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Analysis of hyposmolarity-induced taurine efflux pathways in the bullfrog sympathetic ganglia

S. Sakai*, T. Tosaka

Department of Physiology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan

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Abstract

Hyposmolarity-induced taurine release was dependent on the decrease in medium osmolarity (5–50%) in the satellite glial cells of the bullfrog sympathetic ganglia. Release of GABA induced by hyposmolarity was much less than that of taurine. Omission of external Cl^- replaced with gluconate totally suppressed taurine release, but only slightly suppressed GABA release. Bumetanide and furosemide, blockers of the $Na^+/K^+/2Cl^-$ cotransport system, inhibited taurine release by about 40%. Removal of external Na^+ by replacement with choline, or omission of K^+, suppressed taurine release by 40%. Antagonists of the Cl^-/HCO_3^- exchange system, SITS, DIDS and niflumic acid, significantly reduced taurine release. The carbonic anhydrase inhibitor, acetazolamide, reduced the taurine release by 34%. Omission of external HCO_3^- by replacement with HEPES caused a 40% increase in the hyposmolarity-induced taurine release. Hyposmolarity-induced GABA release was not affected by bumetanide or SITS. Chloride channel blockers, 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and *N*-phenylanthranilic acid (DPC), practically abolished taurine release. Blockers of K^+ channels, clofilium and quinidine, had no effect on the taurine release. The hyposmolarity-induced taurine release was considerably enhanced by a simultaneous increase in external K^+. GABA was not mediated by the same transport pathway as that of taurine. These results indicate that Cl^- channels may be responsible for the hyposmolarity-induced taurine release, and that $Na^+/K^+/2Cl^-$ cotransporter and Cl^-/HCO_3^- exchanger may contribute to maintain the intracellular Cl^- levels higher than those predicted for a passive thermodynamic distribution in the hyposmolarity-induced taurine release.

Keywords: Taurine efflux; Hyposmolarity; Satellite glia; Cl^- channels; GABA

1. Introduction

Taurine (β-aminoethanesulfonic acid) is well known as one of the most abundant amino acids throughout the nervous system (Oja and Kontro, 1983). Taurine has been thought to be an organic osmolyte in many cell types such as astrocytes (Pasantes-Morales and Schousboe, 1989; Pasantes-Morales et al., 1990; Kimelberg et al., 1990b), C6 glioma cells (Jackson and Strange, 1993), Madin-Darby canine kidney (MDCK) cells (Sánchez-Olea et al., 1991), erythrocytes (Kirk et al., 1992) and Ehrlich ascites tumor cells (Lambert and Hoffmann, 1993). Morán et al. (1994) showed that taurine deficient astrocytes exhibited less efficient volume recovery.

Although the mechanism for uptake of taurine has been well defined and is known to involve a Na^+-dependent cotransport system in neuronal (Oja and Kontro, 1983) and glial cells (Sakai et al., 1989), the efflux pathways in situ glial cells is little known. When glial cells are exposed to hypotonic media, they initially swell and then show a regulatory volume decrease (RVD) (Kimelberg and Frangakis, 1985; Olson et al., 1986). RVD is accompanied by net efflux of organic osmolytes (Vitarella et al., 1994; Pasantes-Morales and Schousboe, 1989) or loss of KCl (Hoffmann and Simonsen, 1989).

There is some evidence that the volume-activated

* Corresponding author. Tel.: +81-3-3351-6141 ex. 323; fax: +81-3-3351-6544.

E-mail address: s-sakai@tokyo-med.ac.jp (S. Sakai)

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taurine efflux from fish erythrocytes is inhibited by band 3 inhibitors such as *N*-(azido-2-nitrophenyl)-2-amino sulfonic acid (NAP)-taurine, niflumic acid and stilbene disulfonates, suggesting that the efflux involves the anion exchanger band 3 (Goldstein and Brill, 1991; Goldstein et al., 1990).

Recent studies by Roy (1994) and Sánchez-Olea et al. (1995) showed a possible involvement of anion channels in the volume-activated organic osmolite release. Kirk et al. (1992) proposed the idea that the volume-activated transport of organic osmolites is via a single pathway with the characteristics of a volume-activated 'chloride channel' in flounder erythrocytes. Also, Jackson and Strange (1993) reported that a volume-sensitive anion channel mediates the efflux of structurally diverse organic osmolytes such as taurine and inositol from the C6 glioma cells.

Most of these experiments were performed on cultured cells or erythrocytes. There is little information about whether these systems exist in intact glial cells and whether these systems are involved in volume-activated osmolite release.

We previously reported that [^{3}H]taurine and [^{3}H]GABA are taken up by only satellite glial cells, not by principal neurons, in the frog sympathetic ganglia (Sakai et al., 1989; Tasaka et al., 1989). The purpose of the present study is to define a possible contribution of Cl^- transport systems, namely, the $Na^+/K^+/2Cl^-$ cotransporter, the Cl^-/HCO_3^- exchanger, and Cl^- channels to the hyposmolarity-induced taurine release from the sympathetic ganglia. Our second aim is to determine whether structurally related compounds such as taurine and GABA, could be released by the same systems. The results show that Cl^- channels are strongly related to the hyposmolarity-induced taurine release in peripheral glial cells, and that the release pathways are apparently distinct from that of GABA.

2. Experimental procedures

2.1. Tissue preparation

Lumbar sympathetic chains consisting of the VIIth to Xth ganglia of the bullfrog (*Rana catesbeiana*) were excised and connective tissues were carefully removed. The isolated chain was tied by silk thread at each end. One of the bilateral sympathetic chains was used for the test and the other as a control. Animal welfare was in accordance with the guidelines of Tokyo Medical University.

2.2. Taurine uptake

The uptake studies were carried out as previously described (Sakai et al., 1989). Briefly, to equilibrate, the sympathetic chains were preincubated at 25°C in the medium with or without inhibitors for 30 min and then incubated with the same medium containing [^{3}H]taurine (0.46 μM or 10 μM) for 90 min. After incubation, each chain was rinsed with fresh medium and homogenized in 1 ml of 10% trichloroacetic acid. The homogenate was transferred to a scintillation vial and 0.2 ml of Ethylcellosolve and 10 ml of Aquasol-2 were added. The radioactivity was determined by a Packard liquid scintillation counter (2250CA) at 4°C. The counting efficiency was 45% for ^{3}H. Taurine uptake was expressed as a percentage of the control value.

2.3. Release of taurine and GABA

Prior to the release experiment, the chains were incubated at 25°C with [^{3}H]taurine (0.46 μM) or [^{3}H]GABA (0.26 μM) in Ringer's solution for 90 or 60 min, respectively. Aminooxyacetic acid (AOAA, 10 μM), an inhibitor of GABA transaminase, was added in the medium throughout the GABA experiment. After incubation, each chain was rinsed with Ringer's solution every 3 min for 15 min and transferred to a superfusion chamber (0.2 ml in volume) connected to a peristalic pump, which allowed to superfuse at a flow rate of 0.81 ml/min. After washing with isosmotic solution for 30 min to stabilize basal efflux, fractions of the perfusate were collected at 1 min intervals. An aliquot (0.5 ml) of each sample was transferred to a vial and 5 ml of Aquasol-2 was added. The basal efflux was combined for 5 min. By switching the flow through a three-way valve, the chain was then stimulated with hyposmotic or high K^+ solution for the desired time. The superfusion medium in the chamber was completely replaced with the desired medium within 50 s. Subsequently, the chain was returned to normal Ringer's solution for 10 or 15 min. At the end of superfusion, the chain was homogenized in 1 ml of 10% trichloroacetic acid, and 0.2 ml ethylcellosolve and 10 ml Aquasol-2 were added. The radioactivity of each fraction and that remaining in the chain was determined by a liquid scintillation counter. Results of the perfusion experiments were expressed as percentages of the isotope [^{3}H]taurine left in the chains at various time points. Data in the inhibition studies were expressed as percentages of control in a parallel experiment without inhibitors. Drugs and various media were applied when the chain was transferred to a superfusion chamber and were present during the washing (30 min) and testing periods (25–50 min).

2.4. Solutions

Ringer's solution contained (in mM): NaCl 112,

KCl 2.0, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 2.4, glucose 5, pH 7.4 and osmotic pressure 247.1 mosM. Hyposmotic solutions (5 to 50%) were prepared by reducing the concentration of NaCl, while the other components remained unchanged. The osmolality of the solutions was measured routinely using a freezing-point osmometer (Advanced Instruments, MA, U.S.A.). Reductions of 5, 10, 20, 30, 40 and 50% NaCl corresponded to 235.0, 224.7, 202.3, 179.9, 157.5 and 135.1 mosM, respectively. SD was within 2%. Both isosmotic and hyposmotic Cl^--free media were obtained by replacing NaCl, KCl and $CaCl_2$ with their corresponding gluconate salts. Potassium-free medium was obtained by omitting KCl. High K^+ (20 and 60 mM) media were prepared by substituting NaCl by equimolar amounts of KCl. When the ionic product of $K^+ \times Cl^-$ was kept constant, 60 mM Na^+ was replaced with 60 mM K^+ and the Cl^- concentration was reduced to 3.95 mM. The impermeant anion gluconate was used to replace the omitted Cl^-. In experiments with Na^+-deficient medium, choline chloride was substituted for NaCl. Bicarbonate-free medium was made by substitution of HEPES for $NaHCO_3$.

2.5. Materials

[2-^{3}H(N)]taurine (810.3 GBq/mmol), γ-[2,3-^{3}H(N)]-aminobutyric acid (GABA, 1433.0 GBq/mmol) and Aquasol-2 scintillation cocktail were purchased from New England Nuclear (Boston, MA, U.S.A.). Bumetanide, furosemide 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), anthracene-9-carboxylate (9AC), niflumic acid, and quinidine were obtained from Sigma Chemicals Co. (St Louis, MO, U.S.A.). Clofilium, 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and N-phenylanthranilic acid (DPC) were obtained from Research Biochemicals International (Natick, MA, U.S.A.). All other chemicals were of the purest grade available from regular commercial sources. When ethanol or DMSO was used as the solvent for a given reagent, appropriate control experiments were conducted.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. Statistical analysis was performed using Student's paired *t*-test. A *P* value of less than 0.05 was taken to indicate significance.

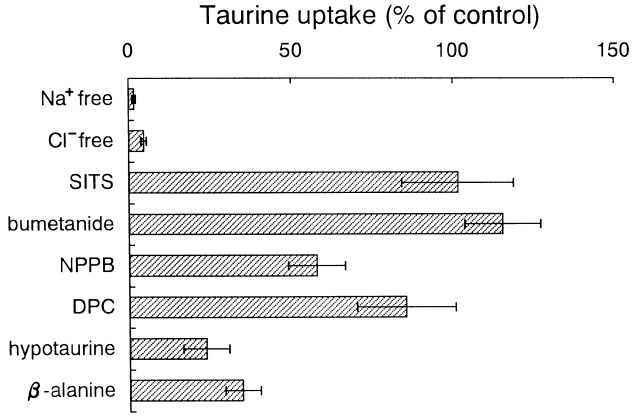

Fig. 1. Effects of Na^+ free (replaced with choline), Cl^- free (replaced with gluconate), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS, 200 μM), bumetanide (100 μM), 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB, 100 μM), *N*-phenylanthranilic acid (DPC, 200 μM), hypotaurine (50 μM), and β-alanine (50 μM) on the uptake of [^{3}H]taurine (0.46 μM, except in cases of hypotaurine and β-alanine in which it was 10 μM) in the bullfrog sympathetic chain containing VII to Xth ganglia. One of the bilateral sympathetic chains was equilibrated with a given medium or a drug from 30 min before the uptake experiment and incubated for 90 min. The uptake in the other was performed in Ringer's solution without any treatment as a control. The results are expressed as percentages of control (means ± S.E.M., $n = 4 \sim 9$, $P < 0.001$ for Na^+ free, and Cl^- free, $P < 0.05$ for NPPB, hypotaurine, and β-alanine).

3. Results

3.1. Taurine uptake

Uptake of [^{3}H]taurine into glial cells of the sympathetic ganglia was dependent on both external Na^+ and external Cl^- (Fig. 1). Chloride channel blockers, NPPB (100 μM) and DPC (200 μM), inhibited the taurine uptake by 42 and 14%, respectively. However, the uptake was unaffected either by 200 μM SITS, an inhibitor of the anion exchanger and Cl^- channels, or by 100 μM bumetanide, a specific blocker of the $Na^+/K^+/2Cl^-$ cotransport system. Structural analogs of taurine, hypotaurine, and β-alanine at concentrations of 50 μM significantly inhibited the [^{3}H]taurine (10 μM) uptake (Fig. 1).

3.2. Hyposmolarity-induced release of [^{3}H]taurine and [^{3}H]GABA

Decreasing the osmolarity of the medium enhanced the release of [^{3}H]taurine from the sympathetic ganglia with a magnitude dependent on the decrease in osmolarity (Fig. 2A). The release reached a peak at 6–9 min after exposure to hyposmotic solutions. During the subsequent minutes, [^{3}H]taurine release gradually declined, despite the persistence of hyposmotic conditions. We used stimulation of 30% hyposmolarity for the following experiment of [^{3}H]taurine release

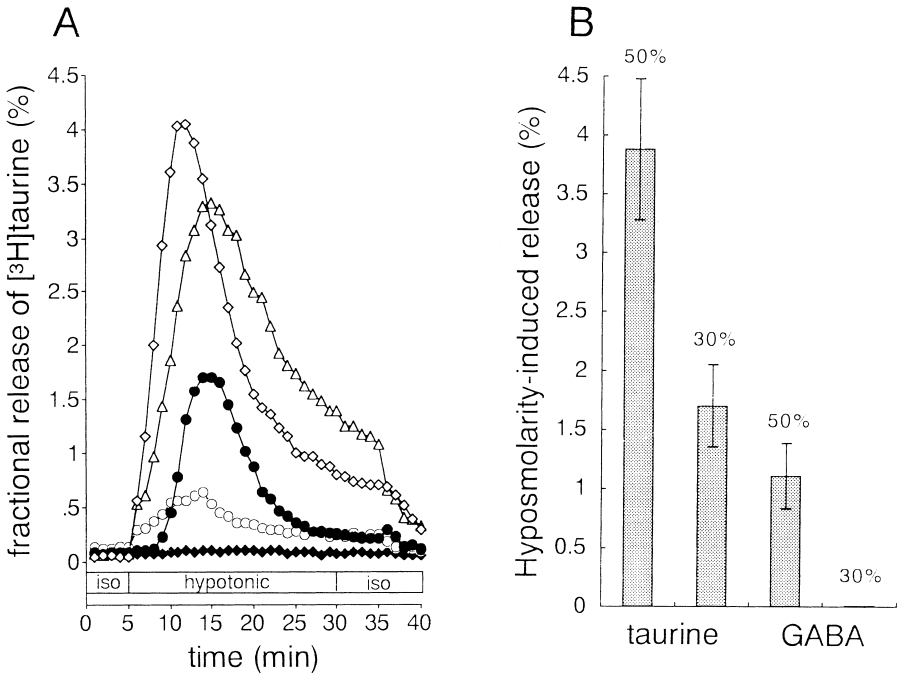

Fig. 2. (A) The times courses of [^{3}H]taurine release from the bullfrog sympathetic chain induced by media with different osmolarities, prepared by 10% (–◆–), 20% (–○–), 30% (–●–), 40% (–△–), and 50% (–◇–) reductions of NaCl. The chain was preloaded with [^{3}H]taurine (0.46 μM) for 90 min. After five washes with fresh Ringer's solution for 15 min, the preparation was mounted on a 0.2 ml superfusion chamber and perfused with isosmotic solution for 30 min to stabilize basal efflux. Samples were collected at 1 min intervals. The bar represents the period of the iso- and hyposmolarity. The experimental procedure was the same in the following figures unless otherwise mentioned. The radioactivity of each sample was expressed as a percentage of [^{3}H]taurine left in the chain at each time point. (B) Comparison of the releases of [^{3}H]taurine and [^{3}H]GABA (0.26 μM) induced by 30 and 50% hyposmotic solutions. The experiment of [^{3}H]GABA release was performed in the same way as described in the [^{3}H]taurine release, except the medium contained 10 μM aminooxyacetic acid (AOAA) and the preloading time was 60 min. Note that the release of [^{3}H]taurine by 30% hyposmotic solution is larger than that of [^{3}H]GABA by 50% hyposmotic solution. Results are means ± S.E.M. ($n = 3 \sim 5$, $P < 0.001$ for 30%, $P < 0.05$ for 50%).

unless otherwise mentioned, since appropriate radioactivity and good reproducibility could be obtained. On the other hand, a reduction in osmolarity (30 and 50%) also induced an increase in GABA release (Fig. 2B), although the release was quite small compared to that of taurine.

3.3. Removal of external Cl^-

Omission of external Cl^- replaced with gluconate

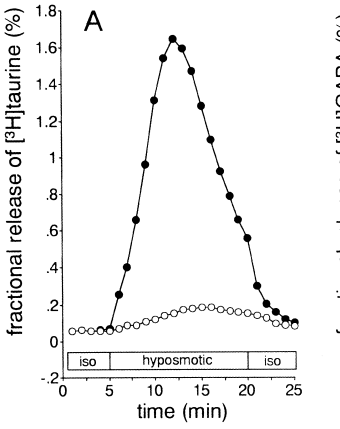

Fig. 3. Effects of Cl^- free on the release of [^{3}H]taurine (A) and [^{3}H]GABA (B) evoked by 30% (A) and 50% (B) reduction of the osmolarity. One of the bilateral sympathetic chains was tested in the Cl^- free medium (–○–) and the other in normal Ringer's solution as a control (–●–). Typical curves in the 6-pair (A) and 3-pair (B) experiments are shown.

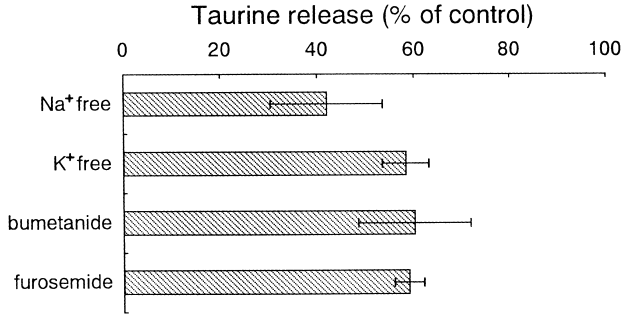

Fig. 4. Effects of Na^+ free, K^+ free, bumetanide (100 μM) and furosemide (200 μM), conditions impairing the $Na^+/K^+/2Cl^-$ cotransport system, on the [^{3}H]taurine release evoked by 30% hyposmolarity. Data are expressed as percentages of control (means ± S.E.M., $n = 3$). All values are significantly different from control ($P < 0.05$).

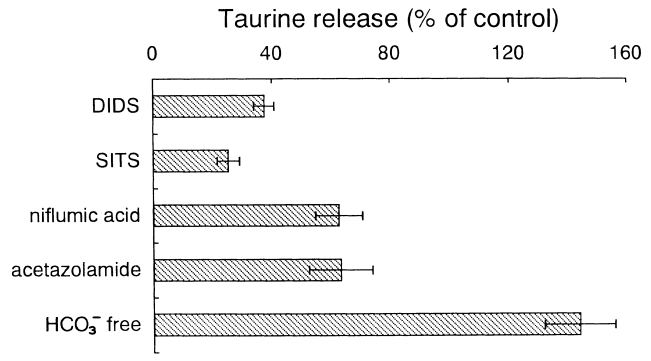

Fig. 5. Effects of an antagonism of the Cl^-/HCO_3^- exchange system by DIDS (200 μM), SITS (200 μM), niflumic acid (200 μM), and of changes in the concentration gradient of HCO_3^- across the membrane by acetazolamide (2 mM) and omission of external HCO_3^- on the hyposmolarity-induced [^{3}H]taurine release. Data (means ± S.E.M., $n = 3$) are expressed as percentages of control. All values are significantly different from control ($P < 0.05$).

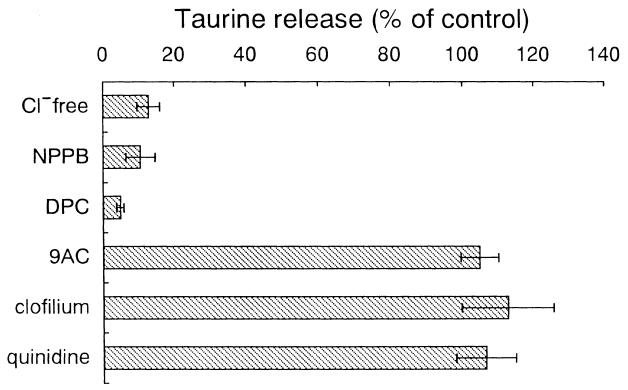

Fig. 6. Effects of Cl^- free, Cl^- channel blockers of NPPB (100 μM), DPC (1 mM), anthracene-9-carboxylate (9AC, 200 μM), and K^+ channel blockers of clofilium (100 μM) and quinidine (100 μM) on the hyposmolarity-induced [^{3}H]taurine release. Data are expressed as percentages of control (means ± S.E.M., $n = 3$). $P < 0.05$ for Cl^- free, NPPB, and DPC.

totally suppressed the hyposmolarity (30%)-induced [^{3}H]taurine release (Fig. 3A), but suppressed only 40% of the hyposmolarity (50%)-induced [^{3}H]GABA release (Fig. 3B). The spontaneous efflux of [^{3}H]GABA was increased significantly by removal of external Cl^-, whereas that of taurine remained unchanged, as indicated by the release prior to the application of low osmotic medium.

3.4. $Na^+/K^+/2Cl^-$ cotransport system

Replacement of external Na^+ with choline inhibited the [^{3}H]taurine release by 53% (Fig. 4). Omission of K^+ from the superfusion medium also inhibited the release by 40% (Fig. 4). The [^{3}H]taurine release was entirely Cl^--dependent (Figs. 3A and 6). We tested the effects of $Na^+/K^+/2Cl^-$ cotransport inhibitors, bumetanide and furosemide, on the [^{3}H]taurine release. Concentrations of the inhibitors known to be effective in blocking the transport systems were used in the following experiments unless otherwise mentioned. Both bumetanide (100 μM) and furosemide (200 μM) inhibited about 40% of the [^{3}H]taurine release evoked by hyposmolarity (Fig. 4). On the contrary, bumetanide had no effect on the hyposmolarity (50%)-induced [^{3}H]GABA release (114.8 ± 13.7%, $n = 3$).

3.5. Cl^-/HCO_3^- exchange system

Omission of external HCO_3^- was tested because it may potentiate the anion (Cl^-/HCO_3^-) exchange system by steepening the outward concentration gradient of HCO_3^-. Substitute of HEPES for external $NaHCO_3$ increased by 40% the hyposmolarity-induced taurine release (Fig. 5). The carbonic anhydrase inhibitor, acetazolamide (2 mM) reduced the taurine release by 34% (Fig. 5).

SITS, DIDS, and niflumic acid were used as the antagonists of the Cl^-/HCO_3^- exchange system (Fig. 5).

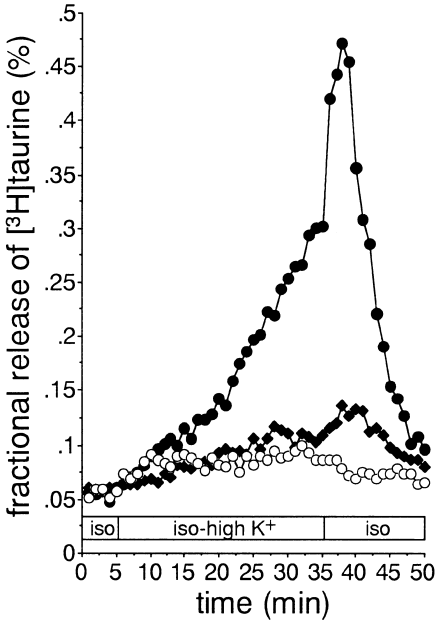

Fig. 7. The time courses and Cl^- dependence of the high K^+-induced [^{3}H]taurine release from the bullfrog sympathetic chain. The chain was preloaded with [^{3}H]taurine (0.46 μM) for 90 min. After five washes with fresh Ringer's solution for 15 min, the preparation was transferred to a superfusion chamber and perfused with Ringer's solution for 30 min to stabilize basal efflux. Samples were collected at 1 min intervals. The bar indicates the period of isosmotic Ringer's solution and high K^+ stimulation. High K^+ media were prepared by substituting NaCl by 20 mM (–◆–) and 60 mM (–●–) KCl. High K^+ (60 mM)-stimulated [^{3}H]taurine release in Cl^- free medium was given by open circles (–○–). Values represented the mean of 4 experiments. S.E.M. was omitted to simplify the figure.

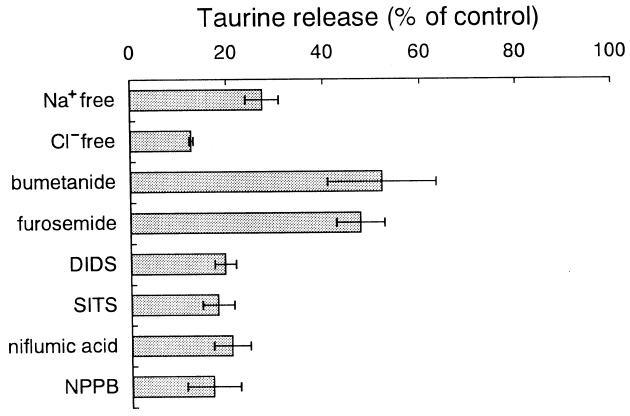

Fig. 8. Effects of Na^+ free, Cl^- free, blockers of the $Na^+/K^+/2Cl^-$ cotransporter, bumetanide (100 μM) and furosemide (200 μM), antagonists of the Cl^-/HCO_3^- exchanger, DIDS (200 μM), SITS (200 μM) and niflumic acid (200 μM), Cl^- channel blocker, NPPB (100 μM) on the high K^+ (60 mM)-induced [^{3}H]taurine release. Data (means ± S.E.M., $n = 3 \sim 6$) are expressed as percentages of control. All values are significantly different from control ($P < 0.05$).

The stilbene derivatives, DIDS and SITS, at a concentration of 200 μM, inhibited the hyposmolarity-induced taurine release by 61 and 76%, respectively. DIDS (200 μM) also suppressed the [^{3}H]GABA release evoked by hyposmolarity (42.3 ± 7.9%, $n = 3$). Niflumic acid (200 μM) inhibited taurine release by 35%. None of these compounds affected the basal efflux of taurine.

3.6. Cl^- and K^+ channels

Chloride channel blockers, NPPB at a concentration of 100 μM and DPC at a high concentration of 1 mM practically abolished the hyposmolarity-induced [^{3}H]taurine release (Fig. 6). DPC at concentrations of 200 and 500 μM inhibited the release by 16 and 57%, respectively. Another Cl^- channel blocker, anthracene-9-carboxylate (Pasantes-Morales et al., 1990) at 200 μM had no effect on the [^{3}H]taurine release. Blockers of K^+ channels, clofilium and quinidine at a concentration of 100 μM failed to modify the release of [^{3}H]taurine evoked by hyposmolarity (Fig. 6).

3.7. High K^+-induced [^{3}H]taurine release

Exposure of sympathetic ganglia to solutions containing high concentrations of K^+ (20 and 60 mM) resulted in concentration dependent increases in the release of [^{3}H]taurine (Fig. 7). At 60 mM K^+, the release of [^{3}H]taurine was markedly enhanced, and peak taurine efflux was seen just after the end of high K^+ stimulation. High K^+ (60 mM) stimulation in Cl^- free medium failed to increase the taurine efflux (Fig. 7). Lowering the Cl^- concentration in the medium to maintain a constant $K^+ \times Cl^-$ product practically abolished the release evoked by 60 mM K^+. The magnitude of K^+ stimulation was about the same both in Cl^- free medium and in medium in which the

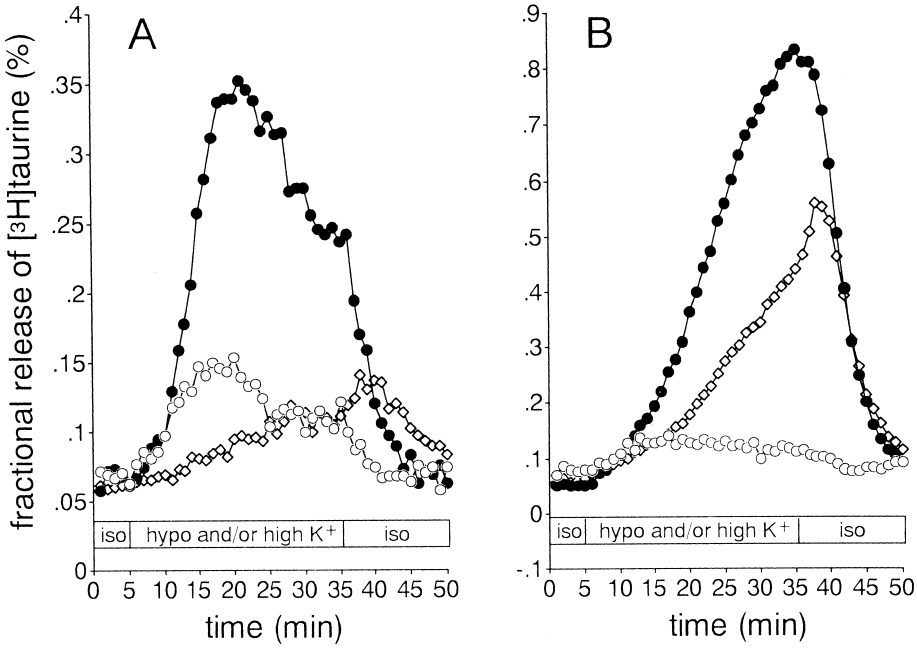

Fig. 9. The additive effect of hyposmolarity and high K^+ on the release of [^{3}H]taurine. (A) The curves indicate the [^{3}H]taurine release evoked by either 10% reduction of osmolarity (–○–) or 20 mM KCl (–◇–), and by a combination of both (–●–). (B) The curves indicate the [^{3}H]taurine release evoked by either 5% reduction of osmolarity (–○–) or 60 mM KCl (–◇–), and a combination of both (–●–). High K^+ media (20 and 60 mM) were prepared by substituting NaCl by equimolar amounts of KCl. Each point is the average of 3–6 experiments. S.E.M. was omitted to simplify the figure.

$K^+ \times Cl^-$ ionic product was constant when the impermeant anion gluconate was used to replace the omitted Cl^-.

3.8. Effect of blockers of osmosensitive taurine release on the high K^+-induced [^{3}H]taurine release

The effect of some blockers of $Na^+/K^+/2Cl^-$ cotransporter, Cl^-/HCO_3^- exchanger, and Cl^- channels on the [^{3}H]taurine release evoked by high K^+ (60 mM) was shown in Fig. 8. DIDS (200 μM), SITS (200 μM), niflumic acid (200 μM), and NPPB (100 μM) decreased the high K^+-evoked [^{3}H]taurine release by 80%. Replacement of external Na^+ with choline depressed the release by 70%. Bumetanide (100 μM) and furosemide (200 μM) also suppressed about 50% of the taurine release.

3.9. Additive effect of high K^+ and hyposmolarity

Additive effect on hyposmolarity-induced [^{3}H]taurine release was observed by reduction of osmolarity (10%) in the high K^+ (20 mM) medium (Fig. 9A). Reducing osmolarity (10%) or increasing external K^+ (20 mM) caused about two-fold increase of the taurine release over the basal efflux. However. the taurine release was greatly increased to six-fold by the combination of hyposmolarity and high K^+ medium. Reduction of osmolarity by 5% which alone did not cause a detectable increase in taurine release, significantly enhanced the taurine release in the high K^+ (60 mM) medium (Fig. 9B).

4. Discussion

4.1. Taurine uptake

The taurine uptake was not mediated by the $Na^+/K^+/2Cl^-$ cotransporter and/or the Cl^-/HCO_3^- exchanger, because SITS and bumetanide had no effect on the uptake. NPPB and DPC moderately inhibited the taurine uptake. A significant inhibition of the [^{3}H]taurine uptake by the Na^+ or Cl^- free medium indicates that the uptake is mediated by the Na^+, Cl^--dependent transporter. The transporter is probably the Na^+, Cl^--dependent taurine transporter, because the taurine uptake was strongly inhibited by hypotaurine and β-alanine (Fig. 1) which are competitive inhibitors in the bullfrog sympathetic ganglia with the Ki/Km of 1.0 and 1.9, respectively (Tasaka et al., 1989).

4.2. Hyposmolarity-induced taurine release

There is considerable evidence that taurine release participates in the processes of RVD after hypotonic swelling (Pasantes-Morales and Schousboe, 1988; Chamberlin and Strange, 1989). In the present study, exposure of the sympathetic ganglia to the hypotonic perfusion medium caused a substantial increase of [^{3}H]taurine release (Fig. 2A). Although the cell volume could not be directly measured in the sympathetic ganglia, the hyposmolarity-induced [^{3}H]taurine release is considered to be a swelling-activated release, because the release increased in proportion to the magnitude of osmolarity reduction, and declined despite the persistence of the hypotonic condition, indicating the restoration of the cell volume.

4.3. Cl^- dependency

Hyposmolarity-induced [^{3}H]taurine release from the glial cells of the sympathetic ganglia was suppressed 90% by the substitution of gluconate of external Cl^- (Fig. 3A). The remaining Cl^- independent [^{3}H]taurine efflux may result from some membrane permeability of gluconate, since the anion permeability of the volume-activated Cl^- current is known to be 1:0.1 for Cl^-:gluconate (Kubo and Okada, 1992). Jackson and Strange (1993) reported that replacement of Cl^- with NO_3^- or SCN^- inhibited the efflux of both taurine and inositol by 20–30% in C6 glioma cells. In contrast, substitution of Cl^- with gluconate or isethionate blocked swelling-induced [^{3}H]taurine efflux by 80–90%. Many anion channels have NO_3^- and SCN^- permeabilities close to or greater than those of Cl^-, whereas isethionate and gluconate permeabilities are usually quite low. Therefore, they have suggested that inhibition by Cl^- removal is dependent on the nature of the substitute used. Recently, Stutzin et al. (1997) have demonstrated that removal of extracellular Cl^-, or its replacement by a less permeable anion, enhanced taurine efflux and decreased the inward current (Cl^- efflux) in HeLa cells. Replacement by NO_3^-, a more permeable anion, had the opposite effect. They have implied that taurine and Cl^- flux might occur through a common channel, with the two solutes interacting within the pore and being affected differentially by Cl^- replacement.

Three routes for transmembrane Cl^- movements such as the furosemide- and bumetanide-sensitive $Na^+/K^+/2Cl^-$ cotransport system, the SITS-sensitive Cl^-/HCO_3^- exchange system, and the voltage-dependent Cl^- channels, which are known to be present in glial cells (Kimelberg, 1990a; Sontheimer, 1994), were examined in relation to the taurine release.

4.4. $Na^+/K^+/2Cl^-$ cotransport system

Hoffmann and Simonsen (1989) reported that the $Na^+/K^+/2Cl^-$ cotransport system is activated in the process of RVI after a hypertonic cell shrinking and

involves a net KCl uptake followed by a concomitant water uptake. In our data, the hyposmolarity-induced taurine release was sensitive to bumetanide and furosemide, and required the inward cotransport of Na^+, K^+ and Cl^-. It is suggested that the $Na^+/K^+/2Cl^-$ cotransport system is activated by a hyposmotic swelling in glial cells of the sympathetic ganglia. Both bumetanide and furosemide inhibited the taurine release by 40% (Fig. 4). The inhibition caused by omission of external K^+ or replacement of Na^+ with choline may have resulted from the suppression of the cotransport system (Fig. 4). Thus, the $Na^+/K^+/2Cl^-$ cotransport system appeared to be partially related to the taurine release.

4.5. Cl^-/HCO_3^- exchange system

Studies by Goldstein and Brill (1991), and Musch et al. (1994) suggested that volume-activated taurine release from fish erythrocytes might involve the anion exchanger band 3. Recently, Fiévet et al. (1995) cloned band 3 protein from the trout and mouse erythrocytes, and expressed the protein in Xenopus oocytes. The data presented evidence that both trout and mouse band 3 showed anion exchange activity, but only trout band 3 elicited anion channel activity and taurine permeability. The presence of Cl^-/HCO_3^- anion exchangers in cultured astrocytes was first described by Kimelberg (1981). The anion exchanger is well known as an important system for the control of brain pH. In the present study, SITS and DIDS, which affect both anion exchangers and Cl^- channels (Sánchez-Olea et al. 1993), markedly reduced taurine release (Fig. 5). Niflumic acid, another inhibiter of the anion exchanger but not Cl^- channels (Sánchez-Olea et al. 1993), inhibited taurine release by 35%.

Acetazolamide, which reduces cellular HCO_3^-, and in turn inhibits the Cl^-/HCO_3^- exchanger, decreased taurine release by 34% (Fig. 5). Replacement of external HCO_3^- with HEPES, which may increase HCO_3^- outward concentration gradient across the membrane, interestingly increased the hyposmolarity-induced taurine release by 40% (Fig. 5). Bourke et al. (1975) have shown that increasing the HCO_3^- concentration of incubation media containing high K^+ (18–71 mM) caused swelling, and that this swelling was mainly associated with increased intracellular Na^+ and Cl^-. However, $NaHCO_3$ did not stimulate tissue swelling and uptake of Cl^- when the extracellular concentration of K^+ approximated physiological levels (~6 mM). We used the concentration of 2.4 mM $NaHCO_3$ and 2 mM KCl which are the exact physiological levels for the frog, therefore tissue swelling by $NaHCO_3$ appears to be less effective. Taken together, the Cl^-/HCO_3^- anion exchanger is thought to be partially related to the hyposmolarity-induced taurine release from the satellite glial cells of the sympathetic ganglia.

4.6. Cl^- and K^+ channels

An anion channel has been suggested to be involved in the swelling-activated release of taurine in the various cell types such as MDCK cells (Banderali and Roy, 1992), flounder erythrocytes (Kirk et al., 1992), human lung cancer cells (Kirk and Kirk, 1993), and C6 glioma cells (Jackson and Strange, 1993). Sánchez-Olea et al. (1995) reported that hyposmolarity-induced taurine release in both cell lines, with or without the anion exchanger, was inhibited by blockers (niflumic acid, DIDS, NPPB and DPC) of the anion exchanger and/or Cl^- channels. They suggested that a red cell-type anion exchanger is not involved in amino acid transport during RVD, and that amino acid and Cl^- may be transported by the same anion channel-like structure.

Electrophysiological studies (McCann et al., 1989; Banderali and Roy, 1992; Kubo and Okada, 1992; Jackson and Strange, 1993) showed that the outwardly rectifying Cl^- channels are activated upon osmotic swelling and are involved in the subsequent cell volume regulation.

In our experiments, specific Cl^- channel inhibitors such as NPPB and DPC completely suppressed the hyposmolarity-induced taurine release (Fig. 6). Furthermore, substitution of gluconate for extracellular Cl^- totally depressed the taurine release (Figs. 2A and 6), suggesting that external Cl^- is necessary for Cl^- channel activity. These results indicate that Cl^- channels may be responsible for the hyposmolarity-induced taurine release. We assume that the Cl^-/HCO_3^- anion exchanger, as well as the $Na^+/K^+/2Cl^-$ cotransport system, contribute to the accumulation of intracellular Cl^- which may function to regulate the hyposmolarity-induced taurine release through Cl^- channels. The K^+ channel blockers quinidine and clofilium did not affect taurine release (Fig. 6). Therefore, K^+ channels are not directly involved in the taurine release.

4.7. Release of GABA and taurine

We examined the possibility that the hyposmolarity-induced release of taurine and GABA shares the same transport systems. From the results of osmotic sensitivity (Fig. 2B), inhibition by Cl^- substitute (Fig. 3B) and pharmacological sensitivity, we conclude that the pathway of the hyposmolarity-induced GABA release is different from that of taurine release. In the case of GABA, exposure of the sympathetic chains to the Cl^- free medium may depolarize cell membranes of the satellite glial cells, causing a significant increase in the

spontaneous release of GABA, as described in brain synaptosomes (Naalsund and Fonnum, 1986). In addition, GABA taken up by glial cells is rapidly metabolized by GABA-T under physiological conditions. Therefore, it is unlikely that GABA is an osmoeffector in this preparation.

4.8. High K^+-induced [^{3}H]taurine release

Increasing extracellular K^+ concentrations is known to induce taurine release and swelling in a variety of nervous tissue preparations (Pasantes-Morales and Schousboe, 1989; Sánchez-Olea and Pasantes-Morales, 1990). In the present study, high K^+-induced [^{3}H]taurine release was almost completely abolished in the Cl^- free medium replaced with gluconate and also in the medium in which the $K^+ \times Cl^-$ ionic product was constant. These results indicate that the high K^+-induced taurine release may occur in response to cell swelling rather than to depolarization. Morán et al. (1991) reported that swelling induced by high K^+ is due to accumulation of K^+ by cells, followed by Cl^- and osmotically obliged water. It is quite likely that swelling induced by high K^+ activates taurine release. We examined the effect of some blockers of the osmosensitive taurine release on the high K^+-induced [^{3}H]taurine release. The inhibitors used were bumetanide and furosemide, which block $Na^+/K^+/2Cl^-$ cotransporter, DIDS, SITS, niflumic acid, which block Cl^-/HCO_3^- anion exchanger, and NPPB, which blocks Cl^- channels. All these compounds exhibited an inhibitory effect on the high K^+-evoked [^{3}H]taurine release as shown in hyposmolarity-induced taurine release (Figs. 4–6). The mechanism of taurine release induced by hyposmolarity and high K^+ may be similar in the bullfrog sympathetic chain.

4.9. Additive effect on the hyposmolarity-induced taurine release

Additive effect on the hyposmolarity-induced taurine release was observed by increasing extracellular K^+ concentration (Fig. 9A and B). It is quite feasible that the additive release of taurine by the combined stimulation of high K^+ and hypotonicity is involved in pathological conditions such as edema, hyponatremia and hypoglycemia, because $[K^+]_0$ around the cell membrane in the nervous system rises subsequently to very high levels (30–80 mM) (Orkand et al., 1966; Hansen, 1985). The hyposmolarity-induced taurine release in glial cells may play an important role in suppression or modulation of the activity of surrounding neurons simultaneously with the volume regulation.

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