



## UvA-DARE (Digital Academic Repository)

### Effects of strongly anisotonic and NaCl deficient solutions on muscimol-and glutamate-evoked whole-cell currents in freshly dissociated hippocampal neurons

Vreugdenhil, M.; Somjen, G.G.; Wadman, W.J.

DOI

[10.1016/0006-8993\(94\)01283-N](https://doi.org/10.1016/0006-8993(94)01283-N)

Publication date

1995

Published in

Brain Research

[Link to publication](#)

#### Citation for published version (APA):

Vreugdenhil, M., Somjen, G. G., & Wadman, W. J. (1995). Effects of strongly anisotonic and NaCl deficient solutions on muscimol-and glutamate-evoked whole-cell currents in freshly dissociated hippocampal neurons. *Brain Research*, 670, 89-96. [https://doi.org/10.1016/0006-8993\(94\)01283-N](https://doi.org/10.1016/0006-8993(94)01283-N)

#### General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

#### Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

*UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)*

Research report

# Effects of strongly anisotonic and NaCl deficient solutions on muscimol- and glutamate evoked whole-cell currents in freshly dissociated hippocampal neurons

M. Vreugdenhil<sup>a</sup>, G.G. Somjen<sup>b,\*</sup>, W.J. Wadman<sup>a</sup>

<sup>a</sup> *Department of Experimental Zoology, University of Amsterdam, 1098 SM Amsterdam, The Netherlands*

<sup>b</sup> *Department of Cell Biology, Duke University Medical Center, Box 3709, Durham, NC 27710, USA*

Accepted 25 October 1994

## Abstract

Sudden exposure of dissociated hippocampal neurons to strongly hypo- or hyperosmotic solutions suppresses voltage gated Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> currents. We investigated whether ligand gated ion currents were similarly shut down by exposure to anisotonic solutions. The effect of hypo-osmotic, NaCl deficient (mannitol-substituted), or hyper-osmotic test solutions delivered from a flow pipette was tested on voltage gated Ca<sup>2+</sup> currents and on currents and conductance changes evoked by brief administration of either the GABA<sub>A</sub>-agonist muscimol or glutamate. Hyper-osmotic solution caused cells to shrink, but cell membrane capacitance did not change. Muscimol-induced conductance increases were depressed by hypo-osmotic and by NaCl deficient solutions and often by hyper-osmotic solution. Voltage gated Ca<sup>2+</sup> currents were depressed by anisotonic, but not by NaCl deficient isosmotic solution. NMDA- and non-NMDA evoked conductance increases were depressed by hyperosmotic solution; hypo-osmotic and NaCl deficient solutions were not tested on glutamate induced currents. Ligand gated currents are suppressed by anisotonic solutions more slowly than are voltage gated channels. The changes caused by anisotonic and NaCl deficient solutions were much greater than expected from calculated electrochemical effects and are probably the result of change in receptor controlled channels.

**Keywords:** Ion channel; Osmotic effect; Glutamate;  $\gamma$ -Aminobutyric acid; Whole-cell patch-clamp; Dissociated neuron; Cell volume

## 1. Introduction

The clinical syndromes caused by reduced or increased osmotic pressure of extracellular fluid are well defined. Dilution of extracellular fluid can lead to convulsions; raised extracellular osmotic pressure (at normal plasma volume) to lethargy [1]. Lowered extracellular osmotic pressure enhances seizure discharges [2,15]. These central nervous syndromes have been attributed to changing water content of the brain. Osmotic flow can occur at two interfaces: first, due to the semipermeability of the blood–brain barrier, the brain as a whole is said to act as an osmometer;

second, under the force of osmotic imbalance water may move across cell membranes. The resulting cell volume changes are partially compensated by volume regulation, mediated by respectively the release or the uptake of solutes [5,6,14].

While the global hydraulic and hemodynamic consequences of osmotic imbalance are well described, our understanding of the cellular mechanisms affecting cerebral responsiveness is incomplete. For example, while the amplitude of excitatory postsynaptic potentials recorded focally (fEPSPs) increases [1,3,7] intracellularly recorded EPSPs were apparently unaffected in hippocampal neurons [3].

Earlier we examined how the resting membrane resistance and the voltage gated ion currents of dissociated neurons respond to osmotic challenge. Unexpectedly, we have found that freshly dissociated hippocampal neurons did not swell when suddenly exposed to

\* Corresponding author. Fax: (1) (919) 684-5481. E-Mail: george\_somjen@cellbio.duke.edu

strongly hypo-osmotic solutions and only rarely shrank in a hyper-osmotic environment. Instead, voltage gated ion currents were reversibly suppressed by both hypo- and hyperosmotic as well as glucose deficient test solutions. We termed this seemingly non-specific response 'channel shutdown' [12].

The experiments reported here continue the already reported study. We now have tested whether anisomotic solutions suppress ion currents induced by synaptic transmitter agents similarly to voltage gated currents. We chose muscimol, a drug which activates specifically the A-receptor for gamma-amino butyric acid (GABA), and glutamate.

Some of our results have appeared in an abstract [13].

## 2. Materials and methods

Isolated cells were prepared as previously described [9,12,17]. Briefly, male rats of 75–150 g were decapitated under ether anesthesia, the brain was rapidly removed and kept in cold solution containing (in mmol/l): NaCl 120, KCl 5, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1, PIPES 10, glucose 25, pH 7.0. The CA1 region of the hippocampus was dissected and cut into small blocks which were incubated for 90 min at 32°C in oxygenated medium with 1 mg/ml trypsin (type XI) added. Cells were dispersed by trituration and perfused with a bathing solution containing: (in mmol/l): NaCl 110, KCl 5.4, CaCl<sub>2</sub> 5, MgCl<sub>2</sub> 1, glucose 25, HEPES 10,

tetraethyl-ammonium chloride (TEA-Cl) 25, 4-aminopyridine (4-AP), 5 mM, tetrodotoxin (TTX) 1 μM, pH 7.4; osmotic pressure 295–310 mosm/l (Vescor vapor pressure osmometer) at room temperature.

Patch pipettes of 1.5–2 MΩ contained: CsF 100, phospho-creatine 20, Phosphocreatine-kinase, 50 unit/ml, Mg-ATP 2, Na-GTP 0.1, leupeptin 0.1, albumin 0.1%. TEA-Cl 20, EGTA 10, CaCl<sub>2</sub> 0.5, MgCl<sub>2</sub> 2, pH 7.3 or 7.1. The Cs<sup>+</sup>, TEA, 4-AP and TTX blocked voltage gated Na<sup>+</sup> and K<sup>+</sup> currents, while the high energy phosphate compounds prevented 'rundown' of voltage gated Ca<sup>2+</sup>, and ligand-mediated currents.

Currents were measured under voltage clamp conditions with a List EPC-7 amplifier; after a Gigaseal (> 0.5 GΩ) was established, the whole-cell mode was entered with mild suction. Electrode capacitance and series resistance were compensated and the cell was held at –100 mV holding potential, unless otherwise mentioned.

Agonists were delivered through a Y-tube application system consisting of a quartz tube with tip of 50 μm o.d. positioned within a glass pipette with tip of 100 μm o.d. positioned < 100 μm from the cell. Hydrostatic pressure continuously supplied the agonist in the inner pipette, while the outer pipette was under negative pressure preventing release of agonist until the suction was cut off by a computer controlled valve. As the agonist solution was ejected, complete exchange of solutions at the tip was estimated at < 50 ms; re-establishing suction restored the bath condition within 0.2–0.3 s; these times were determined by trial

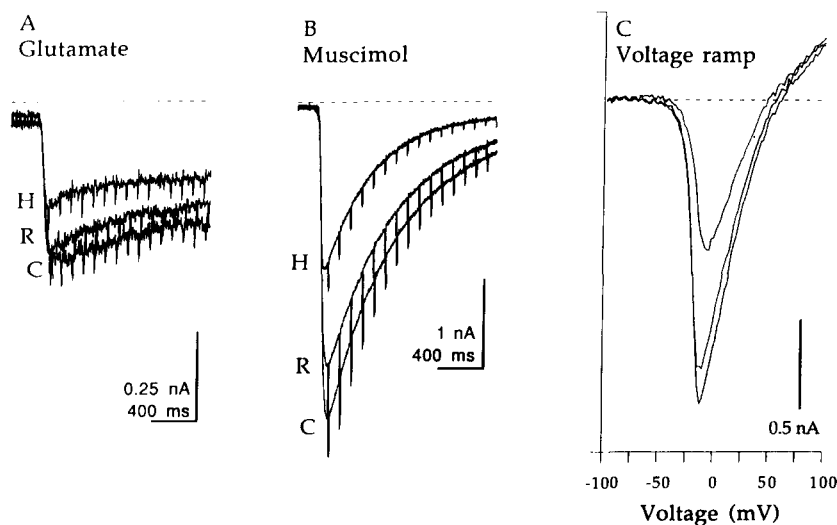


Fig. 1. The effect of raising osmotic pressure on agonist-evoked membrane currents and on voltage-gated Ca<sup>2+</sup> current in a dissociated neuron. A: membrane current evoked by 100 μM glutamate before (C), during (H) and after (R) flooding the environment of the cell with hyperosmotic solution. Downward deflection indicates inward current. The repeated small inward current pulses were evoked by hyperpolarizing voltage steps of –10 mV, used to determine membrane conductance. B: similar superimposed currents evoked in the same cell by 200 μM muscimol. C: voltage gated calcium current evoked by linear ramp depolarization (1 mV/ms) in the same cell. The smallest of the three calcium currents was obtained during hyperosmotic exposure. The holding potential was –95 mV for A and B and –100 mV for C. The traces in A and B were not corrected; C was corrected for linear 'leak'.

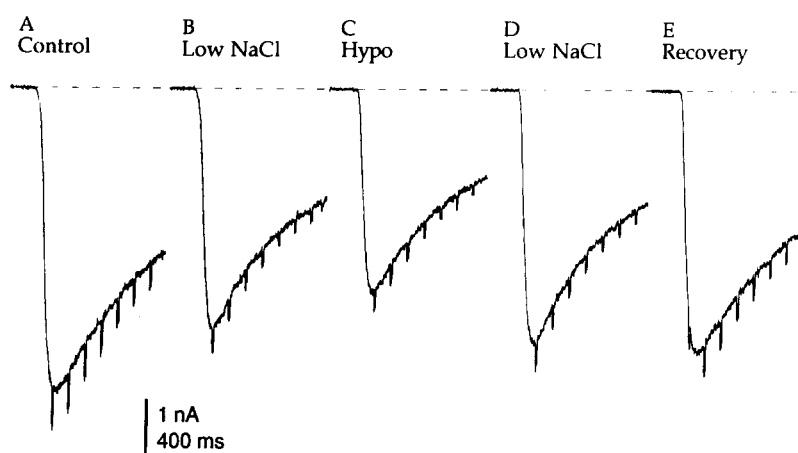


Fig. 2. The effect of low NaCl (B and D) and of hypo-osmotic exposure (C) on muscimol induced current. Conditions similar to those of Fig. 1B, except that traces have been corrected by subtracting 'blank' sweeps to eliminate 'resting' holding current and 'resting' conductance. Holding potential was  $-95$  mV.

perfusion with dye. Glutamate was dissolved in nominally  $Mg^{2+}$ -free solution, sometimes with 1 mM glycine added. The vehicle for muscimol (200  $\mu$ M) or Nalglutamate (100  $\mu$ M) was either control bath medium, or hyper- or hypo-osmotic or NaCl deficient solution.

Hypo-osmotic solution was prepared by reducing NaCl to 11 mmol/l with other solutes identical to the control bath; the measured osmolality was 130–150 mosmol/l. Hyperosmotic solution contained 200 mmol/l sucrose or mannitol in addition to the other, normal constituents (490–510 mosmol/l). These osmotic pressures are outside the range seen in vivo. NaCl deficient isosmotic medium was prepared by substituting 99 mmol/l NaCl by isosmotic mannitol. Cells were first tested for the presence of voltage gated  $Ca^{2+}$  current by step depolarizations [12] (from  $-100$  to 0 mV) or by a depolarizing ramp (from  $-100$  to  $+50$  mV in 150 ms). Agonist solutions were delivered for periods of 0.9 to 1.8 s (constant in any one experiment); with intervals of 56 or 76 s. Just before and during agonist release, holding potential was stepped to  $-90$  mV while the membrane conductance was tested every 100 ms by a 10 ms hyperpolarization of 10 mV. Muscimol was applied at a voltage of  $-95$  mV and  $-35$  mV.

Changes of cell volume were estimated from photographs before, during and after hyperosmotic exposure approximating the cell by a sphere and the attached apical dendrite by a cylinder.

### 3. Results

#### 3.1. Features of the ligand-evoked currents

At  $-95$  mV, the holding current was inward. Administration of either glutamate or muscimol caused a marked additional inward current and a strong en-

hancement of slope conductance (Figs. 1A,B, 2). Based on the literature, it is expected that at a strongly negative potential most of the glutamate-evoked current is carried by  $Na^+$ , while with muscimol it should be  $Cl^-$ . The calculated equilibrium potential for  $Cl^-$  ( $E_{Cl}$ ) for the given chloride concentrations in our experiments is close to  $-46$  mV, so that the muscimol-evoked chloride current at  $-95$  mV should be inward. The experimentally determined reversal potential of the muscimol-induced current was found to be near  $-35$  mV (Figs. 3, 5).

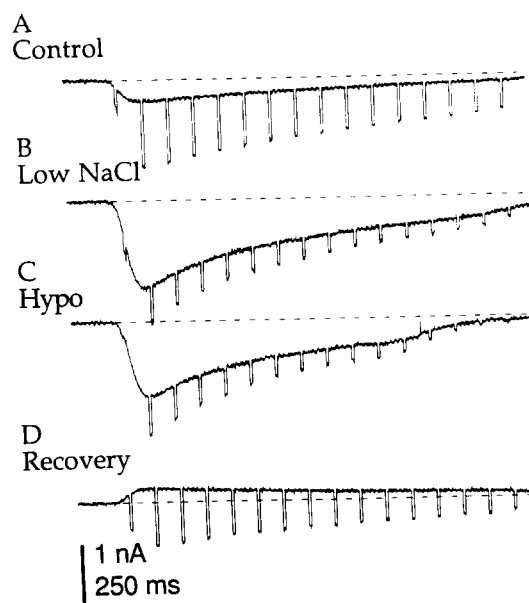


Fig. 3. The effect of low NaCl and of hypo-osmotic exposure on muscimol-evoked current at holding potential of  $-35$  mV. Same cell as Fig. 2. Note increased inward current with concurrently reduced membrane conductance in B and C. The inward current is replaced by small outward current in D.

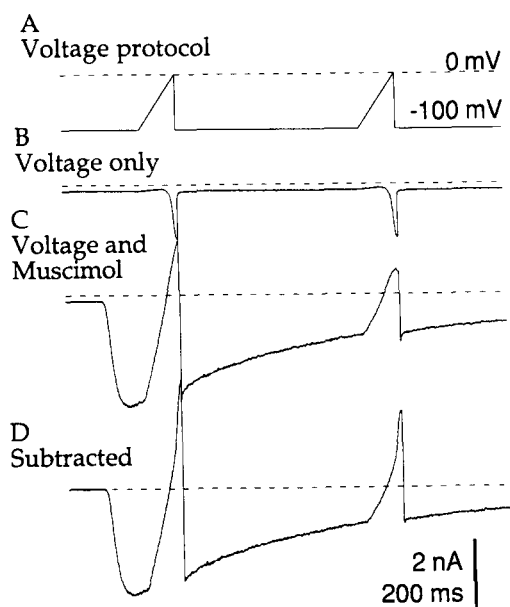


Fig. 4. Illustrating the method to estimate the reversal potential of agonist-evoked current. A: the double ramp voltage command. B: the voltage dependent  $\text{Ca}^{2+}$  currents evoked by the voltage ramps of A without application of agonist. C: the currents evoked by the combined application of voltage ramps and muscimol. D: subtraction of the trace of B from C isolates the muscimol evoked current. The voltage corresponding to the point where the ramp-evoked currents in D crosses zero is the reversal potential of the muscimol-evoked current. Note the current rectification in D.

The glutamate-evoked inward current reversed near 0 mV as expected if the channels are permeable to several cations [8]. In a few cases the antagonist drugs 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphono-valerate (APV) were used to differentiate the NMDA and non-NMDA components of the glutamate-evoked current. On three occasions rapid delivery of glutamate was followed by a very brief large amplitude transient current peak which probably was due to the activation of the AMPA-receptor; in the other trials probably only the activation of a mixture of kainate and NMDA-receptors was observed [8].

During continued presence of agonist the ligand evoked current and permeability increase decayed; we measured peak amplitude of the current ( $I_p$ ) and conductance ( $g_p$ ), as well as the 'late' current and conductances ( $I_l$  and  $g_l$ ) usually at 900 ms but sometimes at 500 ms after the peak.

### 3.2. Effects of hyperosmotic solutions

Fig. 1 illustrates that flooding a cell with hyperosmotic medium depressed the glutamate- and muscimol-evoked currents and the voltage gated  $\text{Ca}^{2+}$  currents. The depression reached its maximum in 1–2 min of anisomotic exposure. Washing with normal control solution was followed by recovery, albeit in-

completely due probably to 'rundown'. The rising phase of the currents are nearly identical in control, anisomotic and recovered condition (Fig. 1A and B) but detailed analysis was not attempted because the rate of rise reflects partly the speed of the application system. As already reported [12], the holding current was reversibly reduced during hyperosmotic exposure (Fig. 1A,B). The traces in Fig. 1A and B have not been corrected, but numerical data reported below refer to measurements corrected by subtracting the 'blank' currents recorded in the absence of ligand.

Voltage gated  $\text{Ca}^{2+}$  currents were always depressed confirming our earlier report [12]; depression began as soon as the anisomotic solution reached the cell. The voltage of activation did not shift (Fig. 1C).

The volume of 5 cells was estimated from photographs and it shrank during hyperosmotic exposure to about 38% of control. Recovery was incomplete (77% of initial control. These values are approximate, due to the irregular shape of the cells, especially during shrinkage). The membrane capacitance, which was compensated after establishing whole cell recording conditions, did not detectably change during hyperosmotic exposure, which indicates that the effective surface area was not changed and shrinkage was the result of wrinkling of the membrane.

Hyperosmotic exposure depressed both the early peak ( $I_p$ ) and the late sustained part ( $I_l$ ) of glutamate-evoked current (Fig. 1A). To estimate the magnitude of the depression, time dependent rundown was compensated by interpolation, and this is likely to result in under rather than over estimation of the degree of depression.

Mean glutamate-induced  $I_p$  during hyperosmotic administration decreased to 66% ( $\pm 4\%$  S.E.M.) of the interpolated control amplitude (range: 51–88%;  $n = 9$ ). Mean  $I_l$  was 74% ( $\pm 7\%$ ) (range: 51–93%  $n = 7$ ). The  $g_p$  was reduced to 67% ( $\pm 3\%$ ) of control (range: 56–79%  $n = 6$ ).

The mean muscimol-evoked  $I_p$  was depressed to 77% ( $\pm 4\%$ ) of control (range: 57–97%;  $n = 6$ ). The decay of muscimol-induced currents accelerated during hyperosmotic exposure. As a result the depression of  $I_l$  was more pronounced than that of  $I_p$ : 46% ( $\pm 5\%$ ) ( $n = 5$ , Fig. 1B).

In Fig. 1B the hyperpolarizing current pulses superimposed on the muscimol-induced inward current decreased markedly during hyperosmotic exposure. Even though the muscimol-induced current was always depressed, the conductances were unchanged in 2 out of 7 cells tested; of these two cells in one case only 1, (the 'late', partially decayed) current was depressed, in the other both  $I_p$  and  $I_l$  decreased. At  $-95$  mV muscimol reduced  $g_p$  to 74% ( $\pm 9\%$ ) (range 48–108%,  $n = 6$ ) and  $g_l$  to 61% ( $\pm 7\%$ ) (range 39–81%,  $n = 5$ ) during hyperosmotic exposure.

To test whether both NMDA- and non-NMDA-dependent currents were affected, glutamate was administered in the presence of either CNQX (10  $\mu$ M,  $n = 6$ ); or APV (50  $\mu$ M) plus  $Mg^{2+}$  (1 mM,  $n = 5$ ). On four of the cells both antagonists were tried in both orders. The APV-resistant as well as the CNQX-resistant residual glutamate-induced current was reversibly and about equally depressed by hyperosmotic exposure: to 66% of the maximum control amplitude in the presence of APV and to 68% with CNQX (n.s.).

### 3.3. Effects of hypo-osmotic and NaCl deficient isosmotic solutions

Voltage gated  $Ca^{2+}$  currents were reversibly depressed by exposure to hypo-osmotic solution, as reported earlier [12]. Low NaCl solution did not cause a depression, but rather a small and reversible increase of the voltage gated  $Ca^{2+}$  currents. In NaCl-deficient solution the activation of the  $Ca^{2+}$  current shifted to a more negative level, but we attribute this shift to the change of junction potential between the NaCl-deficient bath and the salt bridge connecting the bath to ground. The dissociated neurons did not noticeably swell when exposed to hypo-osmotic solution in agreement with earlier observations [12].

Exposure to hypo-osmotic solution reversibly depressed muscimol induced currents evoked at  $-95$  mV and reduced the conductance increase.

Because the hypo-osmotic solutions were also severely deficient of NaCl, their effect was compared to isosmotic, mannitol or sucrose-substituted solutions with equally reduced NaCl concentration. In Fig. 2 muscimol-induced currents at a holding potential of  $-95$  mV are illustrated. When the bath was changed to one low in NaCl, and then muscimol was administered in similarly NaCl deficient vehicle, the muscimol-induced currents and conductance increases were depressed (Fig. 2B). Exposure to hyposmotic solution resulted in a slightly enhanced depression (Fig. 2C). Return to low NaCl solution and then washing with normal solution was followed by partial recovery (Figs. 2D,E).

Fig. 3 illustrates the currents recorded in the same series of exposures at holding potential of  $-35$  mV, delivered in alternating fashion with those held at  $-95$  mV (see section 2). In the control state muscimol evoked a small inward current and a large conductance increase (Fig. 3A). In low NaCl the inward current increased markedly, unlike the depression seen at  $-95$  mV (Fig. 2). Yet the muscimol-induced conductance increases became smaller (Fig. 3B). Then, during expo-

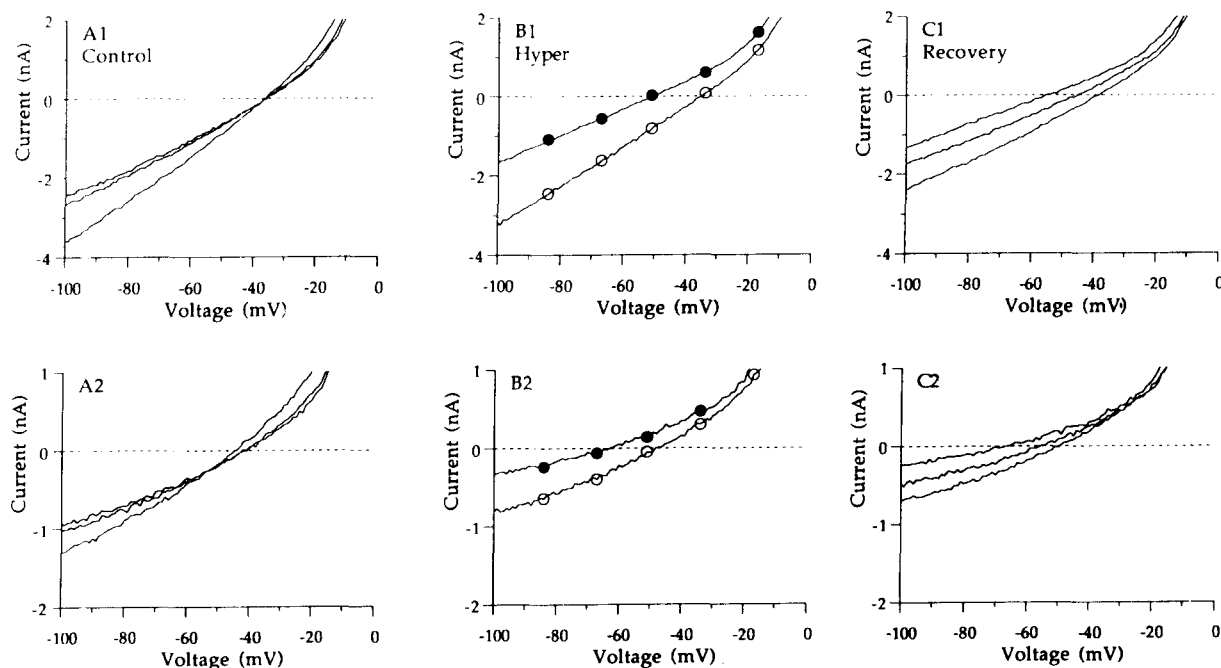


Fig. 5. Ramp portions of traces of isolated muscimol current (method in Fig. 4) before, during and after hypertonic exposure. The upper row (A1–C1) illustrates currents evoked by the first ramp; lower row (A2–C2) are currents corresponding to the second ramp. A1 and A2: Three traces in control condition. Note that, small variation in muscimol administration varied the magnitude of the currents, but not the reversal potential. B1 and B2: traces obtained 56 (open symbol) and 112 s (closed symbol) after start of hypertonic exposure. Note marked left-shift of zero current point. C1 and C2: three consecutive traces obtained during recovery in control solution. The reversal potential of the muscimol-induced current was  $-37$  and  $-43$  mV (A1 and A2) in initial control condition;  $-50$  and  $-60$  mV, when hypertonic effect was maximal (B1 and B2); and  $-39$  and  $-48$  mV at final recovery (C1 and C2).

sure to hypotonic solution the current was slightly smaller than in low NaCl (Fig. 3C). After washing with control solution a small outward current replaced the inward current, and the conductance increases recovered almost completely.

In most cells the difference between the depression caused by low NaCl and by hypo-osmotic medium was not as marked as in the case illustrated in Figs. 2 and 3. At  $-95$  mV holding potential the mean  $I_p$  was reduced to 58% of control (SEM =  $\pm 7\%$ , range 37–83%;  $n = 6$ ) in hypo-osmotic and to 62% ( $\pm 6\%$ , range 41–82%;  $n = 7$ ) in NaCl-deficient medium. Mean  $I_i$  was depressed to 54% ( $\pm 6\%$ ,  $n = 6$ ) in hypo-osmotic and to 59% ( $\pm 6\%$ ,  $n = 7$ ) in low NaCl. The muscimol-induced mean  $g_p$  was depressed to 51% ( $\pm 5\%$ , range 35–68;  $n = 6$ ) in hypo-osmotic and to 58% ( $\pm 6\%$ , range 43–86;  $n = 7$ ) in low NaCl exposure.

### 3.4. Reversal potentials of agonist induced currents

Lowering external chloride concentration  $[Cl^-]_o$  shifts  $E_{Cl}$  to a more positive level and this could explain the increase of the muscimol evoked inward current seen at  $-35$  mV holding potential (Fig. 3). We determined the reversal potential of muscimol induced currents with ramp depolarizing voltage commands (Fig. 4A) given alternately during muscimol administration (Fig. 4C) and in 'blank' sweeps (Fig. 4B). Subtraction of the voltage gated  $Ca^{2+}$  current (Fig. 4B) from the ramp-evoked current during muscimol (Fig. 4C) yielded (assuming no interference) the muscimol-induced current and the reversal potential (potential of zero current) (Fig. 4D).

In two different cells the reversal potential of the muscimol current shifted in low NaCl by, respectively,  $+17$  and  $+18$  mV, and in the same two cells in hypo-osmotic solution by  $+19$  mV. The expected (calculated) shift of the  $Cl^-$  equilibrium potential corresponding to the deficiency in  $[Cl^-]_o$  is 26 mV.

We also tested the reversal potential of muscimol induced currents during hyperosmotic exposure. In these trials the reversal level shifted in the negative direction. In two cells double ramps at 500 ms interval were used. Fig. 5 illustrates one, where the shift of the reversal potential measured early and late during muscimol application amounted to  $-13$  and  $-21$  mV. In the other double ramp the shifts were  $-3$  and  $-6$  mV. In a third cell single ramp near the peak of the muscimol effect revealed a shift of the reversal level of  $-12$  mV.

The glutamate induced current reversed polarity at a few mV negative relative to zero voltage. Its reversal potential did not detectably change during hyperosmotic exposure, even as the evoked current was strongly depressed ( $n = 3$ ).

## 4. Discussion

Our earlier study [12] has led to the conclusion that sudden exposure of dissociated hippocampal neurons to strongly hypo- or hyperosmotic and glucose deficient solutions caused the shutdown of voltage gated  $Na^+$ ,  $K^+$  and  $Ca^{2+}$  channels, and that this was a nonspecific response to severely adverse environments. Even if not specific, the degree of the suppression of diverse currents was not uniform. The sustained components of  $K^+$  and  $Ca^{2+}$  currents were invariably more powerfully affected than the transient currents, and  $K^+$  currents were more strongly depressed than  $Na^+$  current. Hypothetically, we have also suggested that the failure of swelling of dissociated neurons in hypo-osmotic environment may indicate restriction of membrane water permeability [12].

The present experiments extend the concept of channel shutdown to  $GABA_A$ , NMDA and non-NMDA glutamate receptor controlled ion channels by hyperosmotic exposure and for  $GABA_A$  currents also for hypo-osmotic exposure and sudden NaCl deprivation. The muscimol- and glutamate-induced currents were consistently and largely reversibly depressed when a cell's environment was flooded with hyperosmotic solution. There was no difference in the degree of depression between NMDA and non-NMDA receptor controlled channels.

The depression of the ligand-controlled currents differed in certain details from the shutdown of voltage gated currents. While the shutdown of voltage gated currents was maximal in 15 s or less [12] the depression of the ligand controlled currents reached its maximum in about 2 min. We have also tested the effect of ligand administered in anisomotic vehicle, without releasing anisomotic medium from the second 'flow' pipette, so that the cell was exposed to an anisomotic environment together with the ligand but not before. With that method the depression was weak and inconsistent (data not included under Results), probably because of the slow onset of the effect.

In most cases the ligand-induced conductance increase diminished along with the amplitude of the current. The depression may mean that fewer channels open, or that mean opening time shortens, or that channel conductance decreases.

The muscimol-induced current is carried mainly by chloride ions. The hypotonic and low NaCl solutions change both the driving force and conductance for chloride current, and therefore require further discussion. The Goldman-Hodgkin-Katz constant field theory permits calculation of the electrochemical effects [16]. It is generally held that in the whole-cell configuration the intracellular concentration of small ions is clamped by the attached pipette with a time constant of 4–5 s [11]. In the pipette solution  $[Cl^-]_i$  was 25 mM. Reduc-

ing  $[\text{Cl}^-]_o$  from 152 to 53 mM while holding the membrane potential at  $-95$  mV should increase  $I_{\text{Cl}}$  by about 11% and reduce  $g_{\text{Cl}}$  by about 20%. Instead of increasing, the peak inward current was actually decreased (by 42% and 38% in, respectively, hyposmotic and low NaCl solutions), and the muscimol-induced conductance was depressed by 49% and 41%. In these experiments the reversal potential sometimes changed slightly, suggesting that 'clamping'  $[\text{Cl}^-]_i$  was imperfect (compare Fig. 3A and 3D). The resulting error could not, however, explain why the inward current was depressed when it was expected to increase. The difference between expected and observed changes indicates that the channel itself must have been affected. By contrast, near the reversal potential for  $I_{\text{Cl}}$ , theory predicts a reduction of  $g_{\text{Cl}}$  by 46%, which is close to what we observed.

In hyperosmotic solutions the ion concentrations were identical to those in the control solution. Here, however, the shrinkage of cells introduces a complication. Recent data and calculations by Vreugdenhil and Wadman ([16] and unpublished observations) suggest that chloride redistribution contributes to the decay of the  $\text{GABA}_A$ -controlled current. Depletion of intracellular chloride occurs when the  $\text{GABA}_A$ -induced  $\text{Cl}^-$ -flux is outward, as was the case at  $-95$  mV holding potential with the  $[\text{Cl}^-]_i$  used in these experiments. Lowering of  $[\text{Cl}^-]_i$  reduces both driving force of  $I_{\text{Cl}}$  and the effective  $\text{Cl}^-$ -conductance, as derived from the Goldman-Hodgkin-Katz equation. During prolonged activation of  $\text{GABA}_A$  receptors the depletion of  $[\text{Cl}^-]_i$  thus reduces the chloride current and accelerates its decay. The shift of  $E_{\text{Cl}}$  is illustrated by the difference in reversal potentials measured by the early and late ramp-evoked currents in Figs. 5A1 and A2. The decay of the current caused by the decrease of  $[\text{Cl}^-]_i$  is added to the desensitization of the receptor itself.

When cells shrink, the  $\text{GABA}_A$ -induced depletion of  $[\text{Cl}^-]_i$  is accentuated. When a patched cell is exposed to a hypertonic medium  $[\text{Cl}^-]_i$  tends to rise as water flows out of the cell, but in the whole-cell configuration this increase is counteracted by exchange with the pipette solution [11]. With its volume reduced, the  $\text{Cl}^-$  content of the shrunken cells is also reduced and therefore, when  $\text{Cl}^-$ -channels open,  $[\text{Cl}^-]_i$  will be depleted faster. This effect was most clearly seen in the two cases when the peak muscimol-induced conductance increase ( $g_p$ ) remained normal during hyperosmotic exposure, yet the late conductance ( $g_l$ ) became depressed. The degree of decay of the conductance could in these cases can be quantitatively accounted for by the calculated  $[\text{Cl}^-]_i$  depletion [16]. Except for these two cases, the suppression of the muscimol-induced conductances by hyperosmotic solutions was greater than could be explained by the shrinkage of the cells. The discrepancy was especially obvious at  $-35$

mV because at that potential the  $\text{Cl}^-$  current is minimal and therefore  $\text{Cl}^-$  depletion was negligible.

The suppression of muscimol-induced current in isosmotic low NaCl solution was unexpectedly strong. Neither  $\text{K}^+$  nor  $\text{Ca}^{2+}$  currents were affected in isosmotic solutions deficient in NaCl.  $\text{Na}^+$  current diminished only as long as the lowered  $[\text{Na}^+]_o$  reduced the driving force on the current [12]. As we have shown earlier, the shifting electrochemical potential cannot explain the suppression. We must conclude that strong lowering of external NaCl concentration caused partial failure of  $\text{GABA}_A$  receptor controlled channels. The depression could have been caused by lowered total ionic strength rather than the specific loss of  $\text{Na}^+$  or  $\text{Cl}^-$  ions.

As both, hyposmotic and NaCl deficient solutions suppressed muscimol-induced currents, we must ask whether the low osmolarity had an added effect of its own. In the example of Fig. 2 the depression during hyposmotic exposure was moderately stronger than in low NaCl medium, but generally the difference was small, in other words the effect of the two solutions showed partial occlusion. In comparing the effects it should be remembered that the low NaCl solution replaced the bathing fluid, but the hyposmotic solution was delivered from a flow pipette and mixing with isosmotic bath fluid may have attenuated its effect. We conclude that low osmolarity weakly reinforces the effect of NaCl deficiency, but the two kinds of solutions probably counteract the opening of  $\text{GABA}_A$  controlled  $\text{Cl}^-$  channels in a similar manner.

The reversal potential of the muscimol-induced current shifted in the positive direction, but not by as much as expected for the decreased  $[\text{Cl}^-]_o$ . This discrepancy may be explained by the change of junction potential. The calculated shift of  $E_{\text{Cl}}$  would be  $+26$  mV. Correcting for the  $-6$  mV calculated change of the junction potential when  $[\text{Na}^+]_o$  is reduced from 110 to 11 mM and  $[\text{Cl}^-]_o$  from 152 to 53 mM, we arrive at an expected shift of  $+20$  mV. This is close to the observed shift of the reversal potential of the muscimol-induced current (between  $+17$  and  $+19$  mV).

In a hypertonic environment the cells were seen to shrink. This is different from our previous series of experiments, when only one out of 6 closely observed cells decreased in volume [12]. The difference may be attributed to the duration of the exposure. In the previous experiments hypertonic solution was ejected for 30 s or less. In the present series, exposure had to continue for several minutes. Exposure to hypo-osmotic solution caused no visible cell swelling, confirming our earlier observation [12]. The resistance of the freshly isolated neurons to hypotonic swelling has yet to be reconciled with the marked swelling of cells in hippocampal tissue slices [4,7]. It may be that in intact tissue mainly glial cells swell [10], and the dendritic



tree of neurons which is mostly lost during the dissociation procedure.

Another remaining task is a clarification of the very marked growth of excitatory postsynaptic potentials in hippocampal tissue slices caused by hyposmotic solutions [3,7]. The increase of the extracellularly recorded synaptic potential (fEPSP) has been attributed to cell swelling, but the increase of tissue electric resistance accounts for less than half of the observed growth of the fEPSP [7]. We have not yet tested the effect of low NaCl and of hypo-osmotic solution on currents induced by glutamate. At the concentrations used in these experiments the strongly reduced  $[Na^+]_o$  would virtually eliminate the driving force of the glutamate induced current. Separating the effect of changing driving potential from that of possible changes of channel function will require detailed quantitative study.

### Acknowledgements

Supported by the Netherlands Organization for the Advancement of Pure Research (NWO).

### References

- [1] Andrew, R.D., Seizure and acute osmotic change: clinical and neurophysiological aspects, *J. Neurol. Sci.*, 101 (1991) 7–18.
- [2] Andrew, R.D., Fagan M., Ballyk, B.A. and Rosen, A.S., Seizure susceptibility and the osmotic state, *Brain Res.*, 498 (1989) 175–180.
- [3] Ballyk, B.A., Quackenbush, S.J. and Andrew, R.D., Osmotic effects on the CA1 neuronal population in hippocampal slices with special reference to glucose, *J. Neurophysiol.*, 65 (1991) 1055–1066.
- [4] Chebabo, S.R., Jing, J., Aitken, P.G. and Somjen, G.G., The reduction of relative interstitial volume of CA1 in hippocampal tissue slices in hypotonic solutions, *Soc. Neurosci. Abstr.*, 20 (1994) 1510.
- [5] Cserr, H.F., DePasquale, M., Nicholson, C., Patlak, C.S., Pettigrew, K.D. and Rice, M.E., Extracellular volume decreases while cell volume is maintained by ion uptake in rat brain during acute hypernatremia, *J. Physiol.*, 442 (1991) 277–295.
- [6] Gullans, S.R. and Verbalis, I.G., Control of brain volume during hyperosmolar and hyposmolar conditions, *Annu. Rev. Med.*, 44 (1993) 289–301.
- [7] Hester, M.A., Aitken, P.G. and Somjen, G.G., Hypotonic environment enhances synaptic potentials in hippocampal tissue slices, *Soc. Neurosci. Abstr.*, 19 (1993) 715.
- [8] Hille, B., *Ionic Channels of Excitable Membranes*, Sinauer Assoc., Sunderland, MA, 1992.
- [9] Kay, A.R. and Wong, R.K.S., Isolation of neurons suitable for patch clamping from adult mammalian central nervous system, *J. Neurosci. Methods*, 16 (1986) 227–238.
- [10] Kimelberg, H.K., Sankar P., O'Connor, E.R., Jalonen, T. and Goderie, S.K., Functional consequences of astrocyte swelling, *Prog. Brain Res.*, 94 (1992) 57–68.
- [11] Pusch, M. and Neher, E., Rates of diffusional exchange between small cells and a measuring patch pipette, *Pflüger's Arch.*, 411 (1988) 204–211.
- [12] Somjen, G.G., Faas, G.C., Vreugdenhil, M. and Wadman, W.J., Channel shutdown: a response of hippocampal neurons to adverse environments, *Brain Res.*, 632 (1993) 180–194.
- [13] Somjen, G.G., Vreugdenhil, M., and Wadman, W.J. Strongly anisomotic solutions suppress GABA<sub>A</sub> currents in freshly dissociated hippocampal neurons, *Soc. Neurosci. Abstr.*, 20 (1994) 1510.
- [14] Strange, K., Regulation of solute and water balance and cell volume in the central nervous system, *J. Am. Soc. Nephrol.*, 3 (1992) 12–27.
- [15] Traynelis, S.F. and Dingledine, R. Role of extracellular space in hyperosmotic suppression of potassium-induced electrographic seizures, *J. Neurophysiol.*, 61 (1989) 927–938.
- [16] Vreugdenhil, M., *Intrinsic Membrane Properties of CA1 Pyramidal Neurons after Kindling Epileptogenesis*, Dissertation, University of Amsterdam, Amsterdam, 1994.
- [17] Vreugdenhil, M. and Wadman, W.J., Enhancement of calcium currents in rat hippocampal CA1 neurons induced by kindling epileptogenesis, *Neuroscience*, 49 (1992) 373–381.