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Interstitial space, electrical resistance and ion concentrations during hypotonia of rat hippocampal slices

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1. The degree to which mammalian brain cells swell in hypotonic environments has not previously been determined. We exposed hippocampal tissue slices prepared from anaesthetized rats to artificial cerebrospinal fluid from which varying amounts of NaCl had been deleted. Interstitial volume (ISV) change was determined from the volume of dilution of the marker ions tetramethylammonium (TMA^+) or tetraethylammonium (TEA^+). Tissue electrical resistance was measured as the voltage generated by constant current pulses.

2. ISV decreased as a function of lowered extracellular osmolality (osmotic pressure, π_o), indicating cell swelling. After reaching a minimum, ISV recovered partially, suggesting regulatory volume decrease of cells. After restoring normal π_o the ISV expanded, indicating post-hypotonic cell shrinkage. The electrical resistance of the tissue (R_o) increased when π_o was lowered, due to the reduced ionic strength, as well as restricted ISV.

3. To control for low NaCl concentration, reduced NaCl was replaced by mannitol or fructose. In isosmotic, NaCl-deficient solution, ISV showed inconsistent change, and R_o corrected for ionic strength tended to decrease.

4. Extracellular K^+ concentration decreased slightly in low π_o except when spreading depression caused it to increase. Extracellular Ca^{2+} concentration decreased substantially, consistently and reversibly. Administration of isosmotic low-NaCl concentration solutions caused a similar decrease in extracellular Ca^{2+} concentrations. We propose that low Na^+ concentration in extracellular fluid impaired the extrusion of Ca^{2+}.

5. In severely hypotonic solution, ISV was reduced to 25% of its control volume, corresponding to a mean cell volume increase of at least 11%, probably more. From plotting relative changes in ISV against osmolarity we concluded that, within the range tested, hypotonic cell swelling was not opposed by the close approach of plasma membranes of neighbouring cells.

Changes of extracellular osmotic pressure (π_o) represent serious clinical hazards which result in disruption of brain function (Andrew, 1991; Strange, 1992; Gullans & Verbalis, 1993). The clinical syndromes of disturbed π_o are well known. Dilution of extracellular fluid can lead to convulsions and raised π_o can lead to lethargy. Systemic osmotic effects are in part exerted by water displacement across the blood–brain barrier (Katzman & Pappius, 1973), in this way influencing intracranial pressure, but osmotic changes in CNS interstitial fluid also have direct effects on neurones (Dudek, Obenaus & Tasker, 1990; Andrew, 1991; Ballyk, Quackenbush & Andrew, 1991; Hester, Aitken & Somjen, 1993).

Our interest in brain cell swelling stems from our investigations of spreading depression (SD) and the related hypoxic SD-like depolarization (also known as anoxic depolarization) (Somjen, Aitken, Czéh, Herreras, Jing & Young, 1992). Normoxic SD and SD-like hypoxic depolarization are characterized by massive depolarization of neurones and glial cells, and by ion fluxes which result in the gross redistribution of intra- and extracellular ion concentrations as well as swelling of cells at the expense of interstitial spaces (Van Harreveld & Khattab, 1967; Hansen & Olsen, 1980; Nicholson, 1984; Somjen *et al.* 1992). We have estimated that during both normoxic SD and transient hypoxic SD, interstitial space is reversibly reduced to less than 4% of total tissue volume, matching the reduction seen in dying tissue (Jing, Aitken & Somjen, 1994).

In an earlier study aimed at the effects of osmotic change on neuronal ion channel function, we exposed freshly dissociated hippocampal neurones to severely hypo- and

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Table 1. Resistivity and osmotic pressure of experimental solutions

Solution	$[Na^+]$ (mmol l^{-1})	$[Cl^-]$ (mmol l^{-1})	R_o (Ω cm)	ΔR_o (%)	Osmolarity (mosmol l^{-1})	π (mosmol kg^{-1})
ACSF	154	136	83 ± 0·3	0	333	297 ± 0·5
H-40	114	96	111 ± 4	34·5	253	217 ± 2
H-60	94	76	129 ± 1	56·3	213	189 ± 2
H-90	64	46	186 ± 5	115·7	153	134 ± 1
F-40	114	96	116 ± 6	41·0	333	295 ± 2
F-60	94	76	135 ± 2	63·3	333	300 ± 6
F-90	64	46	197 ± 7	138·4	333	300 ± 4
M-40	114	96	115 ± 4	38·7	333	298 ± 1
M-60	94	76	136 ± 3	65·1	333	298 ± 2
M-90	64	46	194 ± 5	135·4	333	297 ± 2

ACSF, control artificial cerebrospinal fluid; H-40, hyposmotic solution with 40 mM NaCl deleted; H-60, 60 mM NaCl deleted; H-90, 90 mM NaCl deleted; F-40, F-60, F-90, isosmotic, fructose-substituted, NaCl-deficient solutions; M-40, M-60, M-90, isosmotic, mannitol-substituted NaCl-deficient solutions. R_o, measured resistivity of the solution. ΔR_o, percentage resistivity increase compared with control ACSF. Osmolarity (osmotic concentration), total osmoles dissolved per litre of solution. π, measured osmotic pressure. Values are means ± S.E.M.

hyperosmotic solutions. To our surprise, these cells did not swell during sudden, brief hyposmotic exposure and did not always shrink in hyperosmotic medium. Voltage-gated ion channels and the channels linked to $GABA_A$ receptors were indiscriminately suppressed in both hypo- and hyperosmotic environments (Somjen, Faas, Vreugdenhil & Wadman, 1993; Vreugdenhil, Somjen & Wadman, 1995). In contrast, hippocampal tissue slices seemed to swell when bathed in hyposmotic medium, as judged by the optical focus of the surface of the slice viewed under a water immersion lens (Somjen *et al.* 1993).

The exact degree to which interstitial volume (ISV) is reduced as a function of lowered osmotic pressure has not previously been determined. In this paper we describe the ISV decrease estimated by the indicator ion dilution technique (Dietzel, Heinemann, Hofmeier & Lux, 1980; Nicholson, 1993), and the related increase in tissue resistance, in rat hippocampal tissue slices. We also examine changes in interstitial ion concentrations associated with lowering of π_o.

Some of our results have appeared as abstracts (Hester *et al.* 1993; Chebabo, Aitken, Somjen & Do Carmo, 1994*a*; Chebabo, Jing, Aitken & Somjen, 1994*b*).

METHODS

Preparation of the slices

Transverse hippocampal slices, 400 μm thick, were prepared from Sprague–Dawley rats (175–250 g) decapitated under ether anaesthesia, as previously described (Dingledine, 1984; Balestrino, Aitken & Somjen, 1986). Slices were maintained in an interface recording chamber which was perfused at a rate of 1·5 ml min^{-1} with artificial cerebrospinal fluid (ACSF) containing (mM): NaCl, 130; NaH_2PO_4, 1·25; $NaHCO_3$, 24; $CaCl_2$, 1·2; $MgSO_4$, 1·2; glucose, 10; at pH 7·4 and 35 °C and saturated with 95% O_2–5% CO_2. Hyposmotic solutions were prepared by deleting NaCl, leaving other ingredients unchanged (see below and Table 1). Recordings were started 90 min after placing slices in the chamber.

Induced osmotic change

To test the effects of low osmotic pressure (π), three solutions with different π values were prepared by omitting 40, 60 or 90 mM of NaCl (Table 1). Isosmotic low-NaCl solutions were prepared by substituting the deleted NaCl by equiosmolar (i.e. slightly less than 80, 120 and 180 mM, adjusted by freezing-point osmometry) fructose or mannitol. For simplicity these solutions are referred to as follows: H-40, H-60 and H-90 designate the three grades of low π, while F-40, F-60 and F-90 or M-40, M-60 and M-90, refer to fructose- or mannitol-substituted low-NaCl solutions, respectively. Table 1 lists osmotic concentrations (sum of osmoles dissolved), as well as the actual osmolarities measured with a freezing-point osmometer (Model 3DII, Advanced Digimatic, Needham Heights, MA 02194, USA).

In most experiments the slices were exposed for 30 min to each of three test solutions, in order of decreasing π or NaCl concentration ([NaCl]). Between administration of the test solutions the slices were allowed to recover in control ACSF for 30 min. This period of time was sufficient for equilibration of interstitial space with modified ACSF (Fig. 1). In other experiments, exposure to a single level of low π lasted for 60 min, and recovery was allowed for 120–160 min.

Extracellular tissue resistance (R_o) measurements

To measure extracellular electrical tissue resistance (R_o) we used the 'four-electrode' method introduced by Li, Bak & Parker (1968) as modified by Traynelis & Dingledine (1989). A pair of nichrome wire electrodes, insulated except at the tip (nominal o.d. 0·038 mm) and held in a glass pipette with a short length of wire protruding, were placed in the stratum radiatum to deliver constant current pulses of 3–4 μA, 10 ms. The pulse was supplied by a photoelectrically coupled Grass PSIU6 constant current stimulus-isolation unit. Constancy of the current in the course of

Table 2. Changes in interstitial volume and tissue resistance in stratum radiatum of CA1 of hippocampal tissue slices

Solution	ISV (Δ%)	n	R_o (Δ%)	n	Corrected R_o (Δ%)
H-40	−41·4 ± 4·6*	6	103 ± 13 **	9	76·0
H-60	−57·9 ± 3·6**	6	135 ± 36 **	9	93·0
H-90	−75·5 ± 2·7 **	6	216 ± 47**	9	121·0
F-40	7·3 ± 31	3	33·4 ± 8·7**	5	−8·3
F-60	−3·4 ± 1·9	3	57·8 ± 9·4**	5	−2·6
F-90	−2·3 ± 0·6	3	94·1 ± 20 **	5	−40·3
M-40	24·0 ± 38	6			
M-60	−26·2 ± 10·4	5	43·7 ± 6·4**	5	−19·9
M-90	−43·1 ± 12·0	6	65·6 ± 6·3**	5	−67·9

ISV, interstitial volume (estimated from TMA^+ or TEA^+ volume of dilution). R_o, measured tissue resistance. Corrected R_o, tissue resistance minus resistivity of solution. All values given as percentage change of mean from control ± S.E.M. n, numbers of slices tested. * $P < 0{\cdot}05$ and ** $P < 0{\cdot}01$, compared with the previous value, e.g. H-40 to control, H-60 to H-90 etc.

osmotic changes was checked by recording the voltage across a resistor in series with the preparation.

The voltage drop generated in the tissue (V_{R_o}) by these current pulses was measured differentially by two micropipettes filled with 150 mM NaCl (4–10 MΩ resistance) which were placed in between, and in a straight line with, the wire electrodes. Current delivery and recording electrodes were inserted to a depth of about 40 μm. The distance between current delivery electrodes was approximately 1 mm, and between the recording micropipettes about 250 μm. As all changes were expressed as a percentage of the control, exact distances were not important.

V_{R_o} pulses varied from about 4 to 22 mV in different experiments and at different osmolalities. These pulses did not evoke a physiological response when the slice was in normal ACSF or moderate hypotonia (see inset in Fig. 5), but a small evoked response sometimes appeared in strongly hypotonic conditions. These small physiological responses did not interfere with measurement of the pulse amplitude at the end ('corner') of the square wave.

The electrical conductivity of solutions is reduced when the concentration of electrolytes is lowered. In order to determine the effect of osmotic cell swelling on R_o, the effect attributable to the reduction of ionic strength must be subtracted from the total change of R_o. Solution resistivities can be calculated from standard tables (Parsons, 1959) from the sums of the conductivities of the electrolytes of the bathing solutions. Since the change in resistivity (ΔR_o) was expressed as the percentage change (Δ%) compared with the control, computed resistivities of the solutions (usually in Ω cm) were also converted to Δ%. The calculated resistivity of the H-40 solution was 34% higher than that of normal ACSF; for H-60 the increase was 61%; and for H-90, 133%. These computed numbers do not take into account interaction among ions in mixed solutions. For this reason the resistivity of the bathing solutions was also measured with a bridge resistivity instrument (Model 31, Yellow Springs Instruments, Yellow Springs, OH, USA). These measured solution resistivities, which are slightly different from the calculated values, are listed in Table 1. The corrected ΔR_os listed in the last column of Table 2 were obtained by subtracting the measured increases of solution resistivity (expressed as Δ%) from the measured (total) mean increases of R_o.

Ion-selective electrodes

Double-barrelled glass microelectrodes were used to record extracellular ionic activity (Somjen, 1981; Nicholson, 1993), with the Fluka ion exchangers for K^+ (ionophore cocktail B: 60398), Ca^{2+} (cocktail A: 21048) and Na^+ (cocktail A: 71178) (Fluka, CH-9470 Buchs, Switzerland) as liquid membranes. To measure TMA^+ and TEA^+ (see below) the WPI IE-190 ion exchanger was used, which is equivalent to the Corning 477317 exchanger, and is about three orders of magnitude more sensitive to TMA^+ than to K^+ (Dietzel *et al.* 1980). Electrodes were calibrated before and after each experiment, and the experiment was rejected in the rare instances when the variation exceeded 10%. The sensitivities ('slopes') for K^+, Na^+ and TMA^+ electrodes ranged from 57 to 61 mV decade^{-1} and for Ca^{2+} electrodes from 26 to 28 mV decade^{-1}. Ionic signals were recorded on chart paper and occasionally also by computer, and transformed off-line to millimolar scale. The reference barrel of the ion-selective electrode served to record DC-coupled extracellular potential (V_o) (Somjen, 1981). Ion-selective electrodes respond to ion activity rather than to concentration, but calibrations were made in terms of the known concentrations of the calibrating solutions, and the results are reported as concentrations (see also Nicholson, 1993). Concentrations remain proportional to activities only as long as the activity coefficient does not change, and activity coefficients are influenced by total ionic strength. Moreover, none of the exchangers are absolutely selective for the measured species of ion and therefore when the concentration of more than one ion is changing, interference with the electrode potential is possible. The effects of ionic strength and imperfect selectivity of the exchangers must be taken into account in using these electrodes. Ordinarily, the standard calibrating solutions were made in ACSF by varying the relevant ion but keeping total ion concentration constant by reciprocal changes in Na^+ concentration ($[Na^+]$) (Somjen, 1981). For the present purpose, in order to check the influence of changing $[Na^+]$ and ionic strength, electrodes were calibrated in normal as well as low π and low [NaCl]. The 'slopes' of calibration curves changed only $\leqslant 1$ mV decade^{-1} for any of the ion exchangers, but in reduced π (and [NaCl]) the calibration curves of Ca^{2+}- and TMA^+-selective electrodes did shift. At the lowest concentrations (H-90 solution) this shift amounted to −2 to −2·5 mV for Ca^{2+}-selective electrodes. In the presence of 0·2 mM TMA^+ the potential of TMA^+ electrodes

changed by −0·8, −2·0 and −5·1 mV when the calibrating bath was changed from normal ACSF to, respectively, H-40, H-60 and H-90 solutions. The data of Figs 3, 7 and 8 and of Table 2 have been corrected by adding these values to baseline voltages. Na^+-selective electrodes were always calibrated in ACSF with varying [NaCl] (and therefore also varying ionic strength), leaving other ingredients unchanged. With NaCl being the dominant extracellular electrolyte this imitated the condition of measuring extracellular Na^+ concentration ($[Na^+]_o$) in the slices, and no correction of the readings of Na^+-selective electrodes was required.

Estimation of relative interstitial volume (ISV)

Changes of relative interstitial volume (ISV) were estimated from the concentration change of a probe ion, either TMA^+ or TEA^+ (Dietzel *et al.* 1980; Nicholson, 1993). These ions enter cells very slowly and therefore become more concentrated in extracellular fluid if cells swell. To provide a baseline concentration, 0·2 mM of the probe ion was dissolved in the bath. At this concentration the pharmacological effect of TEA^+ is weak or absent. Additionally, probe ions were administered by microiontophoresis, and recorded by an ion-selective electrode fastened so that its tip lay 60–150 μm from the tip of the iontophoresis pipette. The electrode assemblies were inserted to a depth of 30–60 μm. The iontophoresis pipettes contained 1·0 M solution of the chloride salt of the probe ion (resistances of 8–20 MΩ). The iontophoresis pulses lasted 0·5–2 s, 50–150 nA amplitude (constant throughout an experiment), once every 100 s. The bridge electrolyte in the shaft of the ion-selective microelectrode was 150 mM TMA-Cl or TEA-Cl without added K^+ or other ions (Jing *et al.* 1994). In the presence of 0·2 mM TMA^+, the electrodes responded by 4–5 mV to a decade change in the K^+ concentration ($[K^+]$), and this could have interfered with $[TMA^+]$ measurements. $[K^+]_o$ decreased slightly and usually transiently during low π_o treatment (see Results) but the error in estimating ISV must have been very small. The 'slope' of the TMA^+ calibration was unchanged in low and high $[K^+]$ as well as in low and high [NaCl] (see above).

The estimation of ISV changes was based on the assumption that all the iontophoretically delivered probe ions remained in the interstitial space for the few seconds until the concentration reached its peak (Fig. 2) and that tortuosity, electrode distance and transport number remained constant during an experiment (Dietzel *et al.* 1980; Nicholson, 1993; Jing *et al.* 1994). Therefore, to estimate changes in ISV (ΔISV), the amplitude of the transient increases of concentration achieved by iontophoretic pulses were measured (Fig. 2). Decrease of the virtual volume of dilution was expressed as the percentage of the control according to the formula:

$$100 \times \{(\Delta[TMA^+]_{contr}/\Delta[TMA^+]_{hypot}) - 1\},$$

where $\Delta[TMA^+]_{contr}$ and $\Delta[TMA^+]_{hypot}$ are the amplitudes of the transient increases of concentration (transient peak − baseline) in control and in hypotonic conditions, respectively (see also Dietzel *et al.* 1980).

Statistical analysis

The variables measured during osmotic manipulations were expressed as a percentage deviation from the mean measured initially in normal solution. Data were compared using the paired Student's *t* test or one-factor ANOVA, as appropriate.

RESULTS

Equilibration of the tissue with test solutions

To verify equilibration of interstitial fluid with bathing fluid, the extracellular Na^+ concentration, $[Na^+]_o$, was recorded with Na^+-selective microelectrodes. A few minutes after switching from normal to hypotonic solution, $[Na^+]_o$ began to decrease, and reached a level corresponding to the concentration of Na^+ in the bathing solution within 20–30 min. One of the reasons for this delay is probably

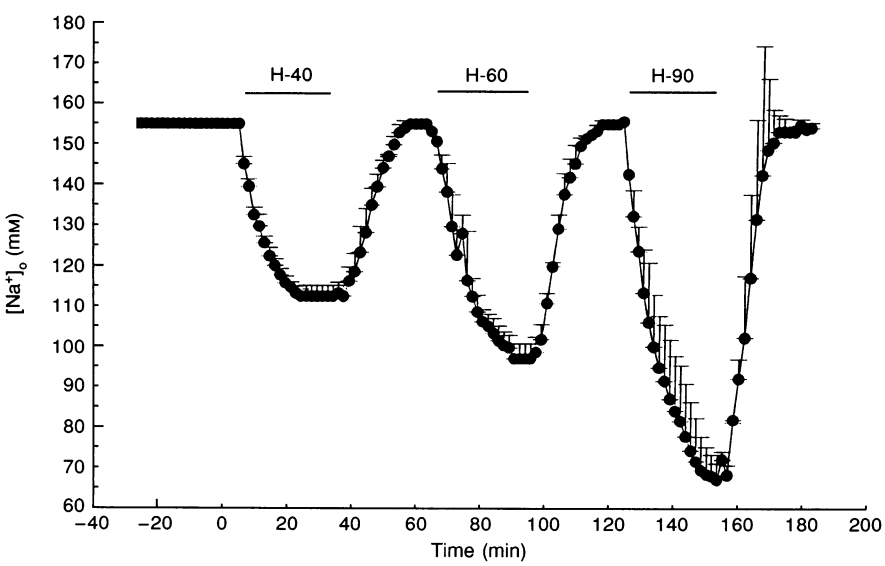

Figure 1. Decrease of $[Na^+]_o$ in a hippocampal slice during perfusion of the tissue chamber by hypotonic solutions

Recording by ion-selective microelectrode, converted to linear millimolar scale off-line. The points show the means ± S.E.M. of three experiments. The horizontal bars indicate the periods of hypotonic perfusion. H-40, H-60 and H-90 indicate the amount of NaCl (in mM) deleted from the solutions (see Table 1).

the slow exchange in the unstirred layer in contact with the slice which rests on a nylon mesh, another is the diffusion within the tissue. Figure 1 shows means and S.E.M. from three experiments.

Changes of TMA^+ space during hypotonic exposure

With a delay similar to that of $[Na^+]_o$, during perfusion with hypotonic solution both the baseline concentration of the marker ion ($[TMA^+]$ or $[TEA^+]$) and the superimposed concentration transients induced by iontophoresis ($\Delta[TMA^+]$) increased (Fig. 2), reaching maximum about 10–25 min after the beginning of the baseline shift or 15–30 min after switching the supply solutions at their source. These changes are consistent with a reduction of the volume in which the probe ion was diluted, in other words with a shrinkage of interstitial volume (ISV), due to swelling of cells. In eight experiments, slices were exposed to three successively lower π_o for half an hour each, with normal control perfusion for half an hour allowing for recovery between exposures. The composition of the three low-π solutions is shown in Table 1. In six of these experiments the iontophoretic and ion-selective electrodes were in stratum radiatum, and in two experiments they were in stratum pyramidale. In five of these experiments TMA^+ was the probe ion and in the other three, TEA^+ was used (all three in stratum radiatum). There was no detectable difference in the results obtained with the two probe ions. The increase of the TMA^+ and TEA^+ signals was dependent on the degree of the decrease in osmotic concentration. The baseline concentration of the probe ions measured in stratum radiatum increased from a mean of 0·2 mM to 0·25 mM in H-40, 0·32 mM in H-60 and 0·59 mM in H-90 solution, which corresponds to estimated changes of ISV by respectively −30, −37 and −66% ($n = 6$). Because of the slow exchange of interstitial fluid with bathing fluid, these values probably underestimate the ISV changes. More reliable are estimates derived from the amplitudes of the transient increases of probe ion concentration achieved by the iontophoretic pulses (see Methods). The maximal changes of ISV calculated from $\Delta[TMA^+]$ and $\Delta[TEA^+]$ in stratum radiatum (as a percentage of control ISV) are plotted against $\Delta\pi_o$ in Fig. 3*A*. The means and S.E.M. of the measurements are shown in Table 2. At the greatest dilution, with the mean osmolality at 134 mosmol kg^{-1} (Table 1), SD episodes frequently interfered with measurements. These bouts of SD induced by hypotonic swelling will be described elsewhere. The values in Table 2 refer to the maximal ISV changes during SD-free intervals. In stratum pyramidale the ISV decreased more than in stratum radiatum (mean of the two experiments: for H-40, −45%; for H-60, −69%; for H-90, −87%).

When low-π perfusion continued beyond 30 min, the TMA^+ signals usually began to subside, suggesting regulatory cell volume decrease (RVD). RVD was not complete within 60 min of exposure. Figure 4 illustrates the variability of this presumably compensatory decrease among preparations. Washing the slice with normal ACSF was always followed by undershooting of the TMA^+ signals,

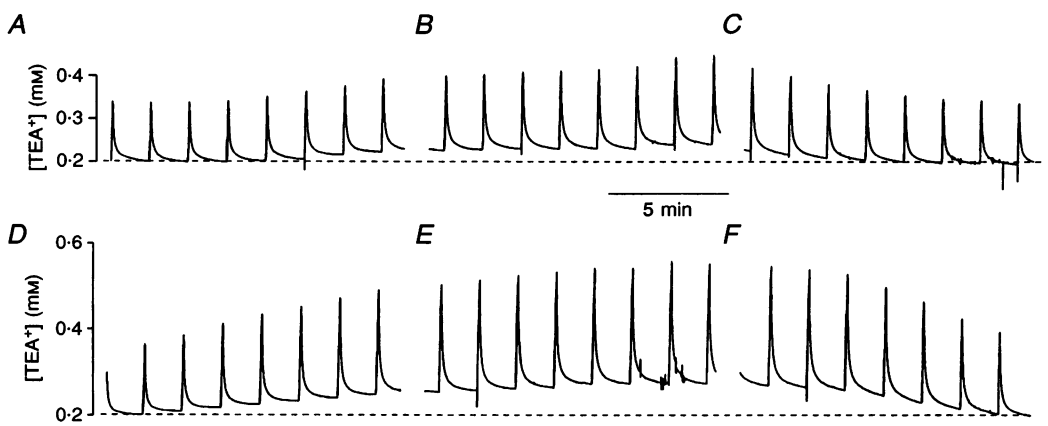

Figure 2. Decrease of interstitial volume (ISV) during exposure of a hippocampal slice to two levels of hypotonia

The tracings show the concentrations of TEA^+ measured by an ion-selective microelectrode in stratum radiatum of CA1 region; TEA^+ concentration is inversely proportional to its volume of dilution (see Methods). TEA^+ (0·2 mM) was present in the bathing solution, and was also released from a nearby iontophoresis electrode by pulses of 1 s, 160 nA, which cause the sharp positive transients. The logarithmic voltage response of the ion-selective electrode was converted to linear millimolar scale by computer, but no other correction was applied. *A*, records taken at the beginning of 30 min perfusion of the tissue chamber with a solution of 220 mosmol kg^{-1} (H-40); *B*, records taken near the end of the 30 min perfusion period; *C*, partial record of the recovery during return to normal ACSF. *D*, *E* and *F*, similar exposure to 184 mosmol kg^{-1} (H-60), later in the same experiment. At the discontinuities of the tracing, parts of the record have been deleted.

indicating expansion of ISV in the initial phase of recovery, corresponding to post-hypotonic cell shrinkage.

The DC voltage trace (ΔV_0) shifted in the negative direction when hypotonic or low-NaCl solution reached the chamber. This shift was reproduced when the electrode was immersed in the bath, and it therefore is attributable to changing junction potential due to lowered [NaCl].

Changes in tissue resistance (R_0) at low π_0

In separate experiments R_0 was measured as the voltage drop (V_{R_0}) generated by constant current pulses, as described under Methods. R_0 increased during administration of hypotonic solution, and the increase was dependent on the degree of lowering of π_0 (Figs 3*B* and 5). When hypotonic exposure was extended to 60 min, R_0 first increased and then partially subsided, in a similar way to the TMA^+ signal (Fig. 6). After restoring normal π_0, R_0 temporarily decreased below its control level.

The increase of R_0 must be attributed to two factors. One is the higher resistivity of low-NaCl solutions and the other is the swelling of cells at the expense of ISV. Due to the high resistance of cell membranes, most of the imposed current flows through interstitial space and only a small fraction through cells. Table 1 shows the measured resistivity of the hyposmotic solutions. To assess the effect attributable to cell swelling, we assumed that the two factors, restriction of conductive path and reduced ionic strength, are additive. Figure 3*B* shows the total ΔR_0 as well as the corrected ΔR_0 (attributed to cell swelling) as functions of π_0. The ΔR_0 values in Table 2 have been similarly corrected for solution resistivity.

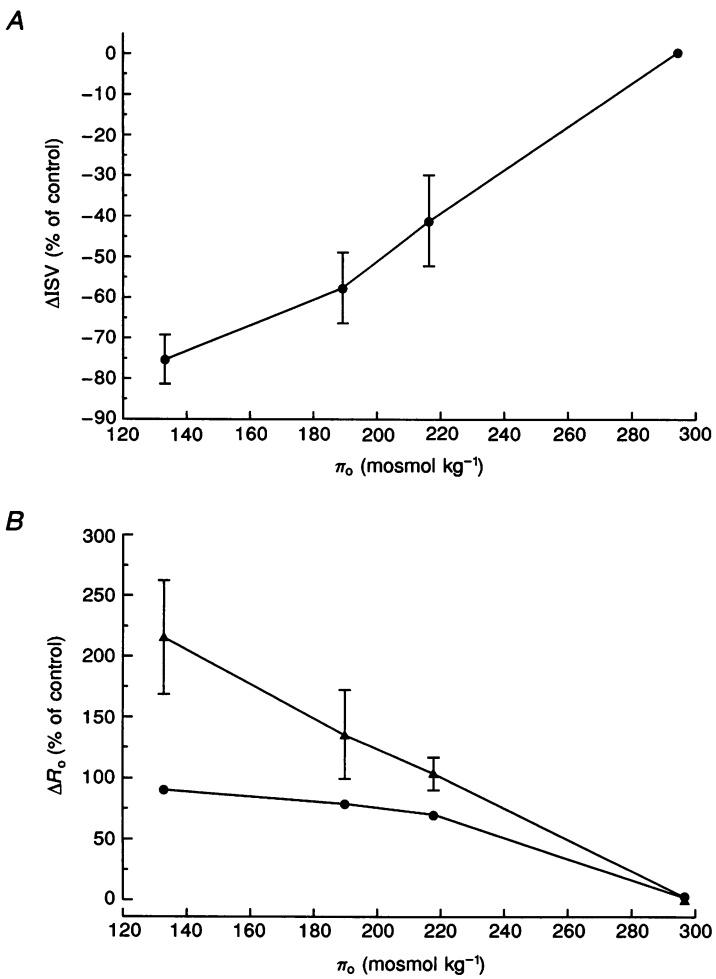

Figure 3. ΔISV and ΔR_0 as functions of π_0

A, mean (± S.E.M.) maximal change of interstitial volume (ISV) as a function of extracellular osmolality (π_0), derived from experiments similar to the one illustrated in Fig. 2. *B*, mean maximal change of total tissue resistance (R_0, ▲), and resistance attributable to cell swelling, corrected for solution resistivity (●), as functions of extracellular osmolality (π_0).

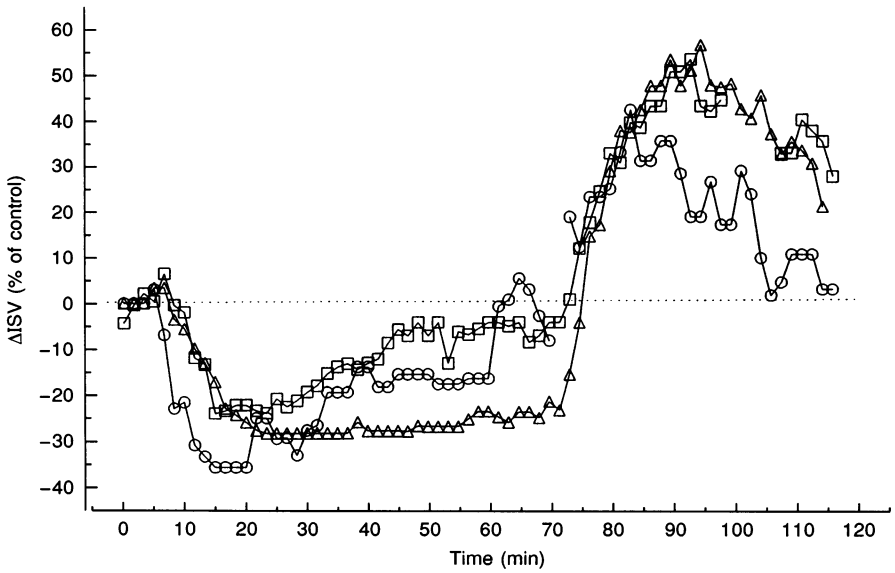

Figure 4. Changes of interstitial volume (ISV) in CA1 stratum radiatum during 60 min exposure to H-40 hypotonic solution, and during recovery

The perfusion was changed from control ACSF solution to H-40 solution at zero time. The ordinate scale represents the change of ISV as a percentage of initial control. The points were derived from recordings similar to those shown in Fig. 2. Different symbols represent 3 different experiments.

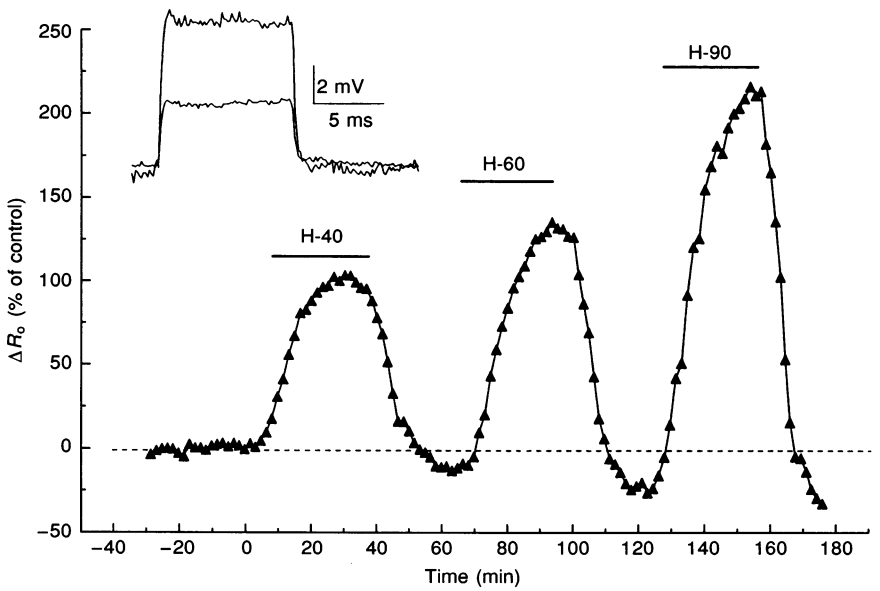

Figure 5. Changes of tissue resistance (R_0) during three successive administrations of increasingly hypotonic solutions

The points show changes of the normalized mean amplitude of the voltage generated by constant current pulses (V_{R_0}) in stratum radiatum of nine hippocampal tissue slices, which are proportional to total (uncorrected) R_0. Horizontal lines indicate times of perfusion of the chamber by hypotonic solutions. The inset shows two superimposed sample recordings of V_{R_0} pulses in control condition and during exposure to H-60 hypotonic solution.

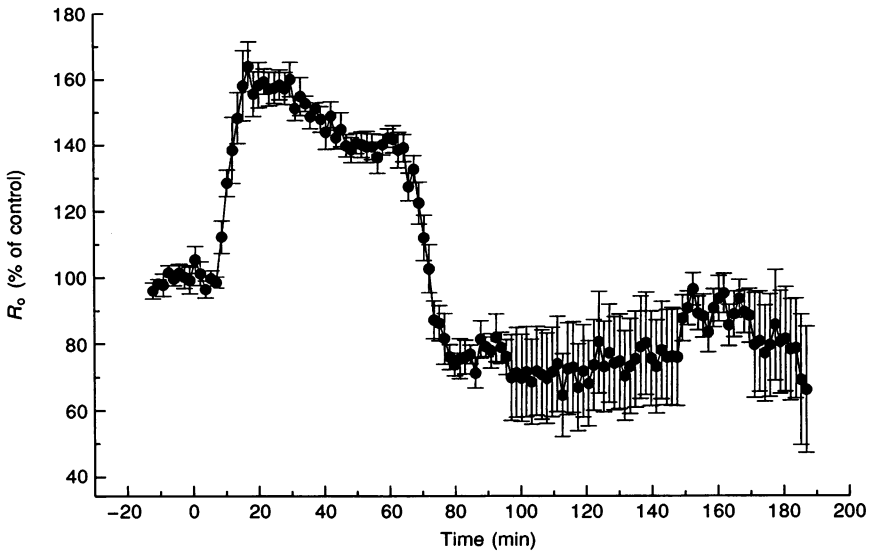

Figure 6. Regulatory volume decrease indicated by R_o

Time course of the change of total (uncorrected) tissue resistance (R_o) during 60 min of exposure to hypotonic solution (H-40) and partial recovery. Means ± S.E.M. of 6 experiments.

Effects of isosmotic NaCl-deficient solutions on R_o and ISV

V_{R_o} also increased during exposure to isosmotic NaCl-deficient solutions, but the increase was less than expected from the resistivity of the bathing solution. With the mild to moderate NaCl-deficient levels, and with fructose as the 'filler' (F-40, F-60 solutions), the discrepancy was small and not significant (Tables 1 and 2). At the severely NaCl-deficient level (F-90 and M-90), however, the ΔV_{R_o} was relatively small, resulting in an apparent decrease of the corrected resistance (Table 2).

The effect of isosmotic, NaCl-deficient solutions was also tested on TMA^+ iontophoretic signals. With fructose substituting for NaCl, the ISV changes were small and inconsistent, as may be expected. When mannitol was substituted for NaCl, the result was similar to fructose in some of the trials, but in several cases, relative ISV seemed

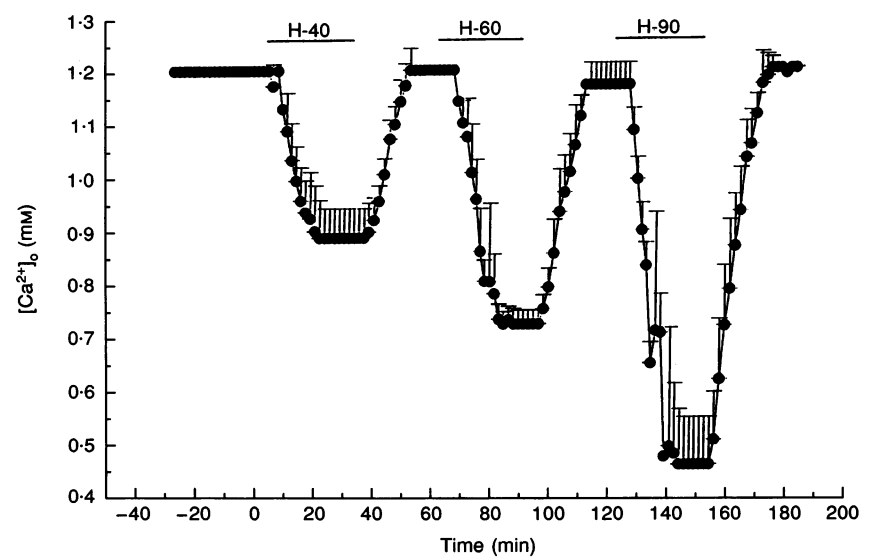

Figure 7

Concentration of extracellular calcium ($[Ca^{2+}]_o$) in stratum radiatum of CA1 region of hippocampal tissue slices during exposure to 3 increasingly hypotonic solutions. $[Ca^{2+}]_o$ was measured with double-barrelled ion-selective microelectrodes. The points show means ± S.E.M. from 3 experiments. The horizontal bars indicate times of administration of hypotonic solutions.

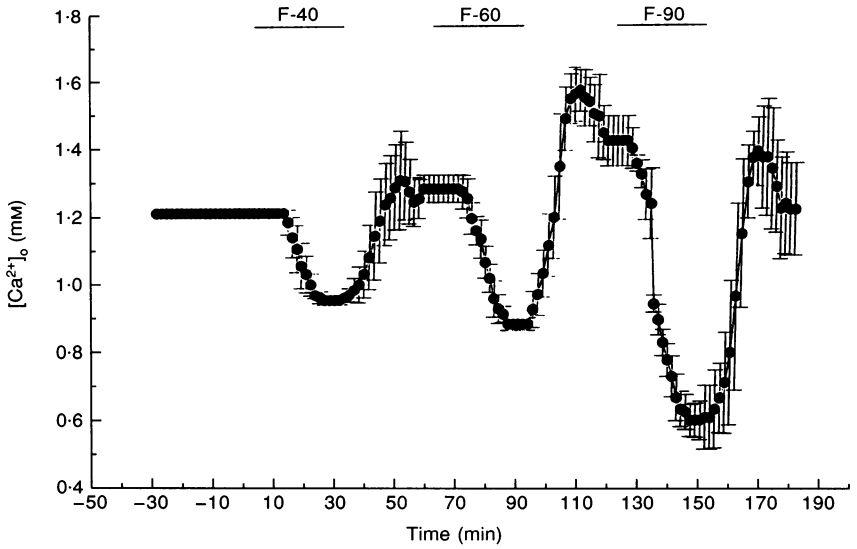

Figure 8

Concentration of extracellular calcium ($[Ca^{2+}]_o$) in stratum radiatum of CA1 region of hippocampal tissue slices during exposure to 3 levels of isosmotic, fructose-substituted NaCl-deficient solutions. Data pooled from 5 experiments.

to decrease considerably. The erratic results obtained with mannitol substitution contrast sharply with the predictability of low π_o and fructose. The large standard errors shown in Table 2 for mannitol substitution reflect this inconsistency.

Changes of extracellular ion concentrations

The changes of $[Na^+]_o$ at three levels of reduced π_o perfusion are illustrated in Fig. 1. Rather unexpectedly, $[Ca^{2+}]_o$ also decreased strongly, consistently, reversibly, and to a degree that depended on the lowering of π_o, even though the concentration of calcium in the hypotonic solutions was identical to that in normal ACSF. Figure 7 shows the mean and standard error of extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) measurements in three experiments. In the most hypotonic solution, $[Ca^{2+}]_o$ decreased to 37·5% of its normal level, reached in about 20 min of exposure. To check the influence of the low [NaCl] of the hypotonic solutions, we also recorded $[Ca^{2+}]_o$ during exposure to isosmotic fructose-substituted NaCl-deficient solutions. Figure 8 illustrates the results of five experiments. In low [NaCl] solutions, $[Ca^{2+}]_o$ decreased almost as much as it did in hypotonic solutions of identical [NaCl] levels. The main difference was the strong, transient overshooting of $[Ca^{2+}]_o$ above the normal level during recovery from low [NaCl] exposure, which was not seen after hypotonic treatments.

When H-40 hypotonic solution was introduced into the tissue chamber, $[K^+]_o$ decreased slightly but consistently (mean of −0·3 mM, $n = 3$) but then it recovered in spite of continued exposure to hypotonic solution. In H-60 and H-90 solutions, the decrease was greater (up to −0·5 mM) and tended to be more maintained. In strongly hypotonic solution, however, episodes of SD erupted and caused $[K^+]_o$ to transiently increase to very high levels. Between SD episodes $[K^+]_o$ tended to remain somewhat low even in H-90 solution.

DISCUSSION

The mildest degree of lowered π_o (H-40) used in this study corresponds to the plasma Na^+ concentration seen in severe clinical hyponatraemia (Katzman & Pappius, 1973). A reservation attaches to this comparison. Brain *in situ* is capable of ion and volume regulation at the blood–brain barrier as well as at cell plasma membranes (Katzman & Pappius, 1973; Cserr, DePasquale, Nicholson, Patlak, Pettigrew & Rice, 1991; Strange, 1992). It is therefore not clear whether plasma $[Na^+]$ is a precise indicator of cerebral $[Na^+]_o$. The blood–brain barrier is absent in tissue slices, and cell membranes may also be less able to maintain regulation, because the constantly refreshed hyposmotic bath fluid may present an overwhelming load. In slices exposed to hyposmotic solution, $[Na^+]_o$ reaches bath level in 20–30 min (Fig. 1), indicating equilibration of interstitial fluid with the bath. This rate of equilibration is similar to that found for K^+ and Ca^{2+} (Dingledine & Somjen, 1981; Balestrino *et al.* 1986).

Interstitial volume and cell volume

ISV, estimated from the apparent volume of dilution of TMA^+ and TEA^+, decreased linearly with decreasing π_o until it reached 192 mosmol kg^{-1} and departed from linearity only slightly at the lowest π_o of 134 mosmol kg^{-1} (Fig. 3*A*). This suggests that, within the range tested, the limit of cell swelling has not been reached. The absolute limit must be the point where the outer surfaces of plasma membranes are pressed together but this extreme situation

may be prevented by the interstitial matrix. In the most severely hypotonic condition applied in this study (H-90), ISV was obliterated to about the same degree as during SD and reversible hypoxic SD-like depolarization induced in slices maintained in ACSF of normal π (Jing *et al.* 1994). In CA1 stratum radiatum, normal interstitial space reportedly amounts to 13·2% of the total tissue volume (McBain, Traynelis & Dingledine, 1990). During the most severe hypotonic swelling in this study, ISV was reduced to 25% of its control volume, which means that ISV has shrunk to only 3·3% of the total volume of the tissue. If all the expansion of the intracellular compartment occurred at the expense of the ISV, then cells expanded on average from 86·8% of the total tissue volume at rest to 96·7% during maximal hypotonia, an increase of 11·4% over the normal total cell volume. This calculation assumes that water moved only from interstitial space into cells, not from the bath into the tissue slice. If the slices took up more water than was supplied from the interstitial space so that total tissue volume increased, then cell swelling must have been greater than 11·4%. In a previous experiment, when thinner hippocampal slices were viewed with a water immersion lens, the surface of the slice was seen to rise out of focus during hypotonic exposure, indicating thickening of the slice as a whole (Somjen *et al.* 1993). The 11·4% just calculated must therefore be regarded as a minimum value.

The swelling need not have been equal among various types of cells, nor even various parts of the same cells. From the ISV and resistance measurements it is not clear which cellular elements swelled the most. Reports concerning other preparations raise the possibility that glial processes and neuronal dendrites may swell more than neurone somata or axons (Van Harreveld & Khattab, 1967; Kimelberg, Sankar, O'Connor, Jalonen & Goderie, 1992; Osehobo, MacVicar & Andrew, 1992; Andrew & MacVicar, 1994). When freshly dissociated hippocampal pyramidal cells were suddenly and briefly exposed to severely hyposmotic solutions, they did not seem to swell at all (Somjen *et al.* 1993). Most of the dendrites are lost during the preparation of cell suspensions. The ability of such dendrite-poor cells to resist hypotonic swelling may mean that the swelling of tissue *in situ* is limited to the dendritic tree and to glial cells. When ion-selective and iontophoretic electrodes were inserted among the cell bodies of stratum pyramidale, the degree of ISV loss was comparable to that measured in the neuropil of stratum radiatum. This does not resolve the issue, however, because glial processes among the cell bodies in stratum pyramidale may have swollen sufficiently to fill the available space, which is smaller in stratum pyramidale than in stratum radiatum, even under normal conditions (McBain *et al.* 1990). In addition, swelling in the adjacent layers, stratum oriens and stratum radiatum, may have compressed the structure of stratum pyramidale. Our earlier measurements of ISV change during SD suggested that, in some cases, TMA^+ entered cells at the height of SD-related cell swelling (Jing *et al.* 1994). It was for this reason that we used TEA^+ in some experiments in this study. There was no discrepancy between ISV measurements made with TMA^+ and with TEA^+ during hypotonic cell swelling. Either both ions were kept out of cells, or both were taken up to the same, presumably very limited, degree.

Tissue electrical resistance and cell volume

That cell swelling is associated with increasing tissue impedance was demonstrated several decades ago (Van Harreveld & Schadé, 1960). The basis for this relationship is the high electrical resistance of cell membranes, which prevents current imposed by an external generator from flowing through cells. Current flow through cells is not zero, however. Ranck (1963) attempted to estimate the admittance of glial cells and neurones, but his model was based on numerous assumptions. More recently, Okada, Huang, Rice, Tranchina & Nicholson (1994) derived the relationship of the 'apparent' (i.e. measured) tissue conductance (σ_a) and the true extracellular conductance (σ_e) calculated from independent estimates of the interstitial volume fraction (α) and tortuosity (λ) in turtle cerebellum *in vitro*. They concluded that σ_e was about two-thirds of σ_a, in other words one-third of the total conductance was transcellular. While the actual fraction in rat hippocampus may differ from that in turtle cerebellum, it seems likely that in measurements such as the ones we report, a substantial fraction of the measuring current flows through cells.

There are two other potential sources of error that could have resulted in underestimation of R_o and its changes. A small fraction of the current delivered to the tissue must have been shunted through the solution in contact with the slice (see also Li *et al.* 1968). The tip of the recording electrodes was at a depth of 40 μm in a slice of 350–400 μm thickness and with the slice at the gas–liquid interface, only the undersurface was in contact with bulk solution. Probably most of the current detected as a voltage drop between the recording electrodes (V_{R_o}) passed through tissue, and the error must have been slight (see also Traynelis & Dingledine, 1989). Another possible source of error would be capacitive reactance of cell membranes in the tissue. In hippocampal slices, Holsheimer (1987), and in turtle cerebellum, Okada *et al.* (1994) found no difference in the impedance to sinusoidal currents of varying frequency within the physiologically interesting frequency range. Therefore the tissue can be considered to be a resistive (ohmic) conductor.

The plot of the increase in the corrected tissue resistance was not a mirror image of the increase in ISV (Fig. 3*A* and *B*). If interstitial space consisted of simple cylindrical conduits whose length remained constant while the diameter changed and if penetration of cells by the current were negligible, then ISV and resistance should have been negatively and linearly correlated. Interstitial space is, however, irregularly tortuous. Moreover, the ionic

permeability of plasma membranes may have changed. Stretch-gated channels have been demonstrated in glial cell membranes but their existence in neurones is in doubt (Kempski, Von Rosen, Weigt, Staub, Peters & Baethmann, 1991; Morris & Horn, 1991; Kimelberg *et al.* 1992; Bowman, Ding, Sachs & Sokabe, 1992). When exposed to the more severely hypotonic solutions, the increasingly stretched glial membranes may have become more permeable to small ions (but not to TMA^+), allowing a larger fraction of current to flow through cell membranes. This could explain why the corrected tissue resistance increased more between normal and 220 mosmol kg^{-1} than from 220 to 140 mosmol kg^{-1} (Fig. 3*B*).

Regulatory volume decrease

ISV and tissue resistance tended to recover partially during prolonged hypotonic exposure. Most probably this is due to regulatory volume decrease (RVD) (Cserr *et al.* 1991; Sarkadi & Parker, 1991; Strange, 1992; Gullans & Verbalis, 1993). The exact time course of RVD in these slices cannot be determined. Because of the slow diffusional exchange (Fig. 1), gradually increasing cell swelling may have competed with incipient RVD. Following hypotonic exposure, after returning the slice to ACSF of normal π_o, ISV overshot and R_o undershot the initial control values (Figs 4, 5 and 6). This is as expected, because cells tend to shrink when normal π_o is restored after having undergone RVD. Post-hypotonic shrinkage is explained by the loss of solutes from cells in the course of RVD, which means that after RVD, normal ACSF is hypertonic relative to the depleted cytosol. Recovery of ISV or R_o was usually incomplete in 60 min, and R_o did not always quite return to control level at the end of 2 h washing with normal ACSF.

As $[K^+]_o$ did not increase except during SD, it is unlikely that RVD was mediated to any large extent by a release of K^+ ions, unless K^+ ions released from cells were transported out of the slice and into the bath by way of the glial network. More probably, organic osmolytes acted as the most important regulators.

The effect of reduced NaCl concentration

In contrast to the consistency in the effects of low π_o and fructose-substituted low [NaCl] solutions, exposure to mannitol-substituted NaCl deficient solutions produced erratic results. In some experiments there was little change in the volume of dilution of TMA^+, in a similar way to fructose-substituted solutions, but in others ISV seemed to decrease. The apparent decrease in ISV suggests cell swelling is contrasted by the decrease of the corrected R_o, suggesting cell shrinkage (Table 2). A possible solution to this paradox could be if glial cells swelled while their membrane became permeable to small ions but not to TMA^+. The increased ion permeability could reduce tissue resistance, and the concomitant swelling would restrict ISV. This explanation is, of course, speculative. There is the question of whether the anomaly is due to the very low $[NaCl]_o$ or the high concentration of mannitol, or the combination. Exposure to the F-90 fructose-substituted solution also caused a decrease in the corrected R_o, but apparently no decrease in the ISV (Table 2). As mannitol is used in clinical practice as well as in research, the matter is of more than academic interest and it invites further study.

Interstitial ion concentrations

One of the most striking observations was the reduction of $[Ca^{2+}]_o$ caused by lowering of both π_o and $[NaCl]_o$. In tissue slices surrounded by a solution with Ca^{2+} of constant concentration, the Ca^{2+} ions that disappeared from interstitial fluid must have entered cells. Net uptake may have resulted in part or in whole by failure of Ca^{2+} extrusion from cells by the Na^+–Ca^{2+} exchange transport. Lowering of $[Na^+]_o$ is expected to decrease the driving force of the exchange pump, and the shortfall should be proportional to the deficit of $[Na^+]_o$. Restoring normal π_o after hypotonic exposure, $[Ca^{2+}]_o$ gradually returned to control level, but following exposure to isosmotic low-NaCl solution, $[Ca^{2+}]_o$ temporarily overshot its control level. This temporary increase of $[Ca^{2+}]_o$ was probably caused by the transport of Ca^{2+} ions from overloaded cells into the extracellular fluid. After hypotonic exposure the interstitial space was expanded due to post-hypotonic cell shrinkage, and the expansion of the available volume into which outflowing Ca^{2+} was received may have prevented the overshooting of its concentration.

Reducing $[Ca^{2+}]_o$ to nearly zero induces epileptiform spontaneous burst firing (Jefferys & Haas, 1982; Dudek *et al.* 1990). Although the reduction of $[Ca^{2+}]_o$ caused by low π_o was more moderate, it could be a factor in the increased risk of seizures typical of hyponatraemia and water intoxication (Traynelis & Dingledine, 1989; Dudek *et al.* 1990; Andrew, 1991). Additionally, if the movement of Ca^{2+} from extracellular fluid into cells and presynaptic terminals raised free Ca^{2+} activity in cytosol, it could cause the release of transmitters, including excitatory amino acids, also favouring abnormal excitation.

The drastic changes of $[Ca^{2+}]_o$ contrasted with the relative stability of $[K^+]_o$ which was disturbed only by the irregularly recurring episodes of SD induced by the lowest π_o. Hypotonia-induced SD will be described in detail elsewhere (Chebabo, Hester, Aitken & Somjen, 1995).

Hypotonic cell swelling and cell viability

Excitotoxic and hypoxic neurone injury has been attributed, in part, to cell swelling caused by the uptake of electrolytes and water induced by profound depolarization (Rothman, 1985). This raises the question whether hypotonic stress damaged the cells in the experiments reported here. In similar experiments, synaptically transmitted responses were increased during hypotonic exposure and depressed thereafter (Ballyk *et al.* 1991; Andrew, 1991; Hester *et al.* 1993; Chebabo *et al.* 1994*b*). Following 30 min treatment by H-40 or H-60 solutions,

synaptic function eventually recovered in most slices. One hour exposure to H-90 solution, however, left orthodromic evoked potentials profoundly depressed for at least 5 h after restoring normal π_o, while at the same time antidromic population spikes did recover (Huang, Aitken & Somjen, 1995). This indicates that even prolonged severe osmotic stress of normally oxygenated hippocampal tissue failed to kill the neurones even though it did cause long-lasting failure of synaptic function.

Andrew, R. D. (1991). Seizure and acute osmotic change: clinical and neurophysiological aspects. *Journal of the Neurological Sciences* **101**, 7–18.

Andrew, R. D. & MacVicar, B. A. (1994). Imaging cell volume changes and neuronal excitation in the hippocampal slice. *Neuroscience* **62**, 371–383.

Balestrino, M., Aitken, P. G. & Somjen, G. G. (1986). The effects of moderate changes of extracellular K^+ and Ca^{2+} on synaptic and neural function in the CA1 region of the hippocampal slice. *Brain Research* **377**, 229–239.

Ballyk, B. A., Quackenbush, S. J. & Andrew, R. D. (1991). Osmotic effects on the CA1 neuronal population in hippocampal slices with special reference to glucose. *Journal of Neurophysiology* **65**, 1055–1066.

Bowman, C. L., Ding, J.-P., Sachs, F. & Sokabe, M. (1992). Mechano-transducing ion channels in astrocytes. *Brain Research* **584**, 272–286.

Chebabo, S. R., Aitken, P. G., Somjen, G. G. & Do Carmo, R. J. (1994*a*). Efeitos da adminstraçao de soluçao hipotônica sobre potencial excitatório pós-sináptico e variaçao iônica extracelular em fatias de hipocampo. *Federation of Brazilian Societies of Experimental Biology* (Abstract).

Chebabo, S. R., Hester, M. A., Aitken, P. G. & Somjen, G. G. (1995). Hypotonic exposure enhances synaptic transmission and triggers spreading depression in rat hippocampal slices. *Brain Research* (in the Press).

Chebabo, S. R., Jing, J., Aitken, P. G. & Somjen, G. G. (1994*b*). The reduction of relative interstitial volume of CA1 in hippocampal tissue slices in hypotonic solutions. *Society for Neuroscience Abstracts* **20**, 1510, 621.6.

Cserr, H. F., DePasquale, M., Nicholson, C., Patlak, C. S., Pettigrew, K. D. & Rice, M. E. (1991). Extracellular volume decreases while cell volume is maintained by ion uptake in rat brain during acute hypernatremia. *Journal of Physiology* **442**, 277–295.

Dietzel, I., Heinemann, U., Hofmeier, G. & Lux, H.-D. (1980). Transient changes in the size of extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. *Experimental Brain Research* **40**, 432–439.

Dingledine, R. (1984). *Brain Slices*. Plenum Press, New York.

Dingledine, R. & Somjen, G. G. (1981). Calcium dependence of synaptic transmission in the hippocampal slice. *Brain Research* **207**, 218–222.

Dudek, F. E., Obenaus, A. & Tasker, J. G. (1990). Osmolality-induced changes in extracellular volume alter epileptiform bursts independently of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. *Neuroscience Letters* **120**, 267–270.

Gullans, S. R. & Verbalis, J. G. (1993). Control of brain volume during hyperosmolar and hypoosmolar conditions. *Annual Reviews of Medicine* **44**, 289–301.

Hansen, A. J. & Olsen, C. E. (1980). Brain extracellular space during spreading depression and ischemia. *Acta Physiologica Scandinavica* **108**, 355–365.

Hester, M. A., Aitken, P. G. & Somjen, G. G. (1993). Hypotonic environment enhances synaptic potentials in hippocampal tissue slices. *Society for Neuroscience Abstracts* **19**, 715, 295.15.

Holsheimer, J. (1987). Electrical conductivity of the hippocampal CA1 layers and application to current-source-density analysis. *Experimental Brain Research* **67**, 402–410.

Huang, R., Aitken, P. G. & Somjen, G. G. (1995). The extent and mechanism of the loss of function caused by strongly hypotonic solutions in rat hippocampal slices. *Brain Research* (in the Press).

Jefferys, J. G. R. & Haas, H. L. (1982). Synchronized bursting of CA1 hippocampal pyramidal cells in the absence of synaptic transmission. *Nature* **300**, 448–450.

Jing, J., Aitken, P. G. & Somjen, G. G. (1994). Interstitial volume changes during spreading depression (SD) and SD-like hypoxic depolarization in hippocampal tissue slices. *Journal of Neurophysiology* **71**, 2548–2551.

Katzman, R. & Pappius, H. M. (1973). *Brain Electrolytes and Fluid Metabolism*. Williams & Wilkins, Baltimore, MD, USA.

Kempski, O., Von Rosen, S., Weigt, H., Staub, F., Peters, J. & Baethmann, A. (1991). Glial ion transport and volume control. *Annals of the New York Academy of Sciences* **633**, 306–317.

Kimelberg, H. K., Sankar, P., O'Connor, E. R., Jalonen, T. & Goderie, S. K. (1992). Functional consequences of astrocyte swelling. *Progress in Brain Research* **94**, 57–68.

Li, C.-L., Bak, A. F. & Parker, L. O. (1968). Specific resistivity of the cerebral cortex and white matter. *Experimental Neurology* **20**, 544–557.

McBain, C. J., Traynelis, S. F. & Dingledine, R. (1990). Regional variation of extracellular space in hippocampus under physiological and pathological conditions. *Science* **249**, 674–677.

Morris, C. E. & Horn, R. (1991). Failure to elicit neuronal macroscopic mechanosensitive currents anticipated by single-channel studies. *Science* **251**, 1246–1249.

Nicholson, C. (1984). Comparative neurophysiology of spreading depression in the cerebellum. *Anais da Academia Brasileira de Ciências* **56**, 481–494.

Nicholson, C. (1993). Ion-selective microelectrodes and diffusional measurements as tools to explore the brain cell microenvironment. *Journal of Neuroscience Methods* **48**, 199–213.

Okada, Y. C., Huang, J.-C., Rice, M. E., Tranchina, D. & Nicholson, C. (1994). Origin of the apparent tissue conductivity in the molecular and granular layers of the *in vitro* turtle cerebellum and the interpretation of current source density analysis. *Journal of Neurophysiology* **72**, 742–753.

Osehobo, P., MacVicar, B. A. & Andrew, R. D. (1992). Glial swelling in CA1 stratum radiatum in response to osmotic and electric stimulation. *Society for Neuroscience Abstracts* **18**, 1125, 475.3.

Parsons, R. (1959). *Handbook of Electrochemical Constants*. Butterworth, London.

Ranck, J. B. (1963). Analysis of specific impedance of rabbit cerebral cortex. *Experimental Neurology* **7**, 153–174.

Rothman, S. M. (1985). The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *Journal of Neuroscience* **5**, 1483–1489.

SARKADI, B. & PARKER, J. C. (1991). Activation of ion transport pathways by changes in cell volume. *Biochimica et Biophysica Acta* **1071**, 407–427.

SOMJEN, G. G. (1981). The why and the how of measuring the activity of ions in extracellular fluid of spinal cord and cerebral cortex. In *The Application of Ion-Selective Electrodes*, ed. ZEUTHEN, T., pp. 175–194. Elsevier, Amsterdam.

SOMJEN, G. G., AITKEN, P. G., CZÉH, G., HERRERAS, O., JING, J. & YOUNG, J. N. (1992). The mechanism of spreading depression: A review of recent findings, and a hypothesis. *Canadian Journal of Physiology and Pharmacology* **70** (suppl.), S248–254.

SOMJEN, G. G., FAAS, G. C., VREUGDENHIL, M. & WADMAN, W. J. (1993). Channel shutdown: a response of hippocampal neurons to adverse environments. *Brain Research* **632**, 180–194.

STRANGE, K. (1992). Regulation of solute and water balance and cell volume in the central nervous system. *Journal of the American Society of Nephrology* **3**, 12–27.

TRAYNELIS, S. F. & DINGLEDINE, R. (1989). Modification of potassium-induced interictal bursts and electrographic seizures by divalent cations. *Neuroscience Letters* **98**, 194–199.

VAN HARREVELD, A. & KHATTAB, F. I. (1967). Changes in cortical extracellular space during spreading depression investigated with the electron microscope. *Journal of Neurophysiology* **30**, 911–929.

VAN HARREVELD, A. & SCHADÉ, J. P. (1960). On the distribution and movements of water and electrolytes in the cerebral cortex. In *Structure and Function of the Cerebral Cortex*, ed. TOWER, D. B. & SCHAD, J. P., pp. 239–256. Elsevier, Amsterdam.

VREUGDENHIL, M., SOMJEN, G. G. & WADMAN, W. J. (1995). Effects of strongly anisosmotic and NaCl deficient solutions on muscimol- and glutamate-evoked whole-cell currents in freshly dissociated hippocampal neurons. *Brain Research* **670**, 89–96.

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