The Regulation of Cytosolic pH in Isolated Presynaptic Nerve Terminals from Rat Brain

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A B S T R A C T Cytosolic pH (pHi) was measured in presynaptic nerve terminals isolated from rat brain (synaptosomes) using a fluorescent pH indicator, 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF). The synaptosomes were loaded with BCECF by incubation with the membrane-permanent acetoxy-methyl ester derivative of BCECF, which is hydrolyzed by intracellular esterases to the parent compound. pHi was estimated by calibrating the fluorescence signal after permeabilizing the synaptosomal membrane by two different methods. Synaptosomes loaded with 15–90 μM BCECF were estimated to have a pHi of 6.94 ± 0.02 (mean ± standard error; n = 54) if the fluorescence signal was calibrated after permeabilizing with digitonin; a similar value was obtained using synaptosomes loaded with 10 times less BCECF (6.9 ± 0.1; n = 5). When the fluorescence signal was calibrated by permeabilizing the synaptosomal membrane to H⁺ with gramicidin and nigericin, pHi was estimated to be 7.19 ± 0.03 (n = 12). With the latter method, pHi = 6.95 ± 0.09 (n = 14) when the synaptosomes were loaded with 10 times less BCECF. Thus, pHi in synaptosomes was ~7.0 and could be more precisely monitored using the digitonin calibration method at higher BCECF concentrations. When synaptosomes were incubated in medium containing 20 mM NH₄Cl and then diluted into NH₄Cl-free medium, pH immediately acidified to a level of ~6.6. After the acidification, pHi recovered over a period of a few minutes. The buffering capacity of the synaptosomes was estimated to be ~50 mM/pH unit. Recovery was substantially slowed by incubation in an Na⁻/H⁺ symporter (Kᵢ = 3 μM), and by abolition of the Na⁺/Na⁺ gradient. pHi, and its recovery after acidification were not affected by incubation in an HCO₃⁻-containing medium; disulfonic stilbene anion transport inhibitors (SITS and DIDS, 1 mM) and replacement of Cl⁻ with methylsulfonate did not affect the rate of recovery of pH. It appears that an Na⁺/H⁺ antiporter is the primary regulator of pHi in mammalian brain nerve terminals.

I N T R O D U C T I O N

Cytosolic pH (pHi) is a key regulator of many cellular processes (e.g., Roos and Boron, 1981), including the release of neurotransmitters from presynaptic nerve

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terminals (Landau and Nachshen, 1975; Cohen and Van der Kloot, 1976; Drapeau and Nachshen, 1988). There is little information, however, about how pH is regulated in presynaptic nerve terminals.

Isolated presynaptic nerve terminals (synaptosomes) are useful for studying many aspects of mammalian nerve terminal activity because they retain the functional and morphological properties of intact neuronal tissue (Blaustein et al., 1977; McGraw et al., 1982). The membrane potential is primarily a K⁺ diffusion potential (Blaustein and Goldring, 1975). Synaptosomes regulate Ca entry and extrusion (McGraw et al., 1982) and maintain a resting level of cytosolic Ca similar to that found in intact cells (0.1–0.2 μM; Nachshen, 1985). In addition, when synaptosomes are depolarized, there is an increase in Ca entry and in the rate of neurotransmitter release (Blaustein, 1975).

In synaptosomes prepared from rat brain, pH is ~7.0 (Richards et al., 1984), well above the equilibrium value expected, given a synaptosomal membrane potential of ~60 to ~80 mV (pH ~ 6.4) (Blaustein and Goldring, 1975; Ramos et al., 1979; Scott and Nicholls, 1980). The synaptosomal pH, must therefore be regulated by acid-extrusion mechanisms.

At least three types of pH-regulatory mechanisms have been described in plasma membranes: (a) a system that requires external Na⁺ and HCO₃⁻ as well as internal Cl⁻ and is inhibited by disulfonic stilbene anion transport inhibitors; (b) a countertransport of Cl⁻ for HCO₃⁻; (c) a countertransport of extracellular Na⁺ and cytosolic H⁺ that is inhibited by amiloride. Recently, it has been shown that there is an amiloride-sensitive Na⁺/H⁺ exchange system in synaptosomes (Sauvaigo et al., 1984). The results of the present study show that it is primarily this system that enables synaptosomes to regulate pH, and to recover from an acid load.

**METHODS**

**Preparation of Synaptosomes**

Synaptosomes were prepared according to the method of Nagy and Delgado-Escueta (1984). In brief, brain samples were homogenized (16 strokes) in ice-cold 0.32 M sucrose with 5 mM HEPES (pH 7.4) and 0.1 mM EDTA. The homogenate was centrifuged at 1,000 g for 10 min. The supernatant was then centrifuged at 12,000 g for 20 min and the supernatant was decanted. The white, fluffy portion of the pellet was resuspended in the medium as above, which contained 7.5% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) and was layered onto a gradient consisting of 10% and 16% Percoll in sucrose medium as above. The gradient was centrifuged at 15,000 g for 20 min. The synaptosomes (banded at the 10%/16% Percoll interface) were removed and diluted with 10 vol of ice-cold Na solution, containing (in millimolar): 145 NaCl, 5 KCl, 3 MgCl₂, 1 CaCl₂, 10 glucose, 0.005 of the heavy metal chelator diethylenetriamine pentaacetic acid, 0.2 pyruvic acid, 0.2 Na₂PO₄, and 10 HEPES; the pH was adjusted to pH 7.4 with NaOH. The diluted suspension was isolated by centrifugation and the pellet was resuspended in the above solution.

**Loading of the Synaptosomes with pH Indicator**

The acetoxymethyl ester of 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF-AM), dissolved in dimethylsulfoxide, was added to the resuspended synaptosomes at concentrations of 5–10 μM. The suspension was warmed to 30°C and, after 30 min, diluted 20-
fold with fresh solution. The diluted suspension was centrifuged at 6,000 g for 8 min and the pellet was resuspended and maintained at 30°C.

Synaptosomes that were incubated with BCECF-AM accumulated substantial amounts of the hydrolyzed free acid. After 30 min of incubation in a solution containing 2.5–10 μM BCECF-AM, the synaptosomes accumulated 60–360 pmol BCECF/mg protein (protein content determined by the method of Lowry et al., 1951). If it is assumed that the internal volume of the synaptosomes is ~4 μl/mg protein (Blaustein and Goldring, 1975), the intraterminal concentration of BCECF was 15–90 μM. In some experiments, the BCECF-AM concentration in the loading solution was reduced to 0.2–0.5 μM, resulting in an ~10-fold-lower intraterminal BCECF concentration.

Measurements of BCECF Fluorescence
For measurement of BCECF fluorescence, an aliquot of the synaptosome suspension was diluted 20-fold in resuspension solution and centrifuged for 45 s at 10,000 g in a centrifuge (Microfuge, Beckman Instruments, Inc., Fullerton, CA). The pellet was resuspended in 100 μl and diluted 20-fold in a 1-cm² quartz cuvette. BCECF fluorescence was measured with a spectrofluorometer (System 3, Farrand, Valhalla, NY) coupled to a chart recorder. The sample was continuously stirred with a Teflon stir bar and magnetic mixer and the sample chamber was thermostatically maintained at 30°C. Excitation and emission wavelengths of 500 nm (5-nm slit) and 530 nm (10-nm slit) were employed. The emitted light was filtered with a Farrand model 3-70 cutoff filter (50% transmission at 506 nm). In experiments where pH was displaced in intact synaptosomes, the rate of recovery of pH was estimated from the slope of the initial 15-s period of the recovery.

Determination of BCECF Leakage from the Synaptosomes
BCECF leakage from the synaptosomes was determined by adding aliquots of BCECF-loaded synaptosomes to 20 vol of test solution. At predetermined times, the samples were spun down in a centrifuge. Triton X-100 (0.25%) was added to the pellet and to the supernatant and BCECF fluorescence in both fractions was measured using a spectrofluorometer. The measurement was compared with the fluorescence from a sample of control synaptosomes, treated in a similar manner, to which was added a known amount of BCECF. The extracellular fluorescence was subtracted from the fluorescence of BCECF-loaded synaptosomes in order to estimate pH.

The loss of accumulated BCECF was initially large (5–10%), which probably reflected the disruption of some synaptosomes during the resuspension procedure. It then proceeded at a slower rate, ~5%/h. Alkalization of the solution with NaOH, but not with Tris, increased the rate of BCECF efflux appreciably. Acidification of the solution, or resuspension in Na-free solution (Na replaced iso-osmotically by N-methylglucamine or choline), had no effect on the rate of BCECF efflux.

Measurement of ⁴⁵Ca Uptake
⁴⁵Ca entry was determined as described by Nachshen and Blaustein (1980). Aliquots of the synaptosome suspension were added to equal volumes of solution containing the tracer. ⁴⁵Ca entry was terminated by rapidly diluting the incubation media with 11 vol of ice-cold Na-free solution (Na replaced by N-methylglucamine) containing 1.0 mM EGTA and the diluted suspensions were filtered through glass-fiber filters (2.4 cm diam, GF/A, Whatman, Inc., Clifton, NJ). The filters were washed twice with the ice-cold Na-free solution and placed in vials containing 10 ml scintillation cocktail. The ⁴⁵Ca content was measured by liquid scintillation spectrometry. The protein content was determined by the method of Lowry et al. (1951).
RESULTS

Determination of pH,

An experiment illustrating how pH, was determined is shown in Fig. 1A. After a stable fluorescence level was obtained, the synaptosomes were permeabilized by the addition of digitonin (200 μM) so that the fluorescence signal could be calibrated. Small aliquots (0.0005–0.0025 vol) of 1 M Tris (not NaOH; see Methods) or HCl solutions were added to the cuvette. The external pH (pH,) was determined after each addition. Fig. 1B (filled symbols) shows a plot of fluorescence vs. pH, after the addition of digitonin (corrected for fluorescence of extracellular dye as described in Methods). The arrow indicates the pH at which the fluorescence should be equal to that observed with intact synaptosomes before the addition of digitonin. This pH value provided an estimate of pH, in the synaptosomes of 6.94 ± 0.02 (mean ± standard error; n = 54) for synaptosomes loaded with 15–90 μM BCECF. A similar value (pH, = 6.9 ± 0.1; n = 5) was obtained for synaptosomes loaded with 10 times less BCECF.

It has been reported (Rink et al., 1982) that the addition of Triton X-100 or digitonin may result in an underestimation of pH,. An additional calibration method was therefore used. This involved depolarizing the synaptosomes by the
addition of gramicidin and nigericin. Gramicidin makes the synaptosome membranes leaky to cations, so that \([K^+]_i = [K^+]_o\) (Blaustein and Goldring, 1975). Nigericin (0.5 \(\mu g/ml\)) is a \(K^+/H^+\) antiporter (Pressman, 1976). After addition of both agents, \(pH_i\) should equal \(pH_o\). It was verified that addition of nigericin and gramicidin did not increase the leakage of BCECF from the synaptosomes above the 5–10% usually found (see Methods); i.e., the dye remained trapped within the synaptosomes.

After addition of gramicidin and nigericin, the \(pH_i\) was varied and fluorescence was measured at different \(pH\) levels (Fig. 2B, filled symbols; corrected for extracellular fluorescence). The \(pH\) corresponding to the fluorescence of synaptosomes before the addition of gramicidin and nigericin was determined (arrow, Fig. 2B). In 12 experiments, \(pH_i\), determined in this manner had a value of 7.19 ± 0.03. This estimate of \(pH_i\) is shifted upward by 0.25 \(pH\) units over the estimate obtained after disruption of the synaptosomal membrane with digitonin. Similar results have been obtained in lymphocytes (Rink et al., 1982) and in proximal tubules (Chaillet et al., 1985).

In another group of 14 experiments, synaptosomes were loaded with 10 times less BCECF and the \(pH_i\) estimated by the gramicidin/nigericin method described above was 6.95 ± 0.09 (not shown). Although it is technically more difficult to estimate because of the lower signal-to-noise ratio, the value of \(pH_i\) obtained at the lower BCECF concentration is probably more accurate since problems such as compartmentalization of the dye should be reduced. It was concluded that
pH in synaptosomes is ~7.0. Calibration by permeabilization with digitonin was an accurate method for monitoring pH, and, more importantly, signals of greater precision could be obtained at the higher dye concentrations using this method. As would be expected, agents that induced synaptosomal acidification or alkalinization (see Figs. 3 and 4) had no effect on pH, after the addition of digitonin (data not shown).

**Determination of the Buffering Capacity of Synaptosomes**

Two different methods were used to estimate the cytosolic buffering capacity of the synaptosomes. The first involved adding NH$_4$Cl (20 mM) to the synaptosomes. As shown in Fig. 3A, this induced an immediate alkalinization of pH, which presumably reflects the inward diffusion of NH$_3$ and its subsequent association with H$^+$ in the cytosol. For each NH$_3$ formed, one intracellular H$^+$ is consumed; therefore,

$$B = \frac{\Delta[\text{NH}_4^+]}{\Delta\text{pH}},$$

(1)

where B is the buffering power, and

$$[\text{NH}_4^+] = [\text{NH}_3] \times 10 \exp(pK_a - \text{pH}),$$

(2)

[NH$_3$] is assumed to equal [NH$_3$] at equilibrium and the pK$_a$ for ammonium is taken as 9.2. In 10 experiments, B was estimated to be 53 ± 8 mM/pH unit.

The second method for estimating B involved acid-loading the synaptosomes using CO$_2$. As shown in Fig. 4A, when synaptosomes were added to a solution...
containing 23 mM NaHCO₃ (pCO₂, 5%; pH₀, 7.4), there was a barely perceptible (0.02 pH unit) acidification of the synaptosomes, produced by an influx of CO₂, which hydrates to yield HCO₃⁻ and H⁺. The largest acidification observed in four similar experiments was 0.1 pH unit. For each HCO₃⁻ formed, one H⁺ is released; therefore,

\[
B = \Delta[HCO_3^-]/\Delta pH_i
\]

and

\[
[HCO_3^-]_i = s \cdot pCO_2 \times 10^{exp(pH_i - pK_a)},
\]

where s is the solubility of CO₂ in cell water (Siesjo, 1962). In four different experiments, B was estimated to be 34 ± 8 mM/pH unit. This value, albeit less accurate, is in reasonable agreement with the estimate found using NH₄Cl, and we therefore conclude that the buffering capacity of the synaptosomes is on the order of 50 mM/pH unit.

**Figure 4.** (A) Synaptosomes were resuspended, added to 20 vol of solution containing 23 mM NaHCO₃, and gassed with 5% CO₂ and fluorescence was immediately measured. (B) Synaptosomes were resuspended in solution containing 25 mM NaHCO₃ and 5% CO₂, as well as 20 mM NH₄Cl. After 3 min, the suspension was mixed with 20 vol of solution with HCO₃⁻ and CO₂, without NH₄Cl, to induce acidification. The initial rate of recovery from acidification was 6.4 milli-pH unit/s. (C) The synaptosomes were resuspended as in B, and mixed with 20 vol of solution containing 23 mM of choline HCO₃ and 5% CO₂. The initial rate of recovery of pH_i was 1.2 milli-pH units/s.

**The Effect of pH₀ on pH_i**

Lowering pH₀ diminishes depolarization-induced Ca uptake (Nachshen and Blaustein, 1979) and Ca-dependent, evoked transmitter release (Landau and Nachshen, 1975; Drapeau and Nachshen, 1988) and increases the rate of spontaneous transmitter release (Landau and Nachshen, 1975; Cohen and Van der Kloot, 1975; Drapeau and Nachshen, 1988). It therefore seemed important to determine whether changes in pH₀ were accompanied by changes in pH_i. The results in Fig. 5 show that pH_i changed little (from 7.0 to 6.5) as pH₀ was lowered from 7.5 to 6.0. The dashed line in Fig. 5 is an “identity” line. The deviation of the data points from this line suggests that pH_i is effectively regulated at both extremes of pH₀.

The effect of pH₀ on the rate of acid extrusion is shown in Fig. 6. As in many other types of cells, the rate of acid extrusion is increased by the lowering of pH₀.
Recovery of Synaptosomes from Acid Load Is Dependent on External Na

When synaptosomes that had been resuspended with a solution containing NH₄Cl (20 mM) were diluted with NH₄Cl-free solution, they immediately acidified (Fig. 3B) and pHᵢ recovered within a few minutes. pHᵢ remained low, however, in Na-free solution (Fig. 3C). The rate of recovery of pHᵢ increased as the concentration of Na in the external solution was increased in the range 0–145 mM (Fig. 7). When synaptosomes were acid-loaded by a 5-min preincubation in Na-free solution containing 20 mM NH₄Cl, the maximum initial rate that was measured in the presence of Na was ~6 milli-pH unit/s. For a cytosolic buffering capacity of ~50 mM/pH unit, this amounted to a proton transfer of ~0.3 mmol/(liter cell water ⋅ s).

Figure 5. The effect of pHᵢ on pHᵢ. Results from five experiments are summarized. Synaptosomes were resuspended and pHᵢ was lowered by the addition of HCl. After the fluorescence signal stabilized (3–4 min), the signal was calibrated with digitonin. The solid line drawn through the points has no theoretical significance. The broken line is a line of identity (pHᵢ = pHᵢᵀ).

Figure 6. The effect of pHᵢ on the rate of acid extrusion. Synaptosomes were acidified to various levels of pHᵢ by loading with 5–40 mM NH₄Cl for times ranging from 30 s to 5 min. The NH₄Cl-loaded synaptosomes were then mixed with 20 vol of NH₄Cl-free solution and the initial rate of acid extrusion was determined. Results from six experiments are summarized. The straight line has no theoretical significance.
The pHi recovery following an acid load depended not only on external Na, but also on the Na gradient. This was shown by abolishing the Na gradient by including veratridine (100 μM, to increase presynaptic Na conductance) and ouabain (1 mM, to block Na extrusion via the Na pump) in the resuspension solution. After this treatment, the synaptosomes were loaded with Na and the rate of recovery from the acid load decreased (Fig. 8B). This was not a direct effect of ouabain and veratridine on the H-extrusion mechanism since, when the synaptosomes were acid-loaded in Na-free solution, veratridine and ouabain had only a slight effect on the subsequent rate of acid extrusion when the synaptosomes were returned to Na-containing solution (Fig. 8C).

Recovery from Acid Load Is Independent of HCO₃⁻ and Cl⁻

In some cells, pH regulation depends on extracellular HCO₃⁻ and Cl⁻. The effect of these anions on pHi and on its recovery after an acid load was investigated.

FIGURE 7. The Na⁺ dependence of acid extrusion. Synaptosomes were acidified to a level of ~6.5, by a 3-min incubation in solution containing 20 mM NH₄Cl and resuspension in solution without NH₄Cl and with varying amounts of Na⁺. Na⁺ was replaced isosmotically with n-methylglucamine (circles) or choline (triangles).

FIGURE 8. Acid extrusion is dependent on the Na⁺/Na₆ gradient. (A) Synaptosomes were resuspended in solution with 20 mM NH₄Cl and after 3 min mixed with 20 vol of solution without NH₄Cl. The initial rate of recovery of pHi was 4.0 milli-pH units/s. (B) Same as in A, except that the resuspension solution also contained 100 μM veratridine and 1 mM ouabain. The initial rate of recovery was 1.3 milli-pH units/s. (C) Synaptosomes were resuspended in Na-free solution with 100 μM veratridine, 1 mM ouabain, and 20 mM NH₄Cl. The suspension was then mixed with 20 vol of Na-containing solution. The initial rate of recovery was 3.6 milli-pH units/s.
pH, in synaptosomes that were maintained in a solution containing 23 mM HCO₃⁻ was 6.86 ± 0.05 (n = 9), a value not significantly different from the control value of 6.94 ± 0.02 that was found in medium without HCO₃⁻. The rate of recovery of pH, was similar in synaptosomes that had been resuspended in solutions with (Figs. 4B and 9A) or without (Fig. 6) HCO₃⁻. Also, pH, recovered only slowly in synaptosomes resuspended in Na-free solution containing 23 mM choline HCO₃⁻ (Fig. 4C).

The effect of Cl⁻ on the rate of recovery of pH, was compared in NH₄⁺-loaded synaptosomes that were resuspended in solutions containing Na⁺ and HCO₃⁻ (46 mM, 10% CO₂) made up with either Cl⁻ or with methanesulfonate as the counteranion. The replacement of Cl⁻ by methanesulfonate did not decrease the rate of recovery (Fig. 9B). In the same experiment, two inhibitors of Cl⁻/HCO₃⁻ countertransport, SITS (Fig. 9C) and DIDS (not shown) (1 mM), were tested and did not decrease the rate of recovery of pH,. These experiments show that an Na-dependent mechanism is the primary process regulating the recovery of pH, from an acid load in synaptosomes.

**Amiloride Inhibits the Recovery of Synaptosomes from Acid Loading**

In most systems that have been studied, Na⁺/H⁺ exchange is inhibited by micromolar concentrations of amiloride. The effect of 0.1 mM amiloride on the rate of recovery of pH, in synaptosomes that were resuspended in medium with HCO₃⁻ (46 mM) is shown in Fig. 9D. There was again a substantial slowing of the recovery of pH..

The effect of amiloride on the Na-dependent recovery of synaptosomes from
an acid load is shown in Fig. 10 (inset). Recovery was almost completely blocked by 0.1 mM amiloride. The amiloride dose-response curve is also shown in Fig. 10. In solutions containing 72.5 mM Na\(^+\), block was half-maximal with an amiloride concentration of \(~3 \mu\text{M}\).

**Recovery of Synaptosomes from Acid Load Is Accompanied by H\(^+\) Extrusion**

The recovery from an acid load could, in principle, be due to internal sequestration of H\(^+\). However, as shown in Fig. 11, the recovery was accompanied by the extrusion of H\(^+\) from synaptosomes. In this experiment, synaptosomes that had not been loaded with BCECF were resuspended in medium that contained a low concentration (0.5 mM) of HEPES buffer and 100 nM BCECF. In Na-containing medium, there was an extracellular acidification that proceeded at a rate of \(~0.3 \mu\text{M}/(\text{mg protein} \cdot \text{s})\) (Fig. 11A). When the synaptosomes were acid-loaded by a 3-min preincubation in medium containing 20 mM NH\(_4\)Cl and resuspended in NH\(_4\)Cl-free medium, the rate of extracellular acidification was greatly increased, to \(~1.5 \mu\text{M}/(\text{mg protein} \cdot \text{s})\) (Fig. 11B). If the intrasynaptosomal volume is 4 \(\mu\text{l}/\text{mg protein}\) (Blaustein, 1975), the proton transfer induced by acid loading is \(~0.3 \text{mmol}/(\text{liter cell water} \cdot \text{s})\). This value is in accord with the initial rate of
recovery from acid loading measured with BCECF-loaded synaptosomes (see Fig. 3 and related text). In addition, acid extrusion was blocked in Na-free medium (Fig. 11 C) and by 0.1 mM amiloride (Fig. 11 D). Thus, it is evident that recovery of the synaptosomes from acid loading is accompanied by the extrusion of H⁺ into the extracellular medium.

**Effect of Ca on pH, and on Recovery from Acid Loading**

In some cells, Ca ions can affect pH, by displacing protons from a common buffer site (Meech and Thomas, 1980; Vaughan-Jones et al., 1983) and by altering the Na⁺ gradient (Deitmer and Ellis, 1980). Since presynaptic nerve terminals undergo significant changes in cytosolic Ca levels during the normal course of their function, the effect of Ca on pH, and the recovery from acidification was examined.

In one set of experiments, synaptosomes were resuspended in Na-free solution containing either 1 mM Ca or no added Ca and 0.1 mM EGTA. In both batches of synaptosomes, there was a lowering of pH. The acidification proceeded at an initial rate of 0.11 milli-pH units/s in the synaptosomes without Ca; at the end of 6 min, pH was 6.46. The acidification proceeded at a similar initial rate, 0.09 milli-pH units/s, in the synaptosomes with Ca; at the end of 6 min, pH was 6.50. In parallel experiments, it was determined that Ca uptake (measured radioiso-
NACHSHEN AND DRAPEAU  Cytosolic pH in Synaptosomes 301
topically) amounted to ~40 nmol/mg protein. Thus, in contrast to observations in other preparations, Ca loading of nerve terminals was not accompanied by acidification. In another set of experiments, synaptosomes were acid-loaded by incubation for 3 min in Na-free medium containing 20 mM NH₄Cl (see above), either with or without Ca, followed by dilution of the synaptosomes into NH₄Cl-free medium with or without Ca. The recovery rate was similar in both batches: 3.75 milli-pH units/s in the synaptosomes without Ca and 3.2 milli-pH units/s in synaptosomes with Ca.

pHᵢ in synaptosomes that were suspended in Na-containing solution without Ca and with 0.1 mM EGTA was 6.97 ± 0.01 (n = 8), not significantly different from the control value of 6.96 ± 0.01 (n = 8) in synaptosomes resuspended in solution with 1 mM Ca.

Veratridine activates presynaptic Na channels, thereby depolarizing the nerve terminals and opening voltage-dependent Ca channels (Blaustein, 1975), and results in a large increase of cytosolic Ca from ~100 nM to >1 t~M (Nachshen, 1984). Nonetheless, addition of veratridine (50–100 μM) to a suspension of synaptosomes incubated in Na-containing medium with 1 mM Ca had no effect on pHᵢ.

In the accompanying article (Drapeau and Nachshen, 1988), it is shown that lowering pHᵢ (to at least 6.0) has no effect on cytosolic Ca (as measured with the Ca-specific fluorescent dye fura-2). Thus, physiological changes in pHᵢ or pCaᵢ in synaptosomes do not appear to affect each other.

DISCUSSION

The purpose of this study was to determine the mechanisms regulating pHᵢ in nerve terminals. pHᵢ and the buffering capacity of synaptosomes were estimated as 7.0 and 50 mM/pH unit, respectively, and these resemble estimates obtained with other preparations (Roos and Boron, 1981, Table 13). The Na⁺/H⁺ antiporter was found to be the primary mechanism for regulating pHᵢ in rat brain synaptosomes and is remarkably similar to the antiporter found in a variety of mammalian cells, e.g., fibroblasts (Moolenaar et al., 1984), lymphocytes (Grinstein et al., 1984), heart cells (Piwnica-Worms et al., 1985), and collecting tubules (Chaillet et al., 1985). Thus, it is activated by Na⁺ and by acidification of the cytosol, and is blocked by micromolar concentrations of amiloride.

In contrast to invertebrate neuronal tissue (squid axon: Russell and Boron, 1982; snail neuron: Thomas, 1982; crayfish stretch receptor: Moser, 1985), where pH regulation is accomplished via an Na⁺/HCO₃⁻/Cl⁻ antiporter, we found no evidence for the presence of either an Na⁺/HCO₃⁻/Cl⁻ antiporter or a Cl⁻/HCO₃⁻ antiporter in nerve terminals isolated from rat brain. It thus appears that the regulation of pHᵢ in nerve endings from rat brain is accomplished by a mechanism common to a variety of vertebrate tissues rather than by a neuron-specific process.

Although an Na⁺/H⁺ antiporter was the only identifiable regulator of pHᵢ, it was noted in several experiments that even in the absence of external Na⁺, or in the presence of amiloride, there was some recovery of pHᵢ from an acid load (cf. Chaillet et al., 1985). One possibility is that intraterminal organelles (e.g., mitochondria) can sequester protons; another possibility is that they are extruded via an as yet unidentified mechanism at the plasma membrane, e.g., an H⁺-
ATPase (Steinmetz and Andersen, 1982). Nonetheless, the conclusion of this study is that the major route for H\(^+\) extrusion from mammalian nerve endings is via exchange for extracellular Na\(^+\).

Ca entry did not affect pH, and its recovery after an acid load. As shown in the accompanying article (Drapeau and Nachshen, 1988), lowering pH had no effect on pCa. These results suggest that the regulation of the cytosolic level of either of these ions in nerve terminals is insensitive to the level of the other ion, although both depend on the Na\(^+\) gradient. The mutually insensitive cytosolic H\(^+\) and Ca\(^{2+}\)-buffering mechanisms contrast with the regulation of transmitter release shown to depend on both pCa and pH (Drapeau and Nachshen, 1988).

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Nachshen and Drapeau  Cytosolic pH in Synaptosomes 303


