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SYMPOSIUM REVIEW

Receptor regulation of osmolyte homeostasis in neural cells

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The capacity of cells to correct their volume in response to hyposmotic stress via the efflux of inorganic and organic osmolytes is well documented. However, the ability of cell-surface receptors, in particular G-protein-coupled receptors (GPCRs), to regulate this homeostatic mechanism has received much less attention. Mechanisms that underlie the regulation of cell volume are of particular importance to cells in the central nervous system because of the physical restrictions of the skull and the adverse impact that even small increases in cell volume can have on their function. Increases in brain volume are seen in hyponatraemia, which can arise from a variety of aetiologies and is the most frequently diagnosed electrolyte disorder in clinical practice. In this review we summarize recent evidence that the activation of GPCRs facilitates the volume-dependent efflux of osmolytes from neural cells and permits them to more efficiently respond to small, physiologically relevant, reductions in osmolarity. The characteristics of receptor-regulated osmolyte efflux, the signalling pathways involved and the physiological significance of receptor activation are discussed. In addition, we propose that GPCRs may also regulate the re-uptake of osmolytes into neural cells, but that the influx of organic and inorganic osmolytes is differentially regulated. The ability of neural cells to closely regulate osmolyte homeostasis through receptor-mediated alterations in both efflux and influx mechanisms may explain, in part at least, why the brain selectively retains its complement of inorganic osmolytes during chronic hyponatraemia, whereas its organic osmolytes are depleted.

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Abbreviations CNS, central nervous system; EAAT3, excitatory amino acid transporter 3; GABA, γ-amino butyric acid; GPCR, G-protein-coupled receptor; KCC, K^+–Cl^- cotransporter; LPA, lysophosphatidic acid; mAChR, muscarinic cholinergic receptor; NKCC, Na^+–K^+–$2Cl^-$ cotransporter; PAR-1, protease-activated receptor-1; PKA, cyclic AMP-dependent protein kinase A; PKC, protein kinase C; PI3K, phosphatidylinositol 3-phosphate; RVD, regulatory volume decrease; S1P; sphingosine 1-phosphate; TauT, taurine transporter; VSOAC, volume-sensitive organic osmolyte and anion channel.

Maintenance of cell volume is an ancient homeostatic mechanism necessary for the survival and proper function of the vast majority of cells. Alterations in cell volume can lead to a number of changes in cell function including excitability, cell-cycle progression, proliferation, apoptosis and metabolic regulation (Okada *et al.* 2009). However, regulation of cell volume is of particular significance to

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the central nervous system (CNS) because of the physical restrictions of the skull. Even small changes in brain cell volume can profoundly influence the spatial relationships between neurons, astrocytes and the extracellular space. A reduction in the latter, as occurs during brain cell swelling, will result in both an increased lateral diffusion and higher extracellular concentrations of neurotransmitters (Sykova, 2004; Thorne & Nicholson, 2006). Larger increases in brain cell volume can compress blood vessels leading to episodes of anoxia and ischaemia, and ultimately to a displacement of the brain parenchyma through the foramen magnum, leading to cardiac and respiratory arrest (Pasantes-Morales *et al.* 2002).

Brain cells can swell either as a result of changes in intracellular ion and water distribution (isotonic swelling, as occurs in stroke or traumatic head injury) or by a reduction in plasma osmolarity (hyposmotic swelling). The most common cause of hyposmotic swelling is hyponatraemia, which is defined as reduction in serum Na^+ concentration from a normal value of 145 mequiv l^{-1} to 136 or below. Hyponatraemia is the most frequently encountered electrolyte disorder in clinical practice and can arise from a diverse array of aetiologies including congestive heart failure, syndrome of inappropriate secretion of vasopressin, liver cirrhosis, psychotic polydipsia or over-hydration, as may occur in athletes and military personnel (Bhardwaj, 2006; Lien & Shapiro, 2007). Although patients with mild hyponatraemia are often asymptomatic, reductions in plasma osmolarity of $>10\%$ can elicit symptoms of nausea and headache whereas seizures, coma, permanent brain damage and death can occur when plasma osmolarity is chronically reduced by $>20\%$. The focus of this review is on the endogenous molecular mechanisms that restore brain volume when neural cells are subjected to hyposmotic stress. In particular, the emergence of a potentially important physiological role for cell surface receptors in this process, in both the efflux and re-uptake of osmolytes, is discussed.

Mechanisms for restoration of neural cell volume

During episodes of hyponatraemia, the reduction in plasma osmolarity results in an influx of water into the brain through the blood–brain barrier and into neural cells across their plasma membranes. However, the increases in brain water are less than expected from a perfect osmometer (Verbalis & Drutarosky, 1988). This reflects the presence of two homeostatic mechanisms that reduce the potential impact of water influx. The first is an increase in the flow of fluid from the interstitial space into the cerebrospinal fluid and from there to the systemic circulation. This phase accounts for the rapid loss of Na^+ and Cl^- ions from brain observed at the onset of hyponatraemia. The second, and more sustained mechanism, is an efflux of osmotically active solutes from the cells, primarily inorganic ions such as K^+ and Cl^- and small organic molecules, such as taurine, glutamate or *myo*-inositol. This in turn leads to the exit of osmotically obligated water and reduces cellular swelling. The mechanism whereby the increase in cell volume is detected by a hypothetical 'volume sensor', and the signal transduction events that elicit volume correction, remain largely unknown, with the exception that a tyrosine kinase has frequently been implicated.

Although organic osmolytes potentially contribute less than their inorganic counterparts to cell volume adaptation (35 and 65%, respectively), organic osmolytes are highly enriched in the CNS and their utilization minimizes changes in membrane potential associated with the efflux of inorganic osmolytes such as K^+ or Cl^-. However, it should be remembered that several of the quantitatively major organic osmolytes are 'neuroactive' and can activate their respective receptors on nearby neurons and glia. Thus, as discussed later, their release under conditions of volume correction is not without neurobiological consequences. The normalization of cell volume that occurs as a result of osmolyte loss is referred to as regulatory volume decrease (RVD). Volume-dependent osmolyte efflux is mediated via a variety of anion/cation channels, including K^+ channels (both Ca^{2+}-dependent and -independent), the K^+–Cl^- co-transporter (KCC) and a putative volume-sensitive organic osmolyte and anion channel (VSOAC; Fig. 1). The latter is yet to be characterized at a molecular level, but is thought to be primarily a swelling-activated Cl^- channel that also mediates the efflux of organic osmolytes (Okada, 2006; Kimelberg *et al.* 2006). Swelling-activated efflux of osmolytes has been monitored in a variety of neural preparations including primary cultures of neurons and astrocytes, neurotumour cell lines, nerve-ending preparations and brain slices (for review, see Fisher *et al.* 2008).

A role for cell-surface receptors in the regulation of volume-sensitive osmolyte efflux

Although volume regulation has previously been considered to be an intrinsic property of cells, it is now evident that many extracellular agonists, acting via cell-surface receptors, can profoundly influence this process (for reviews see Fisher *et al.* 2008; Franco *et al.* 2008; Vasquez-Juarez *et al.* 2008). An indication that GPCRs could potentially be involved in osmoregulation in neural cells was obtained from experiments in which agents that mimic the activation of certain categories of cell-surface receptors, for example by increasing the concentration of cytosolic Ca^{2+} or activities of protein kinase C (PKC) or protein kinase A (PKA), were observed

to potentiate volume-dependent osmolyte release from cells. However, Bender *et al.* (1993) provided the first direct demonstration of the involvement of G-protein-coupled receptors (GPCRs) in volume control when they reported that RVD in cultured astrocytes exposed to hypotonic media was accelerated by the addition of endothelin or noradrenaline (norepinephrine). However, only in the last few years has the potential contribution of cell-surface receptors to the regulation of osmolyte fluxes in the CNS been systematically examined. A significant number of pharmacologically distinct receptors have now been identified to regulate osmolyte fluxes and volume control in a variety of different neural preparations (for summary, see Table 1). Although a diverse array of receptors has been identified, a number of common characteristics are apparent. First, with some exceptions (e.g. AMPA, vascular endothelial growth factor and erythropoeitin receptors), most documented examples of receptor regulation of osmolyte fluxes and/or volume control involve the activation of GPCRs and, where examined, the effects are mediated via pharmacologically distinct receptor subtypes. Under hyposmotic conditions, receptor activation almost invariably leads to a facilitation of osmolyte efflux and, when monitored, an enhancement of RVD (see Ramos-Mandujano *et al.* 2007). However, it remains possible that a category of GPCRs may also serve to negatively regulate osmolyte efflux and volume correction, as has been observed for the adenosine A_{2B} receptor in pituicytes (Pierson *et al.* 2007). The GPCRs coupled to an increase in osmolyte efflux typically respond to neurotransmitters or neuromodulators (e.g. neuropeptides, cholinergic or adrenergic agonists, ATP, thrombin or lysophospholipids) at nanmolar or micromolar concentrations, i.e. well within the concentration range found *in vivo*.

A second characteristic of receptor-stimulated osmolyte efflux is that the release of organic osmolytes and Cl^- can be blocked by inclusion of known inhibitors of VSOAC, such as DCPIB (4-[(2-butyly-6,7-dichloro-2-cyclopentenyl-2,3-dihydo-1-oxo-1*H*-inden-5-yl)oxy] butanoic acid), DDF (1,9-dideoxyforskolin) or NPPB, (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid). This is the same pharmacological profile as is observed under basal (swelling-activated) conditions and indicates that the same or similar channels mediate osmolyte release under both conditions. A third characteristic is that osmolyte efflux is only significantly increased following receptor activation under hyposmotic conditions, whereas little or no increase is observed under isotonic or hypertonic conditions (see Fig. 2*A*). From this observation it can be concluded that GPCRs serve to regulate, rather than initiate, the activity of channels that are involved in osmolyte release. Fourth, as initially described by Mongin & Kimelberg (2002) for ATP-stimulated release of D-aspartate from primary cultures of astrocytes, receptor activation appears to lower the threshold osmolarity at which osmolyte release occurs. An additional example of this phenomenon is shown in Fig. 2 for human SH-SY5Y neuroblastoma cells, a model neuronal cell line. For these cells, no measurable increase in the basal (swelling-activated) release of either taurine or Rb^+ (used as a marker for K^+) over that monitored under isotonic conditions is observed until the osmolarity of the medium is reduced by ∼30%. Similar large, non-physiologically relevant, reductions in osmolarity have frequently been required to reliably monitor osmolyte efflux from other neural preparations under basal conditions. In contrast, the activation of muscarinic cholinergic receptors (mAChRs) on SH-SY5Y cells permits the release of

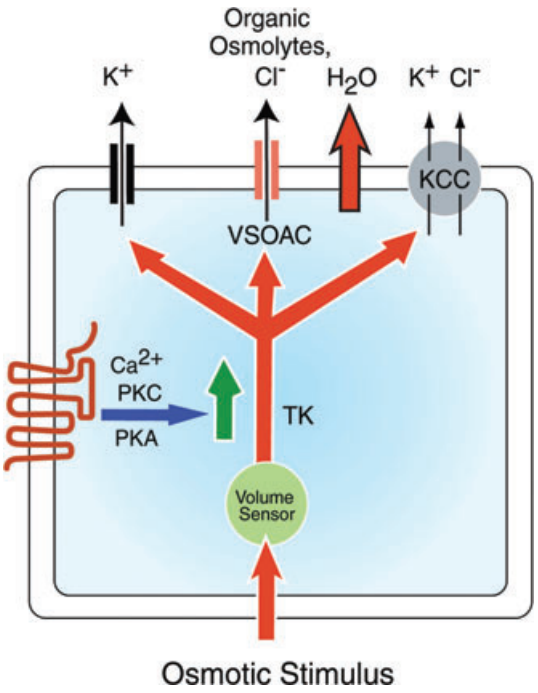

Figure 1. General schematic representation of events leading to swelling-activated (basal)- and GPCR-mediated osmolyte efflux
A reduction in osmolarity results in an influx of water and an increase in cell volume, which in turn triggers a hypothetical 'volume sensor'. It has been proposed that the latter may represent changes in macromolecular crowding and/or changes in the cytoskeleton. Activation of the volume sensor elicits a tyrosine kinase (TK)-dependent activation of K^+ channels (both Ca^{2+}-dependent and -independent), VSOAC and, on occasions, the KCC transporter. This results in the efflux of K^+, Cl^-, organic osmolytes and osmotically obligated water. The latter results in a restoration of cell volume or 'regulatory volume decrease'. Volume-dependent efflux of inorganic and organic osmolytes can also be significantly increased by activation of GPCRs, the magnitude of which is often dependent on Ca^{2+} availability and PKC activity. Note that GPCR activation of osmolyte release is strongly dependent on prior activation of the volume sensor and little or no increase is seen under isotonic or hypertonic conditions. Pharmacological evidence indicates that the same or similar channels mediate osmolyte efflux under basal- and GPCR-stimulated conditions.

Table 1. Receptor-mediated regulation of osmolyte fluxes or cell volume under hyposmotic conditions

Receptor	Subtype	Signalling pathways	Cell type	Parameter	Reference
Adenosine	A_1	PKA	Retina	Cell volume	Uckermann *et al.* 2006
	A_1	PKA/PI3K/Akt	Retina	Cell volume	Wurm *et al.* 2008
	A_{2B}	PKA	Pituicytes	Taurine flux	Pierson *et al.* 2007
Purinergic	P_{2Y}	ND	Retina	Cell volume	Uckermann *et al.* 2006; Wurm *et al.* 2008
	P_{2Y}	Ca^{2+}/PKC/CaM/CaMKII	Cortical astrocytes	D-Asp flux	Mongin & Kimelberg, 2002, 2005
	P_{2Y}	ND	Cortical astrocytes	D-Asp/Cl^- flux	Abdullaev *et al.* 2006
	P_{2Y}	ND	Cortical astrocytes	Cl^- flux	Darby *et al.* 2003
	ND	Ca^{2+}	Pituicytes	Taurine flux	Rosso *et al.* 2004
	ND	ND	Hippocampal neurons	Taurine/Cl^- flux	Li & Olson, 2004
	ND	ND	Substantia nigra	Taurine flux	Morales *et al.* 2007
Bradykinin	ND	Ca^{2+}	Pituicytes	Taurine flux	Rosso *et al.* 2004
Endothelin	ND	PPI hydrolysis	Cortical astrocytes	Cell volume	Bender *et al.* 1993
Erythropoietin	ND	Jak-2/ERK1/2	Retina	Cell volume	Krugel *et al.* 2010
Glutamate	AMPA	ND	Substantia nigra	Taurine flux	Morales *et al.* 2007
	mGluR	ND	Retina	Cell volume	Uckermann *et al.* 2006; Wurm *et al.* 2008
LPA	ND	Ca^{2+}/PKC	SH-SY5Y	Taurine flux	Heacock *et al.* 2006
	ND	ND	SH-SY5Y	K^+ flux	Foster *et al.* 2008
NA	ND	PPI hydrolysis	Cortical astrocytes	Cell volume	Bender *et al.* 1993
	β-AR	PKA	Cortical astrocytes	Taurine flux	Moran *et al.* 2001
mAChR	M3	PKC/Ca^{2+}/Tyr kinases	SH-SY5Y	D-Asp/taurine flux	Heacock *et al.* 2004
	M3	Ca^{2+}/PKC	SH-SY5Y	Inositol flux	Loveday *et al.* 2003
	M3	Ca^{2+}/PKC	SH-SY5Y	Cl^- flux	Cheema *et al.* 2007
	M3	Ca^{2+}/PKC	SH-SY5Y	K^+ flux	Foster *et al.* 2008
S1P	ND	Ca^{2+}/PKC	SH-SY5Y	Taurine flux	Heacock *et al.* 2006
	ND	ND	SH-SY5Y	K^+ flux	Foster *et al.* 2008
PAR	PAR-1	ND	Cortical astrocytes	Taurine flux	Cheema *et al.* 2005
	PAR-1	Ca^{2+}/PKC/CaM/PI3K	Cortical astrocytes	D-Asp flux	Ramos-Mandujano *et al.* 2007
	ND	PPI hydrolysis	Cortical astrocytes	Cell volume	Bender *et al.* 1993
	PAR-1	Ca^{2+}/PKC	SH-SY5Y	Taurine flux	Cheema *et al.* 2007
	PAR-1	ND	SH-SY5Y	Cl^- flux	Cheema *et al.* 2007
	PAR-1	ND	SH-SY5Y	K^+ flux	Foster *et al.* 2008
	PAR-1	Ca^{2+}/PKC	1321N1 astrocytoma	Taurine flux	Cheema *et al.* 2005
	PAR-1	Ca^{2+}/Rho GTPase	1321N1 astrocytoma	ATP flux	Blum *et al.* 2010
Vasopressin	V_{1A}	Ca^{2+}	Pituicytes	Taurine flux	Rosso *et al.* 2004
	V_{1A}	ND	Neocortex	Cell volume	Niermann *et al.* 2001
	V_{1A}	ND	Cortical astrocytes	Cell volume	Sarfaraz & Fraser, 1999
VEGF	VEGFR-2	Ca^{2+}/PLC/PKC*/src*	Retina	Cell volume	Wurm *et al.* 2008

Abbreviations: β-AR, β-adrenergic-receptor; D-Asp, D-aspartate; CaM, calmodulin; CaMKII, calmodulin-dependent protein kinase II; mGluR, metabotropic glutmate receptor; NA, noradrenaline; ND, not determined; PLC, phospholipase C; PPI, phosphoinositide; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor 2. Jak-2, Janus kinase-2 and ERK1/2, Extracellular signal regulated kinases 1 or 2.

osmolytes in a volume-dependent manner at relatively modest reductions in osmolarity (>6% for taurine and >9% for Rb^+). Activation of other GPCRs present on SH-SY5Y cells such as sphingosine 1-phosphate (S1P), lysophosphatidic acid (LPA) and protease-activated-1 (PAR-1) receptors also reduced the threshold osmolarity at which taurine was released (Heacock *et al.* 2006; Cheema *et al.* 2007). The ability of GPCRs to increase not only the magnitude of osmolyte release but also the threshold osmolarity at which the release occurs, may provide a physiological mechanism whereby neural cells are able to respond to reductions in osmolarity in the range that is encountered in hyponatraemia.

Hyposmolarity has also been reported to enhance the release of several potential ligands for GPCRs, thereby increasing the likelihood of their activation (Tuz *et al.* 2004; Tuz & Pasantes-Morales, 2005; Blum *et al.* 2010). One such ligand is ATP, which is released at an increased rate from

cells in response to hyposmotic stress (Corriden & Insel, 2010). ATP then contributes to RVD by triggering the efflux of both inorganic and organic osmolytes, an effect mediated via purinergic receptors. A role for ATP (and also its breakdown product, ADP) in osmolyte homeostasis has been frequently observed for primary cultures of astrocytes and neurons, neurotumour cells and the retina (Table 1). The importance of purinergic mediation of volume control in the retina is indicated by the fact that retinal glial cells do not swell in response to hyposmolarity unless the P_{2Y} receptor is pharmacologically blocked. Thus in response to hyposmolarity, glia (and possibly nearby neurons) are thought to release ATP, which, in an autocrine or paracrine fashion, serves to minimize cell swelling (Wurm *et al.* 2010). In contrast, retinal glial cells obtained from P_{2Y} knockout mice exhibit swelling in response to hyposmolarity, but this can be countered by administration of ATP. At least in retinal glial cells, it appears that the final mediator of volume correction is not ATP itself, but adenosine, derived from the breakdown of ATP. Adenosine is proposed to facilitate K^+ and Cl^- efflux via activation of adenosine A_1 receptors. Consistent with this mechanism is the observation that volume regulation is impaired in glial cells deficient in adenosine A_1 receptors (Wurm *et al.* 2010). In addition to its efflux under hyposmotic conditions, the release of ATP can be further increased following activation of the PAR-1 receptor, mediated by thrombin (Blum *et al.* 2010). Since activation of the PAR-1 receptor itself has previously been documented to facilitate osmolyte release and volume correction (Cheema *et al.* 2005; Ramos-Mandujano *et al.* 2007; Blum *et al.* 2010), it is conceivable that a release of ATP may contribute to some of the effects on osmolyte efflux elicited by activation of other GPCRs. However, not all cells possess P_{2Y} receptors (e.g. SH-SY5Y neuroblastoma or 1321N1 astrocytoma cells) and thus, an obligatory role for ATP release in regulating osmolyte efflux following GPCR activation appears unlikely.

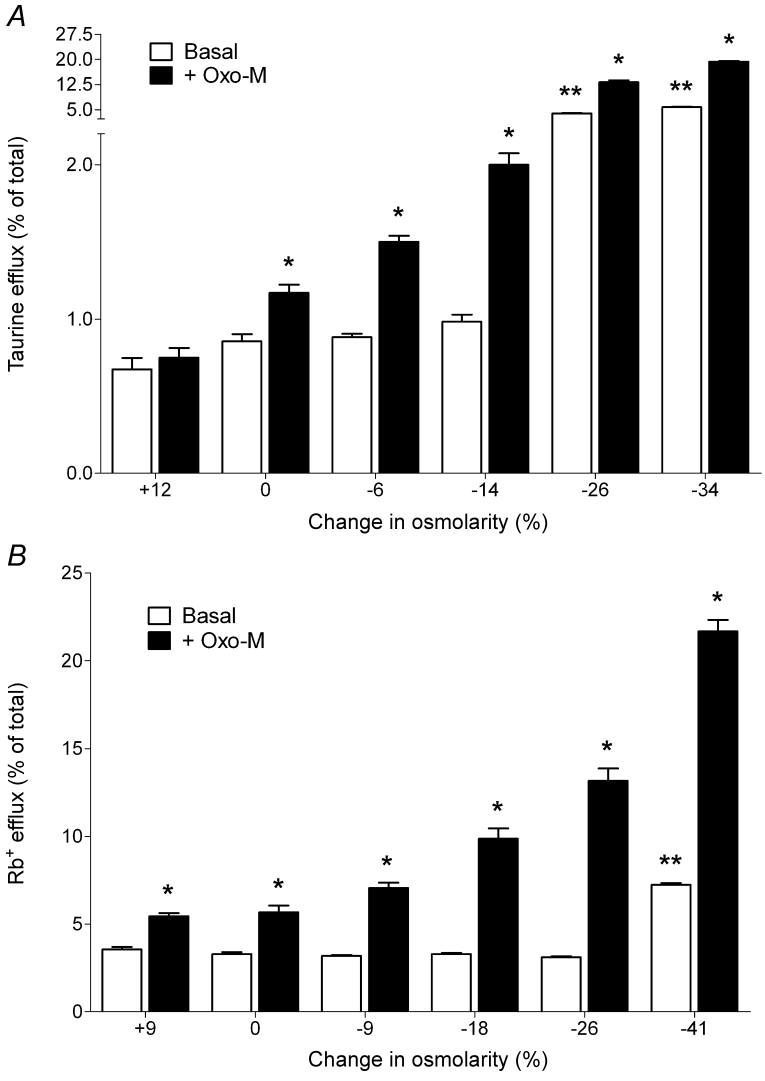

Figure 2. Basal- and oxotremorine-M (Oxo-M)-stimulated efflux of taurine and Rb^+ as a function of osmolarity
SH-SY5Y neuroblastoma cells, prelabelled overnight with either [^{14}C]taurine or $^{86}Rb^+$, were incubated in isotonic, hypertonic or hypotonic media for either 20 min (taurine) or 5 min (Rb^+) in the absence (open bars) or presence (filled bars) of 100 μM Oxo-M, a muscarinic cholinergic agonist. Results are expressed as taurine or Rb^+ efflux (percentage of total soluble radioactivity) and are the means ± S.E.M. for 4–13 experiments. *A*, taurine efflux; *B*, Rb^+ efflux. *Different from basal efflux, $P < 0.05$ by paired Student's *t* test. **Different from basal efflux monitored under isotonic conditions, $P < 0.01$ by one-way ANOVA, followed by Dunnett's multiple comparison test. Data are taken from Heacock *et al.* (2004) and Foster *et al.* (2008).

Signal transduction pathways that mediate GPCR-mediated stimulation of osmolyte efflux

Many of the GPCRs that have thus far been identified to facilitate osmolyte efflux are also those known to regulate Ca^{2+} homeostasis and the activation of PKC. Examples of receptors in this category are the P_{2Y} purinergic, M3 mAChR, PAR-1, LPA, S1P and V_{1A} vasopressin receptors. Accordingly, attenuation of the ability of GPCRs to regulate osmolyte release or modulate volume control is consistently observed following depletion of intra/extracellular Ca^{2+} or inhibition of PKC. If intracellular Ca^{2+} is depleted and PKC inhibited, receptor regulation of osmolyte efflux is essentially abolished. It should be stressed that the Ca^{2+}- and PKC-dependence of receptor-stimulated efflux is in marked contrast to that observed for swelling-activated efflux, which appears to be relatively independent of both parameters. These observations are consistent with the proposal that distinct up-steam mechanisms underlie basal- and receptor-mediated osmolyte efflux, even though the same or similar channels are used for the exit of osmolytes from cells under both conditions (Mongin & Kimelberg, 2005; Heacock *et al.* 2006). Although the evidence for a requirement for Ca^{2+} in osmoregulation is compelling, the source of this Ca^{2+} (extra- or intracellular) appears to be both receptor- and cell-type-specific (Cheema *et al.* 2005; Mongin & Kimelberg, 2005, Heacock *et al.* 2006; Wurm *et al.* 2010). Moreover, no simple relationship exists between the magnitude of increase in Ca^{2+} concentration elicited by activation of individual GPCRs in a specific cell type and the extent of osmolyte release (Heacock *et al.* 2006). An additional complexity is that the release of inorganic osmolytes (K^+, Cl^-) appears less sensitive to the removal of Ca^{2+} than that of organic osmolytes, a result that suggests that distinct biochemical requirements may exist for the two classes of osmolytes within the same cell (Cheema *et al.* 2007; Foster *et al.* 2008). It is also evident that osmolyte release can be facilitated by Ca^{2+}-mobilizing receptors that operate via either phospholipase C-dependent or -independent mechanisms (Heacock *et al.* 2006). The down-stream effectors for Ca^{2+} have yet to be identified but may include calmodulin (Mongin & Kimelberg, 2005; Ramos-Mandujano *et al.* 2007) and/or PKC. There is also evidence for the involvement of two conventional (Ca^{2+}- and diacylglycerol-dependent) isoforms of PKC (α and β) in ATP-mediated D-aspartate release from astrocytes (Rudkouskaya *et al.* 2008).

Other signalling pathways have also been implicated in receptor-mediated osmolyte efflux. These include phosphatidylinositol 3-kinase (PI3K; Ramos-Mandujano *et al.* 2007), tyrosine kinases (Heacock *et al.* 2004; Mongin & Kimelberg, 2005) and protein kinase A. Evidence for involvement of the latter pathway has been obtained for β-adrenergic stimulation of taurine efflux from cortical astrocytes (Moran *et al.* 2001) and for adenosine A_1 receptor-mediated volume changes in retinal glial cells (Uckermann *et al.* 2006; Wurm *et al.* 2010). In summary, it appears likely that cell volume regulation by GPCRs involves the activation of several distinct signalling pathways.

Is osmolyte influx an additional mechanism whereby GPCRs can regulate osmolyte homeostasis?

When monitored under acute conditions *in vitro*, the rate of efflux of inorganic osmolytes from neural cells under both basal- and receptor-stimulated conditions either exceeds, or is comparable to, that observed for organic osmolytes. In contrast, chronic hyponatraemia *in vivo* results in a disproportionately greater loss of organic osmolytes than of inorganic osmolytes from the brain (Melton *et al.* 1987; Pasantes-Morales *et al.* 2002; Massieu *et al.* 2004). Similarly, if primary astrocytes are chronically cultured in a hyposmotic medium, organic osmolytes such as taurine or aspartate are lost from the cells whereas K^+ is retained (Olson, 1999). One potential explanation for this discrepancy is that the influx of inorganic and organic osmolytes, like that of their efflux, is also volume dependent, but that the two classes of osmolyte are differentially regulated by hyposmolarity. In keeping with this possibility, previous studies have indicated that hyposmotic swelling of astrocytes results in a stimulation of K^+ influx (Mongin *et al.* 1994, 1996) whereas, in contrast, a down-regulation of the taurine transporter (TauT) in hippocampal neurons has been reported in response to a reduced osmolarity (Olson & Martinho, 2006). However, whether the influx of osmolytes, like that of their efflux, is also subject to receptor regulation in an osmosensitive manner, remains to be determined. In a recent series of studies, we have systematically examined this possibility by monitoring the influx of three distinct osmolytes, namely K^+, taurine and glutamate in SH-SY5Y neuroblastoma. Agonist occupancy of several GPCRs (mAChR, LPA receptor, PAR-1 and S1P receptor) in this cell line under isotonic conditions resulted in an increase in the uptake of K^+ (monitored as $^{86}Rb^+$ flux) that was further enhanced when the osmolarity was reduced (Foster *et al.* 2008). A similar increase in K^+ influx was also obtained following activation of PAR-1, LPA and S1P receptors in primary cultures of astrocytes, cells that are known to actively regulate K^+ concentrations in the extracellular space. Thus activation of GPCRs appears to facilitate *both* the efflux and influx of K^+ in an osmosensitive manner. However, whereas K^+ channels and the KCC transporter mediate the efflux of K^+, influx occurs via the Na^+–K^+–$2Cl^-$ co-transporter (NKCC), ouabain-sensitive Na^+–K^+-ATPase and to a

lesser extent KCC (Fig. 3*A*). A physiological role for K^+ uptake under hyposmotic conditions was indicated by the observation that activation of mAChRs under either isotonic or mildly hypotonic conditions did not result in a change in the intracellular concentration of K^+, unless the influx of K^+ was concurrently prevented by inclusion of ouabain and furosemide (frusemide) (an inhibitor of NKCC). This result suggests that, under normal conditions, the agonist stimulation of K^+ efflux from cells is countered by an equivalent increase in K^+ influx. Only under more pronounced hypotonic conditions (>30% reduction in osmolarity) were reductions in intracellular K^+ concentration observed, due to the predominance of the efflux pathway (Foster *et al.* 2008).

In contrast to the results obtained for K^+ influx, activation of GPCRs in SH-SY5Y cells (mAChR, LPA, S1P or PAR-1 receptors) results in a reduction in the rate of taurine transport under isotonic conditions, an effect that is further enhanced by hyposmolarity (Fig. 3*B*). Similar results were obtained for primary cultures of astrocytes. The receptor-mediated reduction in taurine transport in SH-SY5Y cells was Ca^{2+} dependent and resulted from a reduction in the V_{max} for transport while the K_m value for taurine uptake remained unchanged (Foster *et al.* 2009). The loss of taurine from cells under hypotonic conditions thus reflects not only an increased rate of efflux through VSOAC, but also its reduced re-uptake from the extracellular space. These observations could explain, in part at least, the dramatic loss of taurine observed from the brain during chronic hyponatraemia (Massieu *et al.* 2004). As TauT-mediated uptake involves the co-transport of two Na^+ ions and one Cl^- ion, the inhibition of taurine re-uptake will also have the net effect of preventing the uptake of four osmolyte equivalents per cycle. Taurine is considered to be a relatively inert organic osmolyte and a significant increase in its extracellular concentration might be expected to have only minimal cellular consequences. However, a similar increase in the concentration of extracellular glutamate, another quantitatively important organic osmolyte within the CNS, would be potentially deleterious, due to the known ability of glutamate to contribute to the rate of propagation of spreading depression, a slow wave of astrocytic and neuronal depolarization that leads to synaptic depression (Basarsky *et al.* 1999). Higher concentrations of extracellular glutamate can also elicit excitotoxic death in neurons. Accordingly, blockade of glutamate release by pharmacological inhibition of VSOAC activity can significantly reduce the infarct size following focal cerebral ischaemia (Zhang *et al.* 2008). To

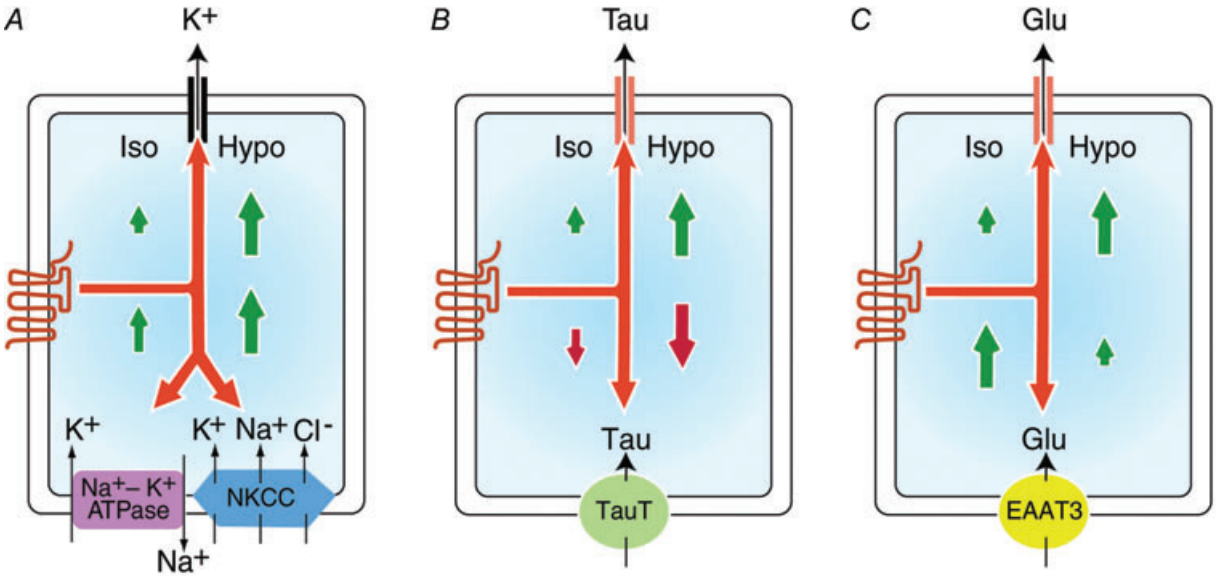

Figure 3. Diagrammatic representation of the ability of GPCRs present in SH-SY5Y cells to regulate the efflux and uptake of K^+ (*A*), taurine (*B*) or glutamate (*C*) under isotonic (Iso) or hypotonic (Hypo) conditions

Although only a limited efflux of osmolytes is observed following the activation of GPCRs under isotonic conditions, the efflux of all three is markedly increased by hypotonicity (as indicated by the relative size of the arrows). The efflux of K^+ is mediated primarily by K^+ channel(s) whereas taurine (Tau) and glutamate (Glu) are released via the VSOAC. K^+ uptake, which is principally mediated by Na^+–K^+-ATPase and the NKCC transporters in this cell line, is facilitated by GPCR activation under conditions of isotonicity, and further enhanced by hyposmolarity. In contrast, under isotonic conditions, GPCR activation results in an inhibition of taurine uptake, which is mediated via the taurine transporter (TauT). This inhibition of TauT is further enhanced by hyposmolarity. Glutamate uptake, which is mediated by EAAT3, is markedly increased by GPCR activation under isotonic conditions. However, receptor-mediated stimulation of Glu uptake is progressively inhibited as osmolarity is reduced.

determine whether similar or distinct mechanisms exist for glutamate and taurine homeostasis, glutamate uptake into SH-SY5Y cells (monitored as D-[^{3}H]aspartate influx) was measured as a function of osmolarity and receptor activation. Glutamate uptake was substantially increased under isotonic conditions by activation of mAChRs and to a lesser extent by endothelin or LPA receptors (Foster *et al.* 2010). The mAChR-mediated enhancement of glutamate uptake, which required the presence of Ca^{2+} and activation of PKC and PI3K, was mediated by a cellular redistribution to the plasma membrane of the excitatory amino acid transporter 3 (EAAT3), a neuron-specific transporter. However, the ability of the mAChR to facilitate glutamate uptake was significantly attenuated by hyposmolarity (>45% following a 21% reduction in osmolarity). As observed for the taurine transporter, the osmosensitive attenuation of glutamate uptake reflects a reduction in the V_{max} for transport without a change in K_m and was accompanied by an altered trafficking pattern of the transporter (Fig. 3*C*; Foster *et al.* 2010). Endothelin-1-stimulated increases in glutamate uptake into C_6 glioma cells (mediated by EAAT3) were similarly attenuated by hyposmolarity indicating that receptor-mediated regulation of glutamate transport may be a general, rather than cell-specific, phenomenon. The regulation of EAAT3-mediated glutamate uptake could potentially have physiological consequences in the intact brain. Thus under conditions of isotonicity or mild hypotonicity, activation of GPCRs may permit EAAT3-containing neurons to maintain relatively low concentrations of extracellular glutamate. However, when osmolarity is further reduced, a combination of glutamate efflux, along with a progressive inhibition of glutamate re-uptake, could result in an increased accumulation of extracellular glutamate.

In addition to glutamate, other quantitatively major organic osmolytes such as aspartate, glycine or GABA are neuroactive and thus their release from osmotically swollen cells, which is enhanced following the activation of GPCRs, can be expected to impact nearby neurons and glia. Even taurine, which is generally considered to be relatively inert, can act as a ligand at glycine, $GABA_A$, $GABA_B$ and *N*-methyl-D-aspartate receptors. Thus the ability of GPCRs to facilitate the release of organic osmolytes constitutes a 'double-edged sword' in that both the beneficial effects of volume reduction and the potentially harmful pathophysiological effects of higher extracellular organic osmolyte concentrations need to be considered. An ability of neural cells to selectively retain or release individual organic osmolytes via differential regulation of their re-uptake might provide a mechanism whereby selectivity of organic osmolyte release is attained. However, the full physiological significance of tonicity-mediated changes in receptor-stimulated osmolyte uptake remains to be directly evaluated *in vivo*.

Summary and conclusions

Regulation of osmolyte fluxes and cell volume following GPCR activation has been documented in both neural and non-neural cells. However, this regulatory mechanism is likely to be of particular physiological significance to the CNS given the prevalence of GPCRs (~300 identified) and the vulnerability of the brain to osmotic disturbances. The identification of GPCRs that not only regulate the efflux of osmolytes, but also their re-uptake, may provide neural cells with the ability to respond to small, physiologically relevant, reductions in osmolarity and to selectively retain or relinquish individual osmolytes.

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