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The Role of Osmotic Pressure and Membrane Potential in K^+-Stimulated Taurine Release from Cultured Astrocytes and LRM55 Cells

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The effects of $[K^+]_o$ on taurine release from glial cells were studied with primary cultures of cerebellar astrocytes and with LRM55 cells, a continuous glial cell line. The characteristics of K^+-stimulated taurine release were virtually identical in the 2 cell types. Both cerebellar astrocytes and LRM55 cells released taurine when stimulated with high-K^+ medium prepared by isosmotically substituting KCl for NaCl, but neither cell type released taurine when stimulated with hyperosmotic high-K^+ medium prepared by adding solid KCl to control medium. The membrane potential of LRM55 cells was measured by intracellular recording and was insensitive to changes in $[K^+]_o$ below 20 mM. LRM55 cells released taurine when stimulated with nondepolarizing concentrations of K^+ (13–22 mM) if the isosmotically prepared high-K^+ medium was used, but the cells did not release taurine when treated with a depolarizing concentration of K^+ (50 mM) if hyperosmotic high-K^+ medium was used. The time course of K^+-stimulated taurine release was quite slow, having a time to peak of 10–15 min. Small changes (2.5–10%) in the osmolarity of the medium strongly affected taurine release by cerebellar astrocytes and LRM55 cells. K^+-stimulated taurine release from both cell types was inhibited when the osmolarity was increased with sucrose or NaCl and was enhanced when the osmolarity was reduced. Similarly, baseline taurine release was suppressed by small elevations in osmolarity and increased by reduced osmolarity. The slow time course and the high sensitivity of K^+-stimulated release to initial osmotic gradients suggest that K^+-stimulated taurine release from astrocytes results from the uptake of KCl and cellular swelling that occurs in isosmotically prepared high-K^+ media.

High concentrations of extracellular K^+ are widely used to elicit the release of neurotransmitters and other compounds from many types of nervous tissue preparations, and it is commonly assumed that release results from K^+-induced depolarization. However, high $[K^+]_o$ can have numerous other effects on tissue that may affect the release of small molecules by cells. In intact brain, elevated $[K^+]_o$ is associated with changes in the distribution of ions between the intra- and extracellular spaces and changes in cell volume (Dietzel et al., 1980; Walz, 1987). *In vitro* elevated $[K^+]_o$ also stimulates energy metabolism, the hydrolysis of glycogen, and leads to changes in cyclic nucleotide levels, protein phosphorylation, and protein synthesis (Bull and Lutkenhoff, 1973; Shimizu et al., 1973; Forn and Greengard, 1978; Lipton and Heimbach, 1978; Hof et al., 1988). Thus, variations in extracellular $[K^+]$ lead to numerous, complex, probably interrelated changes in fundamental cellular activities that may include release processes.

Recent studies have shown that cultured astrocytes and LRM55 cells release taurine when stimulated with elevated $[K^+]_o$ or neurotransmitters (Shain and Martin 1984; Madelian et al., 1985; Shain et al., 1986; Martin et al., 1987; Philibert et al., 1988). The role of the membrane potential in glial release has not been investigated, although studies of neurotransmitter-stimulated taurine release suggest that depolarization is not involved. Neurotransmitters such as β-adrenergic agonists stimulate taurine release by a cAMP-mediated mechanism but do not appear to depolarize the cells (Madelian et al., 1985). Furthermore, glial taurine release is Ca^{2+} independent (Martin et al., 1989; Shain et al., 1989) and, therefore, differs from other depolarization-dependent release processes such as neurotransmitter release at the synapse. Nevertheless, astrocytes characteristically have large negative membrane potentials and are readily depolarized by elevated $[K^+]_o$, a property clearly consistent with depolarization-mediated release. To investigate the role of depolarization, we have examined the ability of elevated $[K^+]_o$ to elicit taurine release under a variety of conditions and have compared K^+-stimulated taurine release by primary cultures of cerebellar astrocytes with release by LRM55 cells, a clonal glial cell line that expresses the astrocytic marker glial fibrillary acidic protein. Like many transformed glial cells, however, LRM55 cells have a relatively low membrane potential that is comparatively insensitive to changes in $[K^+]_o$. Thus, these cells provide a means of studying the effects of manipulating external $[K^+]$, while minimally affecting membrane potential.

Materials and Methods

Materials. 1,2-^{3}H-taurine (35 Ci/mmol) was from Amersham and L-U-^{14}C-lysine (300 mCi/mmol) was from Research Products International (Mount Prospect, IL). (−)Isoproterenol was from Sigma. Ortho-phthalaldehyde was from Pierce. Solvents were HPLC grade. Other compounds were analytical grade or the highest purity available.

Cell culture. Primary cultures of cerebellar astrocytes were prepared

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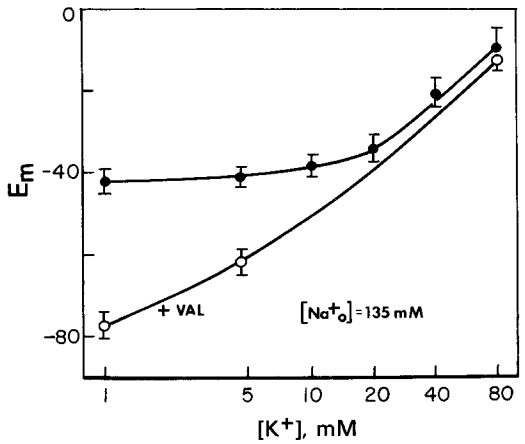

Figure 1. Membrane potential of LRM55 cells as a function of $[K^+]_o$. Each point is the average ± SEM of 5–20 observations. Symbols: (○, 10 μM valinomycin, ●, no valinomycin.

as described by Frangakis and Kimelberg (1984) and grown in Eagle's medium supplemented with 10% fetal bovine serum for 5–7 weeks. LRM55 glial cells are a single cell clone obtained from a rat spinal tumor and possess a number of glial cell properties such as carbonic anhydrase and Cl^- transport (Wolpaw and Martin, 1982, 1984). Immunocytochemical experiments indicate that LRM55 cells contain glial fibrillary acidic protein and that more than 90% of the cells in the cultures of cerebellar astrocytes express this protein (data not shown). Cells are routinely grown in mass cultures in 100 mm dishes in modified Ham's F12 medium (Vogel et al., 1972) supplemented with 5% fetal bovine serum. To prepare for release experiments, astrocytes and LRM55 cells were subcultured onto plastic strips (Cell Support film, Bellco Glass) and allowed to grow to confluence.

Incubation media. Release experiments were conducted with a modified HEPES-buffered Hanks' saline with ascorbate (HHA) that contained (in mM): 128.7 NaCl, 4.09 KCl, 1.125 $CaCl_2$, 0.49 $MgCl_2$, 0.21 $MgSO_4$, 0.93 Na_2HPO_4, 0.45 KH_2PO_4, 11 glucose, 0.5 sodium ascorbate, and 10 HEPES and was adjusted to pH 7.3 with NaOH. Ascorbate was included to prevent the oxidation of isoproterenol when it was used. Modified media containing elevated $[K^+]$ were prepared by substituting KCl for an equimolar amount of NaCl (termed isosmotic replacement) or by dissolving solid KCl in HHA (termed hyperosmotic addition). Low-osmolarity media were prepared by adding deionized water to HHA, if the change was less than 10%; otherwise, the media were prepared by omitting an appropriate amount of NaCl. The osmolarity of the media were checked routinely with a Wescor vapor-pressure osmometer. The calculated osmolarity was 300 mOsm, and the measured osmolarity was 271.6 ± 6 mOsm (mean ± SD, n = 28).

Release experiments. The release of labeled amino acids was measured as previously described (Shain and Martin, 1984; Shain et al., 1986). Briefly, the cells were incubated for about 1 hr with labeled amino acid (generally at a concentration of 10 μCi/ml in HHA, ca. 300 nM) to load the cells with label. The cell support film with adherent cells was then transferred to the superfusion chamber and superfused with HHA at 0.5 ml/min. Drugs or modified medium were applied to the cells at appropriate times by a system of valves controlled by a computer. In some experiments, the superfusate was collected at 1 min intervals with a fraction collector, and the radioactivity in each sample was determined by liquid scintillation counting. In other experiments, the radioactivity in the superfusate was measured for 1 min intervals with an in-line, flow-through scintillation counter (Radiomatic Instruments).

Determination of amino acids. In some experiments, the concentrations of amino acids in the superfusate were measured by automated HPLC. Because of the low concentrations of the amino acids, a modification of our previous methods (Waniewski and Martin, 1986; Spink et al., 1988) was developed (R. A. Waniewski, personal communication). Briefly, automated precolumn derivatization was accomplished by programmed injection of 50 μl of derivatizing reagent followed by 200 μl of superfusate and a second 50 μl injection of derivatizing reagent. The reagents were allowed to react for 0.7 min in a precolumn reaction chamber after which the gradient elution program was begun.

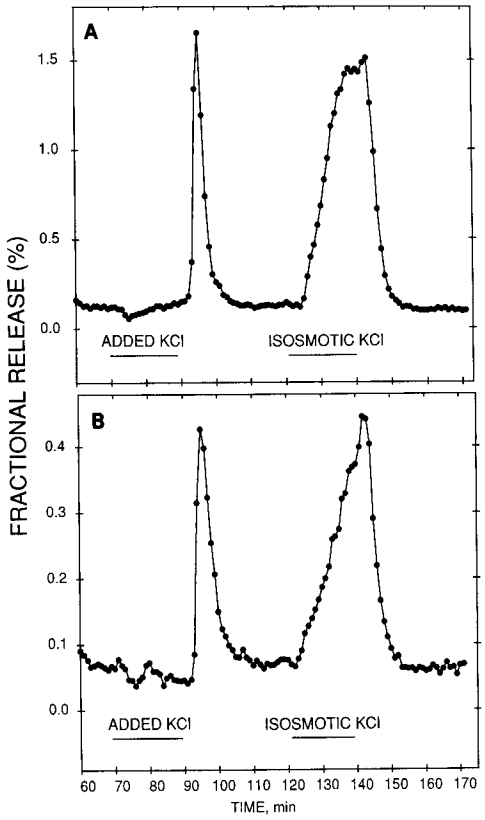

Figure 2. Cerebellar astrocytes and LRM55 cells release taurine when stimulated with isosmotically elevated $[K^+]$ but not with hyperosmotically elevated $[K^+]_o$. The concentration of K^+ was raised to 50 mM by replacing NaCl with KCl (isosmotic replacement) or by adding solid KCl (hyperosmotic addition). *A*, Cerebellar astrocytes; *B*, LRM55 cells.

Electrophysiology. For these experiments, LRM55 cells were grown at a low density to minimize the formation of a glial syncytium, and recordings were made only from single, well-isolated cells. Under normal culture conditions, LRM55 cells are very similar in appearance to astrocytes in primary culture; they have a thin, flat morphology, and will change shape to form rounded process-bearing cells when treated with dibutyryl AMP or β-adrenergic agonists (Shain et al., 1987). Our recordings were made with cultures having the flat morphology (i.e., cells were not treated to form process-bearing cells), and release experiments were done with cells in the same state.

Cells were bathed in HEPES-buffered Hanks' saline during recording and maintained at 34–37°C by a heating block built into the microscope stage. Cells were visualized with interference optics to provide better depth perception. Intracellular electrophysiological measurements were made with glass microelectrodes filled with 3 M potassium acetate. Recordings of membrane potential were accepted as valid only if the potential dropped immediately to a stable value upon penetration and a membrane resistance of about 50 MΩ and reasonable time constant (120 msec) was observed. Because it was difficult to hold any cell longer than 5 min, a recording was considered stable if it did not fluctuate for at least 0.5 min. In most cases, recordings were stable for longer than this. In addition, the recording was not accepted as valid if there was any evidence of cell swelling or the formation of vacuoles.

Results

The membrane potential of LRM55 cells was relatively low in normal HEPES-buffered Hanks' medium (average ± SD = −43 ± 3 mV, n = 20 cells) and was virtually insensitive to changes of $[K^+]_o$ at concentrations below 20 mM (Fig. 1). The cells were substantially depolarized when $[K^+]_o$ was raised to 40 mM or higher, but the slope of the graph (ca. 30 mV/10 fold change in

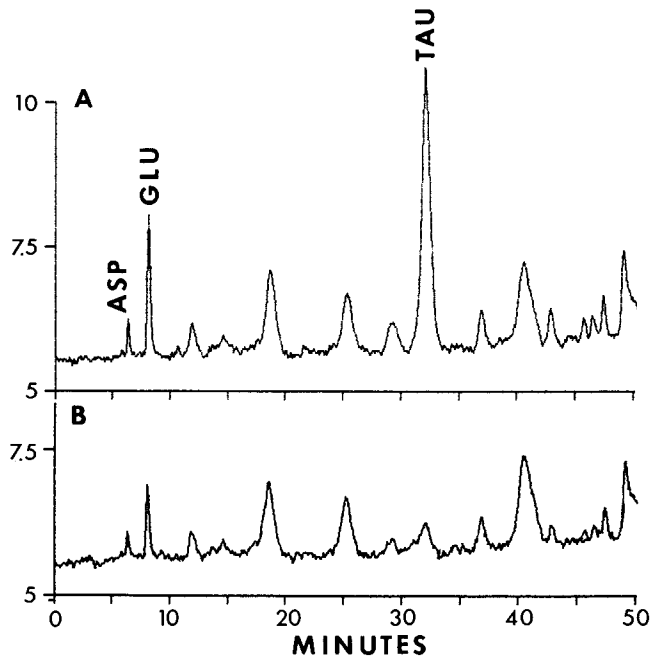

Figure 3. Amino acid analyses of superfusates from LRM55 cells before the application of elevated $[K^+]_o$ (*B*), and at the peak of the response to 50 mM isosmotically elevated $[K^+]_o$ (*A*). The taurine (TAU), glutamate (GLU), and aspartate (ASP) peaks are indicated.

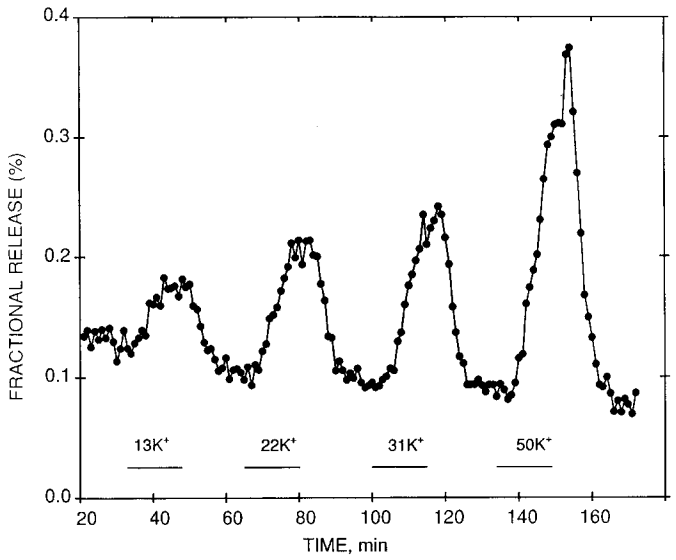

Figure 4. Taurine release from LRM55 cells increases as $[K^+]_o$ is increased. $[K^+]_o$ was elevated isosmotically as described in Materials and Methods.

$[K^+]_o$, Fig. 1) was about half the value predicted by the Nernst equation, if the membrane potential was assumed to be due principally to K^+ permeability. Furthermore, increasing the K^+ permeability of the cell membrane by adding valinomycin, a K^+ ionophore, hyperpolarized the cells at low $[K^+]_o$. These results indicate that ions other than K^+ contribute significantly to the membrane potential in LRM55 cells. More importantly, however, the insensitivity of the membrane potential to $[K^+]_o$ provided an important experimental advantage, as we were able to change $[K^+]_o$ without significantly affecting the membrane potential.

Treatment with high-K^+ medium stimulated taurine release from both LRM55 cells and primary cultures of astrocytes, but only when $[K^+]_o$ was increased isosmotically by replacing NaCl with KCl (Fig. 2, second application). Exposure to high-K^+ medium prepared by hypertonic addition of KCl did not stimulate taurine release, even though $[K^+]_o$ was high enough to depolarize the cells (Fig. 2, first application). A peak of taurine release was observed when the hyperosmotic high-K^+ medium was removed (Fig. 2); this effect is attributable to the change in osmotic pressure following the change of medium (see below). K^+-stimulated taurine release was stable and reproducible; the response did not diminish during repeated applications of 50 mM isosmotically elevated $[K^+]_o$ during a 3-hr perfusion experiment (data not shown). The stimulation of taurine release in isosmotically prepared high-K^+ medium was not attributable to the decrease of $[Na^+]_o$, since taurine release was not stimulated by similar media in which $[Na^+]_o$ was reduced by about 35 mM by isosmotically replacing Na^+ with choline or *N*-methyl-D-glucamine (results not shown). Isosmotic substitution of 35 mM *N*-methyl-D-glucamine for Na^+ did lead to a slight shift in baseline release, but the effect was small compared with the release stimulated by 0.1 μM isoproterenol (IPR).

Taurine was the major amino acid released by LRM55 cells during stimulation with elevated $[K^+]_o$, as shown by analysis of the superfusate by HPLC (Fig. 3). High $[K^+]_o$ also stimulated the release of aspartate and glutamate but to a much smaller degree than taurine; at the peak of release the concentrations of taurine, aspartate, and glutamate were 9.5-, 1.5- and 1.8-fold above their concentrations during baseline release. Although high K^+ did not appear to stimulate the release of other amino acids, the concentration of some was below the detection limit of the HPLC method. Thus, we determined if high $[K^+]_o$would stimulate the release of ^{14}C-lysine, a representative, non-neuroactive amino acid present at a low concentration. Little or no ^{14}C-lysine release was observed with either high $[K^+]_o$ or IPR (data not shown).

As reported for taurine release from other systems (Kaczmarek and Davison, 1972; Oja et al., 1976; Wheler et al., 1979; Smith and Pycock, 1982; Korpi and Oja, 1983; Bernardi et al., 1984; Girault et al., 1986; Hanretta and Lombardini, 1986; Kontro and Oja, 1987), taurine release from cultured glial cells depended strongly on $[K^+]_o$ (Fig. 4). Most importantly, moreover, taurine release was strongly stimulated by low concentrations of external K^+ (13–22 mM) that have little effect on the membrane potential of LRM55 cells (cf. Fig. 1).

The onset of K^+-stimulated taurine release was relatively slow, having a time to peak >10 min (Figs. 2, 4), and the time to peak appeared to increase as the concentration of K^+ increased (Fig. 4). (Times to peak were measured starting 3 min after the application of K^+, since the flow characteristics of the superfusion system result in a 3 min delay between the time a compound is applied and the time it appears in the samples taken for radiochemical measurements.) The times are much longer than the time to peak for IPR-stimulated release, ca. 5 min (Madelian et al., 1985; Shain et al., 1986, 1988; Martin et al., 1987). As reported previously, IPR-stimulated taurine release rises rapidly to a peak and then declines equally rapidly to an elevated steady state which is maintained as long as the drug is applied (Madelian et al., 1985; Shain et al., 1986, 1988; Martin et al., 1987). With prolonged application of elevated $[K^+]_o$ (35 min), K^+-stimulated release also rose to a peak and then de-

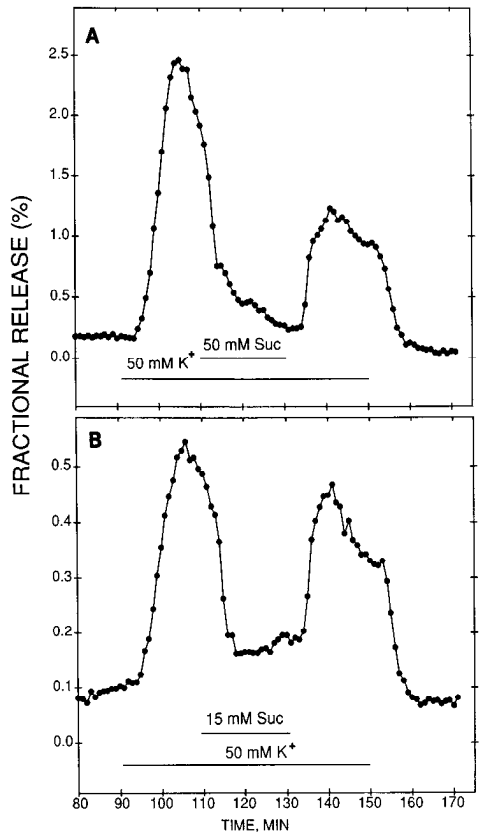

Figure 5. Suppression of K^+-stimulated taurine release from cerebellar astrocytes (*A*) and LRM55 cells (*B*) by sucrose. Taurine release was elicited by applying 50 mM $[K^+]_o$. After a 20 min exposure to 50 mM $[K^+]_o$ the osmotic pressure of the high-K^+ medium was increased by adding 50 mM (*A*) or 15 mM sucrose (*B*) to the medium.

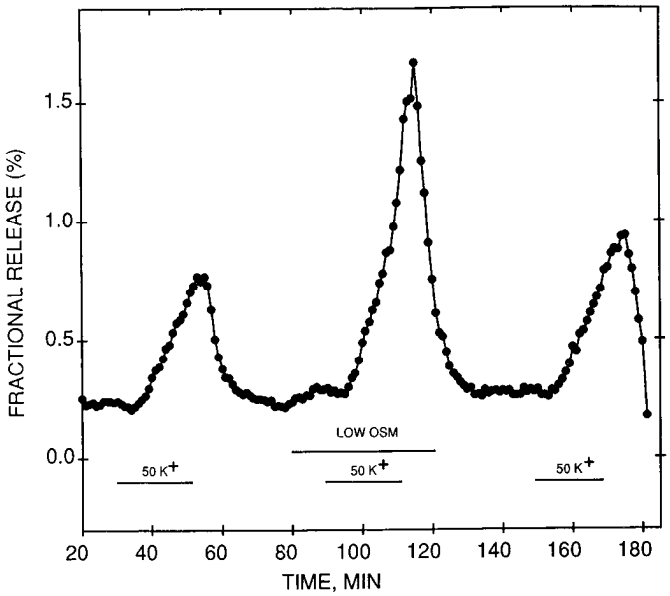

Figure 6. Enhancement of K^+-stimulated taurine release from cerebellar astrocytes by reducing the osmotic pressure of the medium. Taurine release was evoked with successive applications of 50 mM $[K^+]_o$. The osmotic pressure of the medium was reduced by 5% for 10 min before, during, and 10 min after the second application of high-K^+ medium. Media are described in Materials and Methods.

clined, but the decline was slower and much smaller than observed with IPR (data not shown). Although peak rates of release were similar with IPR and K^+, total taurine release was greater with K^+, particularly at higher concentrations, because K^+-stimulated release was sustained at a high rate for a longer period.

The observation that high $[K^+]_o$ stimulates taurine release from astroglial cells when elevated isosmotically but not when added hyperosmotically (Fig. 2) suggested that the transmembrane osmotic pressure difference is an important factor in taurine release. Subsequent experiments indicated that taurine release is extremely sensitive to the osmolarity of the medium. K^+-stimulated taurine release by both LRM55 cells and primary cultures of astrocytes was suppressed by elevating the osmolarity of the medium with sucrose (Fig. 5). Raising the osmotic pressure of the medium with NaCl had the same effect (data not shown). Conversely, reducing the osmotic pressure of the medium greatly enhanced K^+-stimulated taurine release (Fig. 6). Only small changes in osmolarity were necessary to affect the response to KCl; a 5% increase in osmolarity (15 mM added sucrose) suppressed the response to 50 mM $[K^+]_o$ almost completely (Fig. 5*B*), and a 5% decrease in osmolarity substantially increased the response to $[K^+]_o$ (Fig. 6).

K^+-stimulated taurine release was also Cl^- dependent. As shown in Figure 7, K^+-stimulated release was greatly diminished when $[Cl^-]_o$ was reduced to 12.33 mM (second application) from the concentration (136 mM) present during the first and third applications of high-K^+ medium and in the baseline perfusate (HEPES-buffered Hanks'). $[K^+]_o$ was isosmotically elevated from 4.54 to 50 mM during each application. We reduced $[Cl^-]_o$ to 12.33 mM to keep the $[K^+]\cdot[Cl^-]$ product equal to that of control medium and thereby minimize the ion shifts and accompanying swelling resulting from Gibbs-Donnan equilibrium forces.

The baseline rate of taurine release was also affected by small changes in the osmolarity of the medium (Fig. 8). Baseline release was increased by a small reduction in osmolarity (7.5 mOsM) and was suppressed significantly by raising the osmolarity by as little as 9 mOsM, whether osmotic pressure was increased with sucrose or NaCl. The calculated osmolarity of control HHA is 300 mOsM. Thus, changing the osmolarity by only about 2.5% significantly affected baseline taurine release. Similar results were observed with cerebellar astrocytes; the baseline rate of release was 124 ± 2% of control when osmolarity was reduced by 5% (mean ± SEM, $n = 6$) and was 68 ± 3% of control when the osmolarity was increased 20 mOsM by adding sucrose. Similar changes in baseline were observed when the osmolarity was reduced by manipulating sucrose at a constant reduced ionic strength. Thus, the baseline rate of release increased to 160 ± 10% of control when the medium was changed from one containing 7.5 mM sucrose and 97.5% of the normal levels of salts to a medium containing the same levels of salts but no sucrose.

The time course of the shift in the baseline differed somewhat with the change in osmolarity (Fig. 9). When the osmolarity was raised, baseline release immediately decreased and remained low until control HHA was reapplied to the cells. At that time, there was often a peak of release followed by a return to the baseline originally observed with HHA. When the osmolarity of the medium was reduced by a relatively small amount (e.g., 5%), baseline release rapidly increased to a new steady-state rate. With a larger reduction in osmolarity (ca. 10%), release increased rapidly to a peak and then declined to a new, elevated

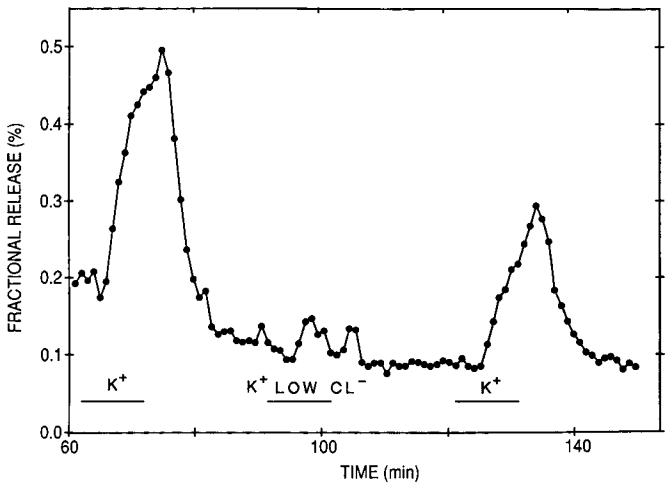

Figure 7. K^+-stimulated taurine release by LRM55 cells is Cl^- dependent. During the first and third applications, release was evoked with isosmotically elevated 50 mM $[K^+]_o$. For the second application, $[K^+]_o$ and $[Cl^-]_o$ were isosmotically adjusted to 50 and 12.33 mM by replacing KCl and partially replacing NaCl with appropriate amounts of potassium gluconate and sodium gluconate. All other components of the medium were unchanged.

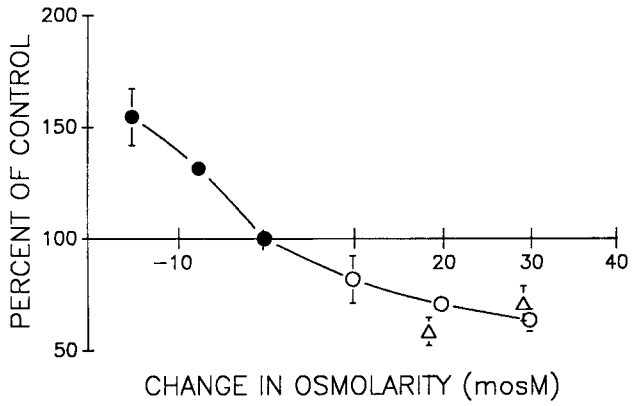

Figure 8. Baseline taurine release by LRM55 cells as a function of the osmolarity of the perfusion medium. The osmotic pressure of the medium was increased by adding sucrose (○) or NaCl (△) and was decreased (●) as described in Materials and Methods. Similar effects were observed with cerebellar astrocytes (see text). The shift in baseline was determined by averaging the fractional release rate over a 10–12 min period before application of the altered medium and after the new steady-state rate of release was attained.

baseline that remained elevated until the medium was changed (30 min in the longest experiments).

Discussion

The high sensitivity of both baseline and K^+-stimulated taurine release to the osmolarity of the medium suggests that physical tension on the cell membrane is an important controlling element in taurine release—that is, treatments that increase membrane tension will increase taurine release, and treatments that decrease membrane tension will decrease release. Exposure to media with low osmolarity causes astroglial swelling (Kimelberg and Goderie, 1988), undoubtedly an increase in membrane tension, and taurine release (our data and Pasantes-Morales and Schousboe, 1988). Similarly, cultured astrocytes swell in isosmotic high-K^+ medium (Walz, 1987; Walz and Mukerji, 1988) and release taurine (our data and Philibert et al., 1988). Conversely, raising the osmotic pressure of the medium, which inhibits taurine release, would be expected to cause cells to shrink and relieve the tension on the membrane. The Cl^- dependence and time course of K^+-stimulated release is also consistent with the swelling and membrane-tension hypothesis. K^+-induced swelling appears to result from the accumulation of high levels of KCl by the cells (Walz, 1987; Walz and Mukerji, 1988), and we observed little or no K^+-stimulated taurine release in a low-Cl^- medium designed to minimize swelling due to Gibbs-Donnan forces. We also have found that KCl uptake by LRM55 cells is relatively slow, requiring more than 10 min to come to steady state at 37°C (Wolpaw and Martin, 1984), a time that correlates well with the 10–15 min time to peak of K^+-stimulated taurine release. This mechanism is also consistent with the apparent increase in time to peak as $[K^+]_o$ is increased, since the time required to complete the ion movements would be greater at higher $[K^+]_o$, where net ion movements are greater (Wolpaw and Martin, 1984).

In recent years, Sachs and coworkers have described transmembrane ion channels that respond to changes in membrane tension in a variety of cell types, including astrocytes (Guharay and Sachs, 1984, 1985; Ding et al., 1988). These channels, termed stretch channels, open when membrane tension is increased and close when the membrane is relaxed. Although all of the stretch channels described to date are cation selective and none appear to have appropriate properties to account for taurine release, they are a well-documented example of a specific, tension-dependent permeability mechanism.

The role of membrane potential in release processes is complex. In neurons and other cells that have vesicular release mechanisms, depolarization opens voltage-gated Ca^{2+} channels leading to an influx of Ca^{2+} that activates the release mechanism. Not surprisingly, this mechanism does not appear to apply to glial taurine release. LRM55 cells readily release taurine when $[K^+]_o$ is isosmotically increased to 10 or 20 mM but are not

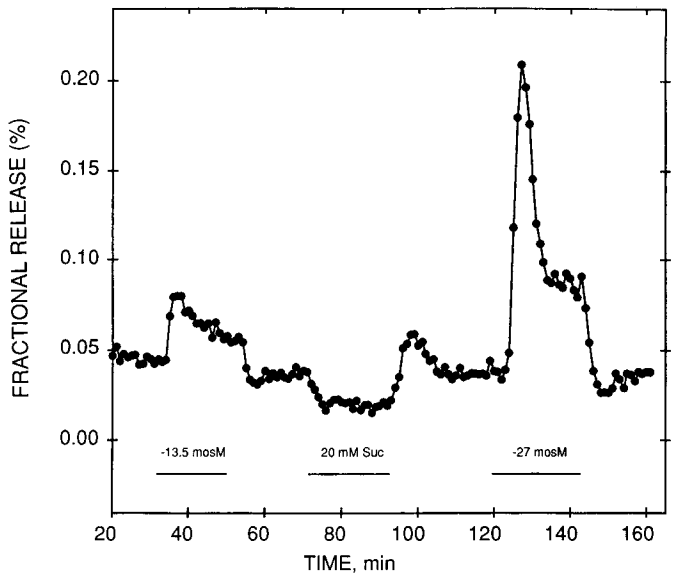

Figure 9. Time course of changes in taurine release from LRM55 cells following changes in osmotic pressure. At the indicated times the osmotic pressure of the medium was increased by adding sucrose (*Suc*) or reduced as described in Materials and Methods.

appreciably depolarized by these concentrations of external K^+. Furthermore, neither LRM55 cells nor cultured astrocytes release taurine when $[K^+]_o$ is hyperosmotically increased to 50 mM, even though the cells are depolarized by this concentration of K^+. Thus, in this sense, K^+-stimulated release is not attributable to depolarization. However, changes in membrane potential may play a role in the ion movements that we believe underlie K^+-stimulated taurine release. It is possible that a small increase of $[K^+]_o$ does depolarize the cells sufficiently to promote Cl^- influx, but the depolarization could not be detected in our experiments because the variability between cells was too great to detect the difference. We could not hold individual cells for a long enough time to measure changes in membrane potential during a change in medium.

Glial taurine release differs in another fundamental way from transmitter release at the synapse, as glial release is not Ca^{2+} dependent (Martin et al., 1989; Shain et al., 1989). We have found that glial taurine release is not blocked by using Ca^{2+}-free medium containing 10 μM EGTA, by replacing extracellular Ca^{2+} with Co^{2+}, Cd^{2+}, or Mn^{2+}, or by the organic Ca^{2+}-channel blockers verapamil, diltiazem, or nifedipine (Martin et al., 1989). Most importantly, the intracellular $[Ca^{2+}]$ of LRM55 cells, as measured with the fluorescent probe FURA-2, is unaffected by elevated $[K^+]_o$ or IPR at concentrations that stimulate release (Shain et al., 1989). We and others (Philibert et al., 1988; Martin et al., 1989) have found that taurine release is inhibited by 10 mM $MgCl_2$ in Ca^{2+}-free medium, but this is attributable to elevated osmotic pressure and is not a sign of Ca^{2+} dependency, since added NaCl and sucrose inhibit similarly.

The characteristics of K^+-stimulated release from cultured astrocyte cells is similar in many ways to K^+-stimulated taurine release in intact tissue preparations, suggesting that release from astrocytes is at least partially responsible for K^+-evoked taurine release from brain tissue. As shown here, K^+-evoked taurine release is relatively slow in astroglial cells, and K^+-stimulated taurine release from brain slices, retina, or *in vivo* lags behind the release of neurotransmitter amino acids such as GABA, glycine, or glutamate (Smith and Pycock, 1982; Girault et al. 1986; Kontro and Oja, 1987). As noted above, glial taurine release is Ca^{2+}- independent. In most other systems, K^+-stimulated taurine release appears to be independent of external Ca^{2+} or to have a large component of Ca^{2+}-independent release. K^+-stimulated taurine release from dorsal medulla (Bernardi et al., 1984) and striatum (Girault et al., 1986) *in vivo* is Ca^{2+} independent, and release from cerebellum *in vivo* is partially Ca^{2+} dependent (Bernardi et al., 1984). With brain slices, K^+-stimulated taurine release was reduced or delayed but not eliminated in studies of Ca^{2+} dependency (Kaczmarek and Davison, 1972; Oja et al., 1976; Wheler et al., 1979; Korpi and Oja, 1983). These similarities suggest that KCl accumulation and cellular swelling contribute to taurine release *in vivo* and in brain slices. It has been known for many years that brain and brain slices swell in isosmotic high-$[K^+]_o$ medium (Varon and McIlwain, 1961; Bourke and Tower, 1966; Bourke et al., 1970).

K^+-stimulated taurine release may be a normal physiological response of astrocytes *in vivo*. $[K^+]_o$ rises significantly during normal neuronal activity and can reach values of 10–12 mM during periods of extreme activity such as seizures (Moody et al., 1974; Sypert and Ward, 1974; Heinemann and Lux, 1977). The increase in $[K^+]_o$ is accompanied by a decrease in extracellular space, indicating that the cells are swelling (Dietzel et al. 1980). This swelling has been attributed to osmotic imbalances across the astrocytic membrane due to the movement of K^+ and anions resulting from spatial buffering and changes in the HCO_3^-/Cl^- balance (Dietzel et al., 1980; Kimelberg and Ransom, 1986). In our view, elevated extracellular K^+ and the associated astrocytic swelling will lead to taurine release, as almost identical conditions cause taurine release from cultured astrocytes. Taurine is also released from brain tissue *in vivo* under other conditions that are accompanied by glial swelling, including seizures, ischemia, hypoglycemia, and perfusion with hypotonic medium (Benveniste et al., 1984; Tossman et al., 1985; Vezzani et al., 1985; Butcher and Hamberger, 1987; Solis et al., 1988; Wade et al., 1988). It has been suggested that this taurine release might have an osmoregulatory function (Pasantes-Morales and Schousboe, 1988; Solis et al., 1988; Wade et al., 1988). Although osmotic sensitivity might suggest osmoregulation, it is not clear that taurine is present or is released in sufficient amounts in brain to substantially change the osmotic balance across the cell membrane (Martin et al., 1990). An alternative mechanism of osmoregulation might be envisaged (e.g., a hormone-like action of taurine), but there is no evidence for such a mechanism. Taurine does have inhibitory effects on certain neurons (Krnjevic and Puil, 1976; Okamoto et al, 1983), and taurine release might have a dampening effect on neuronal activity under conditions where swelling occurs, thereby reducing the further release of K^+ and preventing some additional swelling.

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