Enhancement of Whole Cell Synaptic Currents by Low Osmolarity and by Low [NaCl] in Rat Hippocampal Slices

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Huang, Rong, Daniel F. Bossut, and George G. Somjen. Enhancement of whole cell synaptic currents by low osmolarity and by low [NaCl] in rat hippocampal slices. J. Neurophysiol. 77: 2349−2359, 1997. We recorded whole cell currents of patch-clamped neurons in stratum pyramidale of CA1 region of rat hippocampal tissue slices. Synaptic currents were evoked by orthodromic stimulation while holding potential of the neuron was varied from hyperpolarized to depolarized levels. Extracellular osmolarity (πo) was lowered by superfusion with artificial cerebrospinal fluid in which NaCl concentration ([NaCl]) was reduced. The effect of low extracellular NaCl was tested in additional trials in which NaCl was substituted by isosmolar fructose. Both lowering of πo and isosmotic lowering of extracellular [NaCl] ([NaCl]o) caused reversible increase of excitatory postsynaptic currents. The effect of lowering πo was concentration dependent, and it was significantly stronger than the effect of equivalent isosmotic lowering of [NaCl]o. Inhibitory postsynaptic currents also increased in many but not in all cases. Lowering of πo caused a prolongation of the time constant of relaxation of the capacitive charging current induced by small hyperpolarizing voltage steps. A virtual input capacitance, calculated by dividing this time constant by the input resistance, increased during hypotonic exposure. Isosmotic lowering of [NaCl]o had no effect on time constant or input capacitance. Depolarizing voltage commands evoked spikelike inward currents presumably representing Na+-dependent action potentials generated outside the voltage-clamped region of the cell. These current spikes became smaller in low πo and in low [NaCl]o. Broader, voltage-dependent, presumably Ca2+-mediated inward currents became more prominent during hypotonic exposure. We conclude that lowering of [NaCl]o causes enhancement of excitatory synaptic transmission. Transmission may be facilitated by the uptake of Ca2+ into presynaptic terminals as well as into postsynaptic target neurons, induced by the low [NaCl]o. Lowering of πo enhances synaptic transmission more than does a corresponding isosmotic lowering of [NaCl]. The excess increase recorded from the cell soma in low πo may in part be due to changing electrorheological length caused by the swelling of dendrites.

INTRODUCTION

The osmolarity of extracellular fluid (πo) can decrease because of water loading or salt loss, with serious clinical consequences, including coma and seizures (Avner 1995; Ellis 1995; Thorn et al. 1977). The clinical syndrome of low πo is complicated by transfer of water across the blood-brain barrier and elevation of intracranial pressure, because the barrier is more permeable to water than to most solutes (Bradbury 1979; Yamaguchi et al. 1994). From studies of cerebral tissue slices in vitro it is clear, however, that low πo also has direct effects on brain tissue itself. When such a slice is exposed to a hypotonic bathing solution, the amplitude of extracellularly recorded excitatory postsynaptic potentials (fEPSPS) is greatly enhanced (Andrew 1991; Ballyk et al. 1991; Chebabo et al. 1995a; Rosen and Andrew 1990). Electrographic seizures are facilitated (Andrew et al. 1989; Dudek et al. 1990), but, in the absence of another convulsant stimulus, low πo by itself rarely induces seizure-like discharges (Chebabo et al. 1995a; Huang et al. 1995). More commonly, in hippocampal tissue slices treated by strongly hypotonic bathing solution, recurrent episodes of spreading depression develop. If, however, calcium is withdrawn and magnesium concentration is raised, then similar hypotonic treatment induces recurrent seizures instead of spreading depression (Chebabo et al. 1995a; Huang et al. 1995).

Interpretation of extracellular recordings is complicated by the fact that lowering of πo causes concentration-dependent decrease of interstitial space, resulting in increase of tissue resistance (Rt), which alters the recorded signals. We found that the antidromic population spike amplitude increased in proportion to Rt, but that fEPSP amplitude increased much more than did the antidromic spike and Rt (Chebabo et al. 1995a). Moreover, the duration and waveform of the fEPSPs were altered in ways that changes in the electrical properties of the tissue could not explain. We concluded that synaptic transmission was genuinely enhanced by low πo (Chebabo et al. 1995a). Earlier, Ballyk et al. (1991) found, however, that intracellularly recorded excitatory postsynaptic potentials of hippocampal pyramidal cells did not increase when πo was lowered, even though the same team reported that intracellular excitatory postsynaptic potentials of neocortical neurons were reliably increased by lowering of πo (Rosen and Andrew 1990).

To resolve the apparent discrepancy between extracellular and intracellular recordings in hippocampus, we chose the whole cell patch-clamp method in voltage clamp configuration, because it enables reliable, stable recordings from single cells even while the tissue slice is subjected to insults that cause cell swelling (Czéh et al. 1993). We found a concentration-dependent reversible enhancement of excitatory postsynaptic currents (EPSCs) when bath osmolarity or NaCl concentration ([NaCl]) were lowered.

Abstracts of some of the results have already appeared (Bossut and Somjen 1995; Huang et al. 1996).

METHODS

Tissue slices were prepared from male Sprague-Dawley rats of 85−140 g body wt, as previously described (Dingledine 1984; Somjen et al. 1986). The rats were decapitated under ether anesthesia, and the brains were rapidly removed and dropped into chilled
artificial cerebrospinal fluid (ACSF). Hippocampal 400 μm thick were prepared by a vibrating cutter (Vibratome). In the more recent experiments the dissection was performed in a modified fluid, with NaCl and CaCl₂ replaced by equiosmolar (240 mM) sucrose. Vibrator-cut slices prepared in sucrose ACSF seemed to be in better functional state, inasmuch as strong orthodromic volleys evoked single population spikes, not double spikes or bursts. The slices were transferred into a dual-well Oslo-style interface chamber that was perfused with normal ACSF at a rate of ~1.5 ml/min at 36°C. In the initial experiments, slices in reserve were retained in a holding chamber in ACSF at room temperature saturated by cell, always allowing 0¢.

m stimuli were constant in each experiment but varied among different experiments from 80 to 150 μA, 0.05–0.1 ms. Individual sweeps were delivered at 15-s intervals; 5 or 6 min separated each set of recordings. In the majority of the experiments this protocol was alternated by ramp depolarizations to test the effective threshold of evoking inward current spikes (Czéh et al. 1993). The ramp took the potential from –120 to 0 mV in 95 ms.

After having been placed in the recording chamber, slices were left undisturbed for 90 min. After whole cell recording conditions were established, control data were collected for 20 min, followed by perfusion of the chamber by hypoosmolar solution. After 20–30 min (usually 25 min) of hypoosmotic treatment, normal ACSF perfusion was resumed and recovery from hypotonia was observed for ~20 min (usually >30 min). In some cases a second, and in rare instances a third hypoosmotic treatment was applied to the same cell, always allowing 55–65 min of washing with normal ACSF between treatments. After completion of the study of a cell, all slices that had been exposed to hypotonia were discarded and a fresh slice was taken from the holding chamber.

Data were analyzed off-line by ClampFit 6.0 software (Axon Instruments). Whole cell input resistance (Rᵢ) was determined from the hyperpolarizing current required to raise the clamp potential from Vᵢ to steady ~5 mV. Such hyperpolarizing steps evoked an initial capacitive charging current, the relaxation of which usually could be fitted by a second-order exponential with the use of either the simplex or the Chebyshev algorithm of the ClampFit software. A virtual input capacitance (Cᵢ) was calculated as τᵢ/Rᵢ, where τᵢ is the larger of the two time constants (Jackson 1992; Rall 1969). The shorter time constant (τ₂) was partly suppressed in the trials in which cell capacity compensation had been attempted after whole cell condition was established, and sometimes second-order exponential fitting failed. In these cases a first-order exponential was fitted to the segment of the capacitive current transient beginning 0.8–1.0 ms after the onset of the hyperpolarizing step and ending either 25 or 50 ms later. When both first- and second-order exponentials were fitted for the same cell, the τᵢ values were very similar.

The amplitudes of sympathetically transmitted currents were measured after correction for linear leak current as the difference between peak current and the average baseline (holding) current shortly before the orthodromic stimulus. EPSC peak was defined as the minimum (i.e., negative peak) and inhibitory postsynaptic current (IPSC) as the maximum (positive peak) current measured by the software between two cursors placed to delimit the relevant part of the current trace.

Significance of differences in mean values was calculated with the use of the t-test.

RESULTS

Whole cell currents of voltage-clamped CA1 pyramidal neurons in tissue slices in normal solution

GENERAL PROPERTIES. The current holding the membrane at ~65 mV in the initial control state, 20 min after establishment of whole cell recording, was on average ~35.7 ± 14.6 (SEM) pA (range ~136 to +59 pA; n = 15). The average control Rᵢ was 153 ± 11 MΩ (n = 39).

The standard test protocol consisted of an initial 5-mV hyperpolarizing step followed after 50 ms by a series of 6, 8, or 10 successive 350-ms voltage step commands taking the potential from hyperpolarized to depolarized levels in 15-mV increments. An orthodromic volley activated synaptic transmission 200 or 262 ms after the start of the voltage steps. Figure 1A shows uncorrected sample current recordings from a neuron in control solution, and the Fig. 1C, inset, illustrates part of the voltage protocol. Figure 2A shows a complete family of synaptic current recordings on an expanded scale, after linear leak subtraction and adjustment of “baseline” current.
FIG. 1. Whole cell currents in a hippocampal CA1 pyramidal neuron. The voltage was first stepped from −65 to −70 mV, then in 15-mV increments from −105 to 0 mV, and then back to −65 mV; for clarity, only 3 sweeps are superimposed here, stepping to −105, −45, and 0 mV (see labels next to traces in A). Voltage protocol is shown in the inset below C. Depolarizing voltages evoked trains of spikelike inward currents probably caused by Na⁺-dependent action potentials, as well as prolonged inward current surges that are probably Ca²⁺ currents. In A, st marks the stimulus pulse. The afferent volley evoked by the stimulus caused the synaptic currents, which were inward at hyperpolarizing and outward at depolarizing voltages; the outward synaptic current interrupted the train of current spikes. Currents are shown before leak subtraction.

Synaptic currents recorded in another experiment at 6 voltages are shown on an expanded scale in Fig. 2. A: control recording, 20 min after whole cell condition was established. B: 15 min after switching to hypotonic (H-40) solution. C: 25 min of H-40 superfusion. D: after 30 min of wash with normal artificial cerebrospinal fluid (ACSF).

CAPACITIVE CURRENT. The 5-mV hyperpolarization evoked a brief capacitive transient current that could not be fully eliminated by the capacity compensation circuit. To explore the nature of this current, in nine trials the capacity compensation was not adjusted once whole cell recording was achieved. Figure 3 illustrates the relaxation of the capacitive transient current on an expanded scale in one such trial. The decay of the capacitive current usually appeared to be the composite of two parts showing a change of course 0.8–1.0 ms after its onset, and it could be fitted by a second-order exponential (Jackson 1992; Rall 1969). The average time constants of these nine cells in normal solution were $\tau_1 = 11.59 \pm 0.78$ ms and $\tau_2 = 0.39 \pm 0.04$ ms. In seven other trials in which the cell capacity compensation was attempted after whole cell recording conditions were established, the mean of $\tau_1$ was $11.62 \pm 1.06$ ms ($n = 7$), which is not significantly different. $\tau_2$ was not measured in these seven cells because adjusting capacity compensation in whole cell conditions distorted or eliminated the fast component of the capacitive current.

VOLTAGE-ACTIVATED CURRENTS. Prolonged hyperpolarizing voltage steps usually evoked a slowly activating inwardly rectifying current. With depolarizing voltage steps a small, persistent voltage-dependent inward current became manifest in many but not in all cells, probably a noninactivating voltage-dependent Na⁺ current (French et al. 1990; Hoehn et al. 1993). These low-amplitude slow currents were clearly revealed after subtraction of “leak” current.

Depolarizing ramp or step voltage commands in the medium range evoked series of brief, very large amplitude in-
FIG. 2. Families of synaptic currents of a CA1 neuron (A–C), and extracellular voltage responses (D–F) in CA1 stratum pyramidale. Six current traces are superimposed in each family of tracings (A–C) obtained at membrane voltages of −105, −90, −75, −60, −45, and −30 mV, preset by a voltage protocol similar to the one partially illustrated in Fig. 1C, inset. Currents were corrected for linear leak, and “baseline” was adjusted to 0 current. Each extracellular recording (D–F) is the average of 6 consecutive sweeps, recorded concurrently with the synaptic currents. A and D: control. B and E: during superfusion by hypotonic (H-40) solution. C and F: recovery, after 30 min of wash with normal ACSF. IPSC, inhibitory postsynaptic current; EPSC, excitatory postsynaptic current.

ward current spikes (Fig. 1A, trace labeled −45 mV; Fig. 4A). These spike currents probably represent Na⁺-dependent action potentials generated in the axon, outside the region controlled by the voltage clamp, because they are not seen when the patch pipette contains the local anesthetic drug N-(2,6-dimethylphenylcarbamoylmethyl)-triethylammonium bromide (QX-314) (Czéh et al. 1993). At strongly depolarized potentials, complex and more prolonged inward current transients appeared, with superimposed Na⁺ spikes (Figs. 1 and 4). These broad inward current surges are probably caused by Ca²⁺ currents, because they are not suppressed by cesium gluconate plus QX-314 (Czéh et al. 1993).

SYNAPTICALLY EVOKED CURRENTS. With the potential held at a hyperpolarized level, afferent volleys evoked a synaptically transmitted transient inward current (Figs. 1A and 2A, traces labeled −105 mV). At depolarized potentials the same volley evoked an outward current (Fig. 1A, trace labeled 0 mV; Fig. 2A, at −30 mV). The outward current rose more slowly to a later summit and it decayed more slowly than did the inward current. At a Vₜ of −105 or −100 mV the synaptic inward current reached peak 7.8 ± 0.4 (SE) ms after the stimulus; at Vₜ between −30 and 0 mV the outward current was maximal at 20.0 ± 0.7 ms. The difference (12.2 ms) is highly significant (P < 0.001). With the membrane potential held at −90 or −105 mV, the mean maximal amplitude of the inward current in control solution was about −180 pA; at −15 or 0 mV the average amplitude of the outward current was ~290 pA.

As the Vₜ was moved to less negative levels, the synaptically transmitted inward current amplitude decreased and the outward current amplitude increased. The voltage dependence of the maximum amplitudes of the currents is illustrated in Fig. 2 and in the current-voltage (I-V) plots of Fig. 5. A true reversal potential cannot be determined from the I-V plots, but the polarity, time course, and voltage dependence of these currents identify them as EPSCs and IPSCs, respectively (see DISCUSSION). When an EPSC was evoked at a slightly depolarized Vₜ, it frequently triggered an inward current spike, presumably an Na⁺ spike, especially when augmented by hypotonic treatment (Fig. 2B). By contrast, the IPSC interrupted and suppressed the train of inward spike currents that were evoked by depolarizing voltages (Fig. 1A, traces labeled −45 mV and 0 mV).

Hypotonic treatment causes reversible, concentration-dependent enhancement of EPSCs

Replacing the bath with hypotonic solution caused, as expected, an increase of the extracellularly recorded ortho-
dromic population spike (Ballyk et al. 1991; Chebabo et al. 1995a). At the same time the EPSC recorded from the patch-clamped cell increased markedly in all but one case (Figs. 1, 2, 5, and 6). The current amplitudes in Table 1 show the means ± SE of the EPSC amplitudes measured at the most hyperpolarized potentials. The number of trials with hypoosomotic solutions other than H-40 was limited, but even so it is evident from Table 1 that the increase of EPSC amplitude was concentration dependent. Washing with normal solution resulted in every case in a return toward normal, often followed by posthypotonic depression. The data for recovery in Table 1 are the averages taken after 30 min of wash with normal solution. The mean fEPSC rose during H-40 treatment from the control amplitude of 189–359 pA (190%) , and after 30 min of wash it returned to 194 pA, which is very near the normal value (104%). This nearly normal mean at 30-min recovery conceals the widely varying recovery rates: after 30 min of wash, the EPSC of some cells was still somewhat augmented whereas that of some returned to control and yet others were depressed below control level (undershooting).

The changes of the EPSC during exposure to hypotonic solution and during washing with normal solution coincided with changes in the amplitude of the extracellular population spike, which were similar in direction but greater (Figs. 2 and 6; Table 1).

EPSC amplitude increased in approximately equal proportion at all V_rest at which it was detectable. Plotting EPSC amplitude against holding voltage yields an I-V plot, which became much steeper during hypotonia than it was under control condition or after recovery. Figure 5 illustrates this change of slope in 12 trials on five cells where all conditions of the experiment were uniform. During hypotonic treatment, EPSCs tended to trigger inward current spikes at holding voltages at which they did not have this effect under control condition (Fig. 2). When such current spikes were present, EPSC amplitude could not be measured, and for this reason the N value of Fig. 5 at intermediate voltages is <12. At the strongly hyperpolarized as well as the most depolarized voltages, the Na⁺ current spikes were absent. The steepening of slope during hypotonia was evident in all individual I-V plots, even when intermediate points were missing.

**IPSCs show variable response to hypoosmotic treatment**

In many trials the increase of the EPSC was paralleled by an increase in IPSC amplitude (Fig. 6). In some cells, however, the IPSC reached a maximum after 10–15 min hypotonic exposure and then it started to decrease, and in some cases it was depressed at the end of 25 min of hypotonic treatment. Of 23 trials, at the end of 25 min of treatment with H-40 solution, the IPSC increased reversibly in 15 trials, decreased in 5 cases, and remained unchanged (within 4% of control) in 3 cases. Of the four trials with H-20 solution, the IPSC increased in two, decreased in one, and remained unchanged (98%) in one. In both trials with H-60 solution the IPSC remained unchanged. As a result of this variability, the increase of the mean IPSC amplitude is statistically not significant (Table 1). Yet in the cases when the IPSC did reversibly increase, its amplitude grew much above the range of variation seen in the initial control state (e.g., Fig. 6); in other words, the change was significant (i.e., “real”) in that cell. Moreover, whenever the IPSC amplitude increased during hypotonia, the slope of its I-V plot steepened, similarly to that of the EPSC, indicating increased slope conductance. In the few cases when the IPSC was depressed at the end of hypotonia, it became even smaller during wash with normal solution without signs of recovery suggesting possible “rundown.”

**Isosmotic low-NaCl solution also induces enhancement of EPSCs**

In our previous study, extracellular fEPSPs were reversibly increased during exposure to solutions in which NaCl
variability, the mean values were not significantly different (Table 1).

Cells maintained in normal solution

In control cells observed for extended periods without change of $\pi_o$ or [NaCl], EPSC and IPSC amplitudes fluctuated somewhat in random fashion, but they showed no consistent trend in any direction (Table 1).

$C_N$ increases during hypotonic treatment but not during low [NaCl]

In the initial series of experiments the capacity compensation dials of the amplifier were repeatedly adjusted during recording, to obtain optimal capacity cancellation. In later experiments cell capacity compensation was adjusted only before recording was started. In the final experiments, cell capacity was not compensated at all, to measure $C_N$ and to follow its changes during treatments.

The longer of the two time constants of the decay of the capacitive charge current, $t_1$, (Jackson 1992; Rall 1969) was consistently prolonged during hypotonic treatment (Fig. 3, Table 2). Wash with normal ACSF after hypotonia partially reversed this increase (Table 2). In a few instances in which recovery was observed for $\approx 55$ min, if $t_1$ was still increased after 30 min of wash, it returned to near normal values at the end of $\approx 1$ h.

If resistance stays constant, prolongation of $t_1$ indicates increase of $C_N$. $R_N$ changes were inconsistent during hypotonia but, on average, $R_N$ did increase. The virtual capacitance obtained from $t_1/R_N$ also increased during H-40 treatment and it recovered partially after 30 min of wash with normal ACSF (Table 2).

There was no increase of $t_1$ or of $C_N$ during exposure to isosmotic low [NaCl] (F-40) solution (Table 2). Time-dependent changes of $t_1$ and of $C_N$ during prolonged observation in normal ACSF were small and inconsistent.

In the trials in which whole cell capacity compensation was not applied, $t_2$ also increased during H-40 hypotonic treatment, from 0.39 ± 0.04 ms to 0.53 ± 0.08 ms ($n = 8$, $P < 0.01$).

Other changes caused by hypotonic treatment

The amplitude of the brief inward current spikes decreased when either $\pi_o$ or [NaCl] was lowered (Figs. 1, B and C, and 4B). This is as expected if these current spikes represent Na$^+$ dependent action potentials.

Although the putative Na$^+$ spike currents decreased, the broader, presumably Ca$^{2+}$-mediated inward current transients became more prominent (Fig. 1, B and C).

The apparent threshold potential at which ramp depolarization triggered putative Na$^+$ current spikes was $-36 \pm 1.3$ mV ($n = 15$). During exposure to H-40 hypotonic solution, the apparent trigger potential shifted inconsistently, with the mean change being $-1.6 \pm 1.4$ mV (not significant). The current corresponding to the trigger was normally 161 ± 17.7 pA ($n = 15$). In all but three cases of H-40 exposure, the ramp-current triggering inward spikes shifted to more positive levels, on average by 38 ± 11.4 pA (Fig.

was replaced by equiosmolar mannitol or fructose (Chebabo et al. 1995a). Similarly, in six patch-clamped cells, EPSC amplitudes increased during treatment with fructose-substituted low-NaCl (F-40) solution. EPSC consistently increased in all trials but, on average, not by as much as in comparably NaCl-deficient hypotonic (H-40) solution (Table 1). The mean EPSC increment (difference between treated and control amplitudes) amounted to 170 ± 25.7 pA during H-40 hypotonia and to 88 ± 40.1 pA during F-40 low-NaCl treatment. Despite the wide variability, the difference between the two conditions is significant ($P < 0.005$). IPSCs were usually also augmented by treatment with F-40 solution but, because of
HYPOTONIA AND LOW [NaCl] ON EPSC

FIG. 5. Current-voltage (I-V) relation of EPSCs. Amplitudes of the inward synaptic currents (presumed EPSCs) at 6 holding potentials, in control conditions, after 25 min of hypotonic exposure (H-40), and after 30 min of wash with normal ACSF. Straight lines: regression of current on voltage for the control data and for hypotonia (regression for recovery deleted for clarity). Points: mean values of 12 trials in 5 cells (from 5 animals).

Changes of threshold current and of threshold voltage were not always correlated.

Electrochemical potential shifts

Electrochemical junctions were present between the bath and the ground connection, between the extracellular electrode and interstitial fluid, and between the patch pipette solution and the cytosol. The junction potential change in the extracellular pipette is the sum of the junctions at the pipette tip and at the ground connection. The change of the Cl\(^-\) potential of the Ag/AgCl ground wire can be calculated to be −9.2 mV (wire more positive, bath more negative) (Kruyt and Overbeek 1969). The calculated shift of the junction between the 150 mM NaCl of the extracellular micropipette and interstitial fluid is 2.05 mV (pipette more positive). During perfusion with H-40 solution, the actual extracellular baseline potential change was −3.9 ± 0.5 mV (n = 15); during F-40 perfusion, which is electrochemically equivalent, change was −10.5 ± 0.42 mV (n = 5). It seems that voltages of unidentified source were added to the junction potentials. The potential of the ground electrode does not influence the recording when the pipette voltage is referred to the extracellular tissue potential instead of “ground.” EPSC amplitude increased during H-40 perfusion to 203 ± 16% of control amplitude (n = 16) when the voltage was clamped with reference to bath ground, and to 191 ± 23% (n = 7) with reference in the tissue. The IPSC grew to 127 ± 10% of control when referred to bath and to 138 ± 15% with reference in the tissue. The differences are small, amounting to less than the SE.

If the neuronal cytosol was diluted during hypotonic swelling, then the cytosol/patch pipette junction would also be altered. The holding current, which was usually but not always negative (mean −35.7 pA, range −136 to +59 pA), consistently became more positive, on average by 33.5 ± 5.5 pA (n = 15), during H-40 hypotonia. Some of the change of holding current can be attributed to the shift of the summed junction potentials and perhaps to other uncontrolled factors. Because linear leak was subtracted and baselines were adjusted, changes of holding current did not influence measured EPSC and IPSC amplitudes.

DISCUSSION

Identification of the synaptic currents

Timing, polarity, and voltage dependence identified the two synaptic whole cell currents. The inward current was maximal at hyperpolarized V\(_h\)S, where the driving potential of the glutamatergic EPSCs is known to be strongest, whereas the outward current grew as the cell was depolarized, as expected for γ-aminobutyric acid (GABA)-controlled IPSCs, whether mediated by Cl\(^-\) or by K\(^+\) (Kaila 1994; Otis et al. 1993). Moreover, in all cases the outward current reached maximum later than did the inward current. The EPSC evoked by Schaffer collateral–commissural volleys is known to be transmitted monosynaptically, whereas IPSCs evoked by the same volley require at least one intermediate synapse if the stimulating electrode is placed >0.5 mm from the recording point (Andersen et al. 1964a,b; Schwartzkroin and Mueller 1987; Turner 1990; Zhu and Krnjević 1994).

Even though the IPSC reached maximum later than did the EPSC, the two overlapped in time. While both were activated, the two conductances must have interfered with each other. Even though at hyperpolarized potentials there was no detectable outward current, the GABA-controlled Cl\(^-\) and/or K\(^+\) conductance presumably curtailed the glutamate-activated inward current. At depolarized potentials the glutamate-controlled conductance must have limited the GABA-induced outward current. From the reported time courses of the two currents (Andersen et al. 1964a,b; Schwartzkroin and Mueller 1987; Turner 1990) it seems that
FIG. 6. Changes with time in the maximal amplitudes of the EPSC, IPSC, and the extracellular population spike. Horizontal bar: duration of exposure to hypotonic (H-40) solution. Data from a single experiment.

the lingering EPSC could subtract more from the amplitude of the IPSC than the IPSC could from the EPSC.

True reversal (0 current) potentials could not be determined for the synaptic currents, in part because of the overlapping time courses and in part because of the location of the synapses. Synapses on the dendritic tree lie outside the effective range of the voltage clamp (Rall 1969).

Interpretation hypotonia-induced synaptic current changes. Possible sources of error

The increase of EPSC amplitude during exposure to low $\pi_o$ was robust, reproducible, concentration-dependent, and reversible. Isosmotic low [NaCl] treatment caused a similar if on the average less powerful effect. These results are especially striking, because the reduced extracellular $[\text{Na}^+]_{o}$ would be expected to lessen both the driving force and the conductance of glutamate-induced inward current. The observed increases of whole cell current must therefore underestimate the actual change in channel function.

The measured change of the EPSC could be subject to some error due to the change in junction potential. A change in junction potential shifts the $I$-$V$ plot along the abscissa but does not alter its slope. The slope of the $I$-$V$ plots of EPSC amplitude became much steeper under the influence of hypotonic exposure, indicating a change in slope conductance. Moreover, the change of EPSC amplitudes measured during hypotonia either with reference to ground or to tissue interstitium were not significantly different.

Changes in IPSC amplitude were inconsistent. Inasmuch as the peak of the IPSC was dominated by GABA$_A$-induced current (Kaila 1994; Schwartzkroin and Mueller 1987), the
Neuron dendrites swell in hypotonic solution

While studying neurons freshly dissociated from rat hippocampal CA1 region, we were surprised that brief exposure to very strongly hypotonic solution did not cause the cells to swell (Somjen et al. 1993). A more detailed recent series of experiments revealed that the volume of most isolated hippocampal neurons does increase when they are placed in hypotonic solution, but the swelling usually begins only after a latent period that can sometimes last several minutes (Somjen et al. 1996).

Earlier we also estimated the swelling of cells in hippocampal tissue slices, with the use of the shrinkage of interstitial volume as an index of cell volume expansion (Chebabo et al. 1995b). Graded lowering of $\pi_o$ appeared to cause concentration-dependent cell swelling in hippocampal slices, and there were also indications of regulatory volume decrease during prolonged hypotonia. Because of the slow exchange of the interstitial fluid of the slice with the bath, the cells in the slice experience a more gradual change of $\pi_o$ than do isolated cells. Both the washout of Na$^+$ and the shrinkage of interstitial volume take 20–25 min to complete (Chebabo et al. 1995b), similarly to the changes in extracellular electrical signals (Chebabo et al. 1995a) and synaptic currents (Fig. 6).

Measurements of interstitial volume (Chebabo et al. 1995b) did not distinguish, however, between the swelling of neurons and of glial elements. In the present experiments, the calculated $C_N$ of the patch-clamped neurons increased reversibly during exposure to low $\pi_o$, but not during exposure to isotonic low-[NaCl] solutions (Table 2). The question is, can we infer hypotonic swelling?

Rall (1969; also Jackson 1992) gave the theoretical treatment of current or voltage steps applied to the soma of a neuron with an extended dendritic tree. In our trials, $-5$-mV step hyperpolarization evoked a transient current the relaxation of which usually could be fitted by a second-order exponential. This capacitive current could not be eliminated by the compensation circuit of the amplifier. The amplitude of the capacitive current was smaller with cell capacity compensation than without, but $\tau_1$ was not affected by cell capacity compensation. This longer time constant is probably related to Rall’s $\tau_1$, representing an aggregate time constant of the dendritic tree (Rall 1969), mostly outside the reach of the clamp circuit. Hypotonic treatment caused both $\tau_2$ and $\tau_1$ to increase, but $\tau_2$ could not reliably be measured after cell capacity compensation.

To derive $C_N$, $\tau_1$ had to be corrected by $R_N$. The measured $R_N$ includes the uncompensated fraction of the access resistance, plus leak resistance (which is the voltage-independent resistance of the plasma membrane shunted somewhat through the pipette-to-membrane seal), plus the resistance of the extracellular tissue (and bath) to ground. Of these components, resistance of the cell membrane is by far the greatest. The leak current flows not only through the soma membrane but also, accessed by way of dendritic cytoplasm, reduced [Cl$^-$], is expected to lessen the driving force and conductance of the IPSC, similarly to the effect of low [Na$^+$] on the EPSC. Nevertheless, more often than not, the IPSC also increased (Fig. 6), in a few cases even more than the EPSC. A problem with evaluating the data is the overlap of the inward and outward currents. Although each must have influenced the other, the EPSC probably spilled over into the IPSC more than the reverse. A powerful increase of the EPSC could therefore obscure an increase in the IPSC. This reasoning is reinforced by the observation in a few cells in which the IPSC was considerably increased after 15 min of hypotonic treatment, but barely or not at all different from control at the end of 25 min. It seems likely that hypotonia augments the IPSC, but to definitely resolve this question, monosynaptically elicited IPSCs will have to be recorded in isolation after pharmacological suppression of EPSCs (Zhu and Krnjević 1994).

### Table 1. Synaptic current amplitudes

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<th>PS</th>
<th>EPSC</th>
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<tr>
<td>%</td>
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<tr>
<td>Control</td>
<td>$-82 \pm 12$ (4)</td>
<td>154 $\pm$ 23 (4)</td>
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| H-20      | $-146 \pm 36$ (4)* | 179 $\pm$ 42 (4) | 115  
| Recovery  | $-112 \pm 52$ (4) | 137 $\pm$ 56 (4) | 120  
| Control   | $-189 \pm 30$ (25) | 325 $\pm$ 61 (23) |  
| H-40      | $-359 \pm 49$ (25)$^\dagger$ | 390 $\pm$ 73 (23) | 118  
| Recovery  | $-195 \pm 31$ (24) | 104 $\pm$ 68 (22) | 88   
| Control   | $-219$ (2) | 335 (2) |  
| H-60      | $-663$ (2) | 338 (2) | 101  
| Recovery  | $-153$ (2) | 70 (2) | 21   
| Control   | $-194 \pm 55$ (6) | 347 $\pm$ 112 (5) |  
| F-40      | $-282 \pm 39$ (6)$^*$ | 145 $\pm$ 170 (5) | 153  
| Recovery  | $-226 \pm 33$ (6) | 116 $\pm$ 104 (5) | 107  
| Control   | $-143 \pm 67$ (5) | 106 $\pm$ 17 (5) |  
| 25 Min later | $-127 \pm 15$ (5) | 89 $\pm$ 20 (5) | 113  
| 50 Min later | $-142 \pm 14$ (5) | 99 $\pm$ 23 (5) | 136  

All ± values are means ± SE, with number of currents in parentheses. PS, Orthodromic population spike amplitude; % of control; EPSC, synaptic inward current; IPSC, synaptic outward current; Control, 20 min after establishing whole cell conditions; H-20, 25–30 min hypotonic exposure, 20 mmol/l NaCl deleted, osmolarity ($\pi$) = 261 mosM/kg; H-40, hypotonic solution, 40 mmol/l NaCl deleted, $\pi$ = 230 mosM/kg; H-60, hypotonic solution, 60 mmol/l NaCl deleted, $\pi$ = 195 mosM/kg; F-40: isosmotic low-NaCl solution, 40 mmol/l NaCl deleted, $\pi$ = 305 mosM/kg; recovery, after 30–35 min washing with normal solution. $^*$Different from control value of the same group, $P < 0.025$. $^\dagger P < 0.001$.

Values are means ± SE; number of measurements is in parentheses. $\tau_1$, input time constant; $R_N$, input resistance; $C_N$, input capacitance; Control, 20 min after establishing whole cell conditions; H-40, 25–30 min after start of perfusion with hypotonic solution, 40 mmol/l NaCl deleted, $\pi$ = 229 mosM/kg; F-40, isosmotic NaCl-deficient solution, 40 mmol/l NaCl deleted, $\pi$ = 305 mosM/kg; recovery, after 30–35 min washing with normal solution. $^*$Different from control of the same group, $P < 0.005$.

### Table 2. $\tau_1$, $R_N$, and $C_N$

<table>
<thead>
<tr>
<th>$\tau_1$, mS</th>
<th>$R_N$, M$\Omega$</th>
<th>$C_N$, pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$11.0 \pm 0.89$ (14)</td>
<td>$167 \pm 9.5$ (14)</td>
</tr>
<tr>
<td>H-40</td>
<td>$15.0 \pm 1.38$ (14)*</td>
<td>$181 \pm 15.6$ (14)</td>
</tr>
<tr>
<td>Recovery</td>
<td>$12.4 \pm 1.42$ (13)</td>
<td>$157 \pm 14.0$ (13)</td>
</tr>
<tr>
<td>Control</td>
<td>$11.8 \pm 1.34$ (6)</td>
<td>$204 \pm 30$ (6)</td>
</tr>
<tr>
<td>F-40</td>
<td>$11.6 \pm 1.89$ (6)</td>
<td>$255 \pm 47$ (6)</td>
</tr>
<tr>
<td>Recovery</td>
<td>$11.9 \pm 1.55$ (6)</td>
<td>$191 \pm 26$ (6)</td>
</tr>
<tr>
<td>Control</td>
<td>$10.3 \pm 1.53$ (5)</td>
<td>$162 \pm 25$ (5)</td>
</tr>
<tr>
<td>25 Min later</td>
<td>$10.0 \pm 1.95$ (5)</td>
<td>$150 \pm 12$ (5)</td>
</tr>
<tr>
<td>50 Min later</td>
<td>$9.6 \pm 2.13$ (5)</td>
<td>$181 \pm 24$ (5)</td>
</tr>
</tbody>
</table>
through the wide expanse of the dendritic membranes. Although the calculated \( C_n \) cannot be equated with the absolute value of cell membrane capacitance, the changes in \( C_n \) can be taken to reflect changes in membrane capacitance, very probably due mainly to changes in dendritic surface area.

We conclude that the increased \( C_n \) indicates a hypotonic expansion of the surface area of the neuronal dendritic tree, perhaps also of the neuronal soma. It is generally held that biological membranes can be stretched only slightly (Evans and Hochmuth 1978). Cell swelling without stressing the membrane is attributed either to the unfolding of previously bent or pleated membrane, or to the addition (“traffic”) of “spare” membrane from cell organelles to the plasma membrane. Smoothing of a wrinkled membrane would add to the capacitance only if the pleating was so tight that fluid was excluded between apposed membrane surfaces.

**Possible mechanisms of the EPSC increase**

Swelling of dendritic shafts (reduced core resistance) and increased “resting” membrane resistance (reduced leak) would expand the dendritic space constant and thereby increase the impact of distant synapses at the soma (Turner 1984). There was evidence for dendritic swelling during hypotonia, but without more detailed data it is difficult to model the magnitude of such electrotonic effects. In addition, because cell swelling reduces interstitial space and perhaps also the width of the synaptic cleft, it could bring presynaptic terminals closer to postsynaptic membranes. Moreover, swelling of persynaptic glia could hinder the diffusion of transmitter away from the synaptic cleft. These latter two effects could combine to raise transmitter concentration at the receptive surface.

None of these geometric and physical factors could, however, play a part in the enhancement seen with isosmotic low [NaCl], which causes no increase of \( C_n \) (Table 2) and little or no change in interstitial volume (Chebabo et al. 1995b). The data in Table 1 as well as our previous measurements (Chebabo et al. 1995a) show the enhancement of the EPSC caused by F-40 solution to be about half of that caused by H-40 solution. It is tempting to suggest that the physical effects of swelling account for half the hypotonic enhancement, and the reduced [NaCl] accounts for the remainder. We must then seek an explanation for the part of the effect that is due to the low [NaCl].

Increased CNS excitability induced by low [Cl\(^-\)] can be attributed to reduced synaptic inhibition (Andersen et al. 1964a,b; Chamberlin and Dinglede 1988; Kaila 1994). In our trials in which both [Na\(^+\)] and [Cl\(^-\)] were reduced, IPSCs were, however, more often increased than decreased or unchanged, and therefore reduced inhibition could not contribute to the increased EPSC.

We must conclude that there was a genuine enhancement of synaptic efficacy, well demonstrated by the increased whole cell conductance associated with the peak EPSC (Fig. 5) and in many cases also with the IPSC. This may seem in conflict with findings in isolated CA1 neurons, where muscimol-induced currents were depressed when the cell was exposed to low \( R_p \) or low [NaCl], (Vreugdenhil et al. 1995). Enhancement of IPSCs could be due to presynaptic facilitation, and competition between postsynaptic depression and presynaptic enhancement could contribute to the variability of IPSC changes. We have no comparable data for glutamate-induced currents, but Paolletti and Ascher (1994) reported that NMDA-induced currents in cultured mouse neurons were enhanced by low osmolality.

A possible clue for the mechanism of enhanced synaptic efficacy could be found in the lowering of [Ca\(^{2+}\)]\(_i\), caused by both low \( \pi_o \) and low [NaCl] (Chebabo et al. 1995b). Lowering of [Ca\(^{2+}\)]\(_i\), indicates uptake of Ca\(^{2+}\) ions by cells, and recently we confirmed that intracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]\(_i\)) measured with the fluorescent indicator FURA-2 is initially depressed but then raised well above control level during hypotonia in neurons in hippocampal slices (Somjen et al. 1997). The uptake could in part or whole be due to suppression or reversal of the Ca/Na exchange transport. Elevated [Ca\(^{2+}\)], could enhance synaptic efficacy both pre- and postsynaptically. Presynaptic uptake of Ca\(^{2+}\) could enhance synaptic transmission because an increase of baseline [Ca\(^{2+}\)] in the terminals could facilitate impulse-evoked transmitter release (Katz and Miledi 1968; Moore and Hines 1986). Contrary to this idea, in isolated cells hypotonia suppresses whole cell calcium currents (Somjen et al. 1993), but these neurons lose their dendritic tree during cell preparation and calcium channels in dendrites and in presynaptic terminals may behave differently from those in the soma membrane. Besides, we have recently seen that not only baseline [Ca\(^{2+}\)], but also [Ca\(^{2+}\)], responses evoked by depolarizing pulses in neurons in hippocampal slices, are initially depressed but later enhanced during hypotonia (Somjen et al. 1997). In the present experiments there was also evidence of enhancement of Ca\(^{2+}\)-mediated voltage-dependent inward currents, illustrated in Fig. 1, B and C. If Ca\(^{2+}\) currents are similarly augmented in presynaptic terminals as well, this would lead to an increased release of transmitter substances. In an earlier study we found indications of enhanced presynaptic Ca\(^{2+}\) uptake during hypotonia, but the data were not entirely conclusive because of technical problems (Chebabo et al. 1995a).

In addition to a presynaptic effect, it has been reported that a rise in [Ca\(^{2+}\)], potentiates the postsynaptic response of pyramidal neurons (Kullman et al. 1992). Responses of [Ca\(^{2+}\)], would be dampened by the EGTA in the pipette solution in patch-clamped cell somata, but much less so in the dendritic branches where the potentiating effect would take place.

**Clinical significance**

Clinically, acute hypotonia of extracellular fluid is by necessity always associated with hyponatremia. The normal range of Na\(^+\) levels in the blood of human subjects is 136–145 meq/l, but overt signs of hyponatremia start only when [Na\(^+\)], drops below 125 meq/l (Thorn et al. 1977). In the H-20 solution used in these experiments, [Na\(^+\)], (in the form of NaCl, NaHCO\(_3\) and NaH\(_2\)PO\(_4\)) was 134 mmol/l, in H-40 solution it was 115 mmol/l, and in H-60 solution it was 95 mmol/l. In human patients the mildest level of hypotonia corresponding to the H-20 solution would, therefore, probably be asymptomatic, the middle level (H-40) corresponds to overt clinical hyponatremia-hypotonia, and the severest level (H-60) represents danger of death (Avner 1995; Ellis


