosis induced by toxic stimuli. Cell Death Differ 2005;12:1134–1140.
Franco R, Torres-Marquez ME, Pasantes-Morales H: Evidence for two mechanisms of amino acid osmolyte release from hippocampal slices. Pflugers Arch 2001;442;791–800.
Godart H, Ellory JC, Motais R: Regulatory volume response of erythrocytes exposed to a gradual and slow decrease in medium osmolality. Pflugers Arch 1999;437:776–779.
Gunthorpe MJ, Benham CD, Randall A, Davies JB: the diversity in the vallinoid (TRPV) receptor family of ion channels. Trends Pharmacol Sci 2002;23:183–191.
Heller-Stilb B, van Roeyen C, Rascher K, Hartwig HG, Huth A, Seeliger MW, Warskulat U, Haussinger D: Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. FASEB J 2002;16:231–233.
Hochman DW, Baraban SC, Owens JWM, Schwartzkroin PA: Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. Science 1995;270:99–102.
Hribar M, Bloc A, Medilanski J, Nüsch L, Eder-Colli L: Voltage-gated K^+ current: a marker for apoptosis in differentiating neuronal progenitor cells? Eur J Neurosci 2004;20:635–648.
Huang Z, Hou Q, Cheung NS, Li QT: Neuronal cell death caused by inhibition of intracellular cholesterol trafficking is caspase dependent and associated with activation of the mitochondrial apoptosis pathway. J Neurochem 2006;97:280–291.
Inoue H, Mori S, Morishima S, Okada Y: Volume-sensitive chloride channels in mouse cortical neurons: characterization and role in volume regulation. Eur J Neurosci 2005;21:1648–1658.
Isaacson JS, Murphy GI: Glutamate-mediated extrasynaptic inhibition: direct coupling of NMDA receptors to $Ca(^{2+})$-activated K^+ channel. Neuron 2001;31:1027–1034.
Kimelberg HK, Goderie SK, Higman S, Pang S, Waniewsky RA: Swelling-induced release of glutamate, aspartate, and taurine from astrocytes cultures. J Neurosci 1990;10:1583–1591.
Lambert IH: Regulation of the cellular content of the organic osmolyte taurine in mammalian cells. Neurochem Res 2004;29:27–63.
Lang F, Föller M, Lang KS, Lang PA, Ritter M, Gulbins E, Vereninov A, Huber SM: Ion channels in cell proliferation and apoptotic cell death. J Membr Biol 2005;205:147–157.
Lang F, Madlung J, Siemen D, Ellory C, Lepple-Wienhues A, Glubins E: The involvement of caspases in the CD95(Fas/Apo-1)- but not swelling-induced cellular taurine release from Jurkat T-lymphocytes. Pflugers Arch 2000;440:93–99.
Lauritzen I, Zanzouri M, Honoré E, Duprat F, Ehrengruber MU, Lazdunski M, Patel AJ: K^+-dependent cerebellar granule neuron apoptosis. J Biol Chem 2003;34:32068–32076.
Lepple-Wienhues A, Szabo I, Laun T, Kaba NK, Gulbins E, Lang F: The tyrosine kinase p561ck mediates activation of swelling-induced chloride channels in lymphocytes. J Cell Biol 1998;141: 281–286.
Lohr JW, Grantham JJ: Isovolumetric regulation of isolated S2 proximal tubules in anisotonic media. J Clin Invest 1986;78:1165–1172.
Maeno E, Ishizaki Y, Kanaseki T, Hazama A, Okada Y: Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. Proc Natl Acad Sci USA 2000;1997: 9487–9492.
McLaughlin B, Pal S, Tran MP, Parsons AA, Borone FC, Erhardt, JA, Aizenman E: p38 activation is required upstream of potassium current enhancement and caspase cleavage in thiol oxidant-induced neuronal apoptosis. J Neurosci 2001;21:3303–3311.
Morán J, Hernández-Pech X, Merchant-Larios H, Pasantes-Morales H: Release of taurine in apoptotic cerebellar granule neurons in culture. Pflugers Arch 2000;439:271–277.

Mori S, Morishima S, Takasaki M, Okada Y: Impaired activity of volume-sensitive anion channel during lactacidosis-induced swelling in neuronally differentiated NG108–15 cells. Brain Res 2002;957: 1–11.
Nagelhus EA, Lehmann A, Ottersen OP: Neuronal-glial exchange of taurine during hypo-osmotic stress: a combined immunocytochemical and biochemical analysis in rat cerebellar cortex. Neuroscience 1993;54:615–631.
Norman DJ, Feng L, Cheng SS, Gubbay J, Chan E, Heintz N: The lurcher gene induces apoptotic death in cerebellar purkinje cells. Development 1995;121:1183–1193.
Okada Y, Shimizu T, Maeno E, Tanabe S, Wang X, Takahashi N: volume-sensitive chloride channels involved in apoptotic volume decrease and cell death. J Membr Biol 2006;209:1–9.
Ordaz B, Tuz K, Ochoa LD, Lezama R, Peña-Segura C, Franco R: Osmolytes and mechanisms involved in regulatory volume decrease under conditions of sudden or gradual osmolarity decrease. Neurochem Res 2004a;29:65–72.
Ordaz B, Vaca L, Franco R, Pasantes-Morales H: Volume changes and whole cell membrane currents activated during gradual osmolarity decrease in C6 glioma cells: contribution of two types of K^+ channels. Am J Physiol 2004b;286:C1399–C1409.
Pal S, Hartnett KA, Nerbonne JM, Levitan ES, Aiznman E: Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. J Neurosci 2003;23:4798–4802.
Pal SK, Takimoto K, Aizenman E, Levitan ES: Apoptotic surface delivery of K^+ channels. Cell Death Differ 2006;13:661–667.
Pasantes-Morales H, Franco R: Astrocyte cellular swelling: mechanisms and relevance to brain edema; in Aschner M, Costa LG (eds): The Role of Glia in Neurotoxicity. ed 2, CRC Press, 2005, pp 173–190.
Pasantes-Morales H, Morales-Mulia S: Influence of calcium on regulatory volume decrease: role of potassium channels. Nephron 2000;86:414–427.
Rosen AS, Andrew RD: Osmotic effects upon excitability in rat neocortical slices. Neuroscience 1990;38:579–590.
Saly V, Andrew RD: CA3 neuron excitation and epileptiform discharge are sensitive to osmolality. J Neurophysiol 1993;69:2200–2208.
Saransaari P, Oja SS: Mechanisms of D-aspartate release under ischemic conditions in mouse hippocampal slices. Neurochem Res 1999;24:1009–1016.
Schwartzkroin PA, Baraban SC, Hochman DW: Osmolarity, ionic flux, and changes in brain excitability. Epilepsy Res 1998;32:275–285.
Small DL, Tauskela J, Xia Z: Role for chloride but not potassium channels in apoptosis in primary rat cortical cultures. Neurosci Lett 2002;334:95–98.
Staub F, Mackert B, Kempski O, Peters J, Baethmann A: Swelling and death of neuronal cells by lactic acid. J Neurol Sci 1993;19:79–84.
Szabo I, Lepple-Wienhues A, Kaba KN, Zoratti M, Gulbins E, Lang F: Tyrosine kinase-dependent activation of a chloride channel in CD95-induced apoptosis in T lymphocytes. Proc Natl Acad Sci USA 1998;95:6169–6174.
Takatani T, Takahashi K, Ouzumi Y, Shikata E, Yamamoto Y, Ito T, Matsuda T, Schaffer SW, Fujio Y, Azuma J: Taurine inhibits apoptosis by preventing formation of the Apaf-1/caspase-9 apoptosome. Am J Physiol Cell Physiol 2004;287:C949–C953.
Tuz K, Ordaz B, Vaca L, Quesada O, Pasantes-Morales H: Isovolumetric regulation mechanisms in cultured cerebellar granule neurons. J Neurochem 2001;79:143–151.
Tuz K, Pasantes-Morales H: Hyposmolarity evokes norepinephrine efflux from synaptosomes by a depolarization- and Ca^{2+}-dependent exocytoic mechanism. Eur J Neurosci 2005;22:1636–1642.
Tuz K, Peña-Segura C, Franco R, Pasantes-Morales H: Depolarization, exocytosis and amino acid release evoked by hyposmolarity from cortical synaptosomes. Eur J Neurosci 2004;19:916–924.
Wei L, Xiao AY, Jin C, Yang A, Lu ZY, Yu SP: Effects of chloride and potassium channel blockers on apoptotic cell shrinkage and apoptosis in cortical neurons. Pflugers Arch 2004;448:325–334.
Yu SP: Regulation and critical role of potassium homeostasis in apoptosis. Prog Neurol 2003;70: 363–386.

Yu SP, Farhangrazi ZS, Ying HS, Yeh CH, Choi DW: Enhancement of outward potassium current may participate in beta-amyloid peptide-induced cortical neuronal death. Neurobiol Dis 1998;5:81–88.
Yu SP, Yeh C-H, Grottron F, Wang X, Grabb MC, Choi DW: Role of the outward delayed rectifier K^+ current in ceramide-induced caspase activation and apoptosis in cultured cortical neurons. J Neurochem 1999;73:933–941.
Yu SP, Yeh C-H, Sensi SL, Gwag BJ, Canzoniero LMT, Farhangrazi ZS, Ying HS, Tian M, Dugan LL, Choi DW: Mediation of neuronal apoptosis by enhancement of outward potassium current. Science 1997;278:114–117.

Prof. Herminia Pasantes-Morales
Instituto de Fisiología Celular, UNAM
Apartado Postal 70–600
04510 Mexico D.F. (Mexico)
Tel. +52 55 5622 5588, Fax +52 55 5622 5607, E-Mail hpasante@ifc.unam.mx