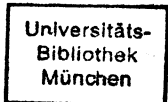


Canadian Journal of Physiology and Pharmacology

An International Journal

Published by
THE NATIONAL RESEARCH COUNCIL OF CANADA



Journal canadien de physiologie et pharmacologie

Une revue internationale

Publié par
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Cellular mechanisms of potassium homeostasis in the mammalian nervous system¹

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Received July 21, 1986

GRAFE, P., and BALLANYI, K. 1987. Cellular mechanisms of potassium homeostasis in the mammalian nervous system. *Can. J. Physiol. Pharmacol.* **65**: 1038–1042.

Double-barrelled ion-sensitive microelectrodes were used to measure changes in the intracellular activities of K^+ , Na^+ , and Cl^- (aK_i , aNa_i , aCl_i) in neurones of rat sympathetic ganglia and in glial cells of slices from guinea-pig olfactory cortex. In sympathetic neurones, carbachol and γ -aminobutyric acid (GABA) produced a reversible decrease of aK_i . The decrease of aK_i during carbachol was accompanied by a rise of aNa_i , whereas in the presence of GABA decreases of aK_i and aCl_i were seen. The reuptake of K^+ released during the action of carbachol was completely blocked by ouabain, whereas furosemide inhibited the aK_i recovery after the action of GABA. In glial cells, in contrast to the observations in the sympathetic neurones, aK_i and aCl_i increased, whereas aNa_i decreased when neuronal activity was enhanced by repetitive stimulation of the lateral olfactory tract. It was found that barium ions and ouabain strongly reduced the activity-related rise of intraglial aK_i in slices of guinea-pig olfactory cortex. These data show that mammalian neurones as well as glial cells possess several K^+ uptake mechanisms that contribute to potassium homeostasis. Ouabain, furosemide, and Ba^{2+} are useful pharmacological tools to separate these mechanisms.

GRAFE, P., et BALLANYI, K. 1987. Cellular mechanisms of potassium homeostasis in the mammalian nervous system. *Can. J. Physiol. Pharmacol.* **65**: 1038–1042.

On a utilisé des microélectrodes à double branche, sensibles aux ions pour déterminer les variations des activités intracellulaires K^+ , Na^+ et Cl^- (aK_i , aNa_i , aCl_i) dans des neurones des ganglions sympathiques de rat et dans des cellules gliales de tranches de cortex olfactif de cobaye. Dans les neurones sympathiques, le carbachol et l'acide γ -aminobutyrique (GABA) provoquent une diminution réversible d' aK_i . La diminution d' aK_i en présence de carbachol fut accompagnée d'une élévation d' aNa_i , alors qu'en présence de GABA, on observa des diminutions d' aK_i et d' aCl_i . La réabsorption de K^+ libéré durant l'action du carbachol fut complètement bloquée par l'ouabaïne, alors que le furosémide inhiba le rétablissement d' aK_i après l'action de GABA. Dans les cellules gliales, contrairement aux observations dans les neurones sympathiques, aK_i et aCl_i augmentèrent, alors qu' aNa_i diminua lorsque l'activité neuronale fut augmentée par la stimulation répétée du pédoncule olfactif latéral. On a constaté que les ions baryum et l'ouabaïne réduisaient fortement l'élévation reliée à l'activité de l' aK_i intraglies dans les tranches de cortex olfactif de cobaye. Ces résultats montrent que les neurones et les cellules gliales de mammifère possèdent plusieurs mécanismes d'absorption de K^+ qui contribuent à l'homéostasie potassique. L'ouabaïne, le furosémide et le Ba^{2+} sont des éléments pharmacologiques utiles pour distinguer ces mécanismes.

[Traduit par la revue]

Introduction

Enhanced neuronal activity in the central nervous system is accompanied by transient elevations in the extracellular K^+ activity (aK_e) (Somjen 1979; Nicholson 1981; Sykova 1983). The rise of aK_e is the consequence of K^+ released from neurones via voltage- or Ca^{2+} -dependent, transmitter-gated, and leaky K^+ channels. Mammalian neurones possess at least two mechanisms for K^+ reuptake. One is the ouabain-sensitive Na^+-K^+ pump, which can be activated, for example, by the acetylcholine-induced rise in the intracellular Na^+ activity and the other one is a furosemide-sensitive, Na^+ -dependent KCl cotransport, which is activated during the action of γ -aminobutyric acid (GABA) (Ballanyi et al. 1984; Ballanyi and Grafe 1985).

Glial cells, on the other hand, accumulate K^+ during an increase of aK_e (literature reviewed by Walz and Hertz 1983). However, the mechanisms of K^+ uptake into glial cells are not yet understood in detail. Several factors such as spatial buffering (Orkand et al. 1966; Dietzel et al. 1982; Coles and Orkand 1983; Gardner-Medwin 1983), Na^+-K^+ pump activity (Kukes et al. 1976; Walz and Hertz 1982; Grisar et al. 1983), KCl cotransport (Kimelberg and Frangakis 1985; Walz and Hinks 1985), or the presence of Ba^{2+} -sensitive K^+ channels (Walz et al. 1984) have

been proposed to contribute to the K^+ uptake process. Recently, we have used double-barrelled ion-sensitive microelectrodes to measure changes in the intracellular activities of K^+ , Na^+ , and Cl^- (aK_i , aNa_i , aCl_i , respectively) in glial cells of slices from guinea-pig olfactory cortex during repetitive stimulation of the lateral olfactory tract (Ballanyi et al. 1987). These experiments showed that Ba^{2+} and ouabain are useful pharmacological tools to study the mechanisms underlying the K^+ uptake in glial cells.

The present paper reviews our intracellular measurements with ion-sensitive microelectrodes from mammalian neurones and glial cells. The data are summarized in a model about cellular mechanisms contributing to the K^+ homeostasis in the mammalian nervous system.

Methods

Sympathetic neurones

Experiments were performed on neurones of superior cervical ganglia of rats. Ganglia were isolated, desheathed, and continuously superfused at 30°C in a recording chamber (Ballanyi and Grafe 1985) with a solution containing (in mM): NaCl, 118; KCl, 4.8; $NaHCO_3$, 2.5; KH_2PO_4 , 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; and glucose, 10.

Cortical glial cells

Experiments were performed on slices of the olfactory cortex of guinea pigs. After decapitation, the brain was rapidly removed and surface slices (approximately 500 μ m thick) were cut using a plastic guide and a bow cutter. Slices were placed in a perspex chamber (vol. 2 mL) and superfused at 25°C with a solution of the following composition (in mM): NaCl, 118; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4 , 1.2; $MgCl_2$, 1.0; $CaCl_2$, 1.5; and glucose, 10 (equilibrated with 95% O_2 – 5% CO_2 ; pH 7.4). Drugs were added to the superfusion fluid. Cortical neurones

¹This paper was presented at the Ion-selective Microelectrodes and Excitable Tissue Symposium (Toronto, Ontario, July 8–11, 1986), a Satellite Symposium of the 30th IUPS Congress, and has undergone the Journal's usual peer review.

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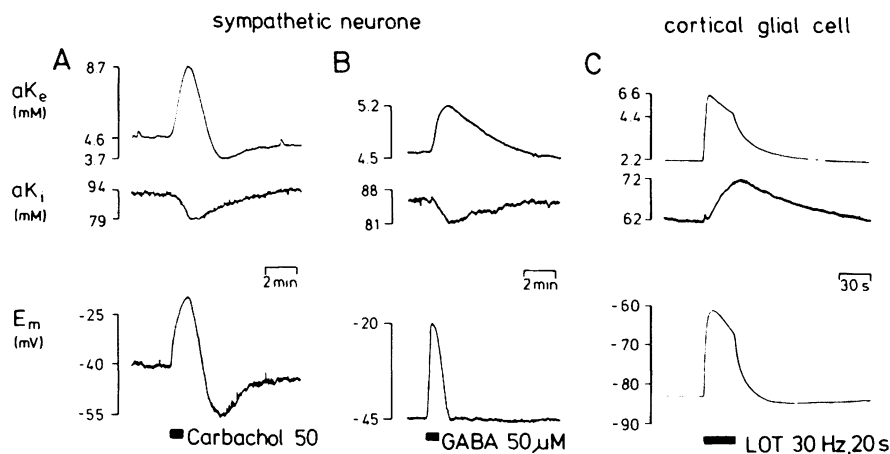


FIG. 1. An overview of changes in membrane potential (E_m) and in intra- and extra-cellular K^+ activity (aK_i , aK_e , respectively) accompanying the actions of carbachol, GABA, and repetitive stimulation on neurones and glial cells. (A and B) Experiments with isolated rat sympathetic ganglia. (C) An experiment with a guinea-pig olfactory cortex slice. For further discussion see text. LOT, lateral olfactory tract.

were activated by electrical stimulation of the lateral olfactory tract with platinum wire electrodes.

Ion-sensitive microelectrodes

The properties and the methods used for the construction and calibration of double-barrelled ion-sensitive microelectrodes are described in detail elsewhere (Grafe et al. 1985; Ballanyi et al. 1987). The ligands used for the K^+ -, Cl^- -, and Na^+ -sensitive microelectrodes were Corning 477317, IE-170 (WP-Instruments), and Fluka 71176, respectively. All values of intracellular Na^+ , K^+ , and Cl^- are given in activities (Meier et al. 1980). Intracellular impalements were achieved by means of a piezo-driven micromanipulator (built by M. Frankenberger, Munich).

Results

Intracellular K^+ activity in mammalian neurones and glial cells

Figure 1 summarizes observations from experiments in which intracellularly and extracellularly positioned K^+ -sensitive microelectrodes were used simultaneously. Parts A and B are from experiments on rat sympathetic ganglia. One can see that both carbachol and GABA depolarize the membrane of sympathetic neurones. This effect is accompanied in each case by a rise of aK_e and a decrease of aK_i . The post-carbachol K^+ reuptake is accompanied by an undershoot of aK_e and membrane hyperpolarization. Neither phenomenon occurs after the end of the GABA application. This indicates that two different mechanisms might contribute to the aK_i recovery after carbachol and GABA. This conclusion was confirmed by pharmacological observations: ouabain blocked completely the post-carbachol aK_e recoveries, whereas furosemide inhibited the K^+ reuptake after the action of GABA (Ballanyi et al. 1984).

In contrast to the neurones, glial cells of guinea-pig olfactory cortex accumulate K^+ during a period of enhanced neuronal activity. Figure 1C illustrates that stimulation of the lateral olfactory tract (30 Hz, 20 s) resulted in a glial depolarization of about 20 mV during an increase of aK_e of about 4 mM (Ballanyi et al. 1987). During the depolarization aK_i increased by about 10 mM.

Activity-related changes of intragial ion activities

The base-line level of aK_i as measured in 12 cells with a mean membrane potential (E_m) of -84.4 ± 3.3 mV was 65.9 ± 6.6 mM (mean \pm SD). Therefore a close correspondence between the calculated E_K of -85.6 mV (aK_e at rest = 2.2 mM) and the glial E_m is revealed. Intragial aNa_i had a resting level of $25.2 \pm$

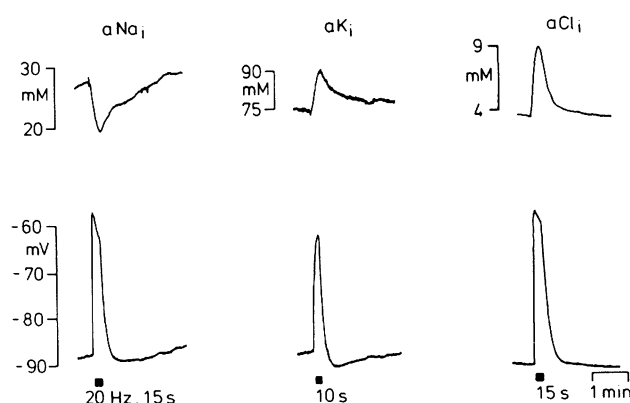


FIG. 2. Changes of intragial ion activities during repetitive stimulation of the lateral olfactory tract (20 Hz for 10–15 s). The figure combines recordings from three different glial cells in which a K^+ -, Na^+ -, or Cl^- -sensitive microelectrode was used to record changes of these ion activities in a glial cell. For a quantitative analysis of such recordings see text.

4.6 mM ($E_m = -83.0 \pm 2.8$ mV; $n = 12$) and the corresponding aCl_i was 6.0 ± 1.5 mM ($E_m = -86.9 \pm 3.8$ mV; $n = 23$; calculated under the assumption of a pure Cl^- selectivity of the Cl^- -sensitive ligand). Figure 2 illustrates how the intracellular activities of these ions changed when the lateral olfactory tract was stimulated repetitively (20 Hz) for 10–15 s. The average rise of aK_i was 16.1 ± 6.1 mM (12 glial cells), aNa_i decreased by 6.0 ± 1.9 mM ($n = 12$), and aCl_i increased by 6.0 ± 1.5 mM ($n = 23$). In conclusion, these observations demonstrate that activity-related glial K^+ accumulation is accompanied by a rise of intragial aCl_i and by a fall of aNa_i .

K^+ uptake mechanisms in cortical glial cells

To differentiate between several possible mechanisms of K^+ uptake into cortical glia, the action of Ba^{2+} was explored. Barium ions are known to block K^+ channels (Hille 1984) and have been previously used as a tool to differentiate between passive and active K^+ uptake mechanisms in skeletal muscle fibres (Sjodin and Ortiz 1975). These ions are also known to interfere with the K^+ homeostasis of cultured glial cells (Walz et al. 1984). In our experiments, three clear effects of Ba^{2+} on the glial cells were observed: (i) Ba^{2+} induced a membrane depolarization and led to a change from a K^+ -induced membrane

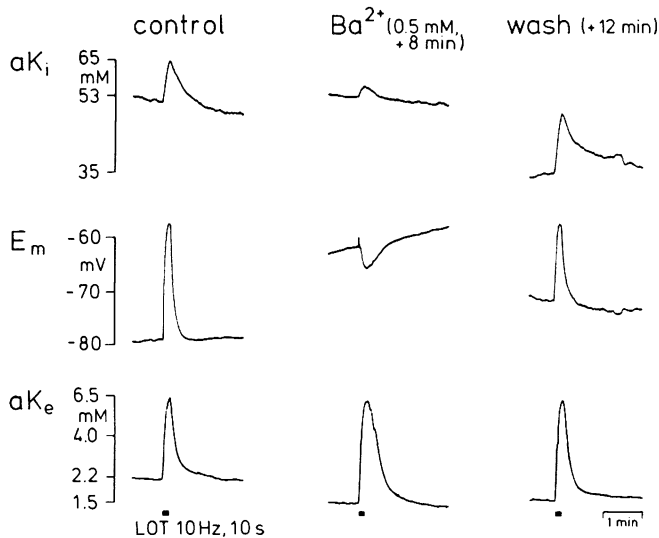


FIG. 3. Effects of barium on stimulus-induced increases in intracellular K^+ activity (aK_i), extracellular K^+ activity (aK_e), and glial membrane potential (E_m). The figure shows three sections taken from a continuous recording from a single glial cell. The left column shows the typical rise of aK_i during a stimulus-induced increase of aK_e in normal solution. In the presence of Ba^{2+} (0.5 mM, central column) an even greater rise in aK_e led to a much smaller increase of aK_i , and was accompanied by a membrane hyperpolarization. Right column shows changes with stimulation during the recovery from Ba^{2+} . LOT, lateral olfactory tract. (From Ballanyi et al. 1987, reprinted with kind permission of The Physiological Society.)

depolarization into a K^+ -induced hyperpolarization, (ii) Ba^{2+} raised the intragial aK_i base-line level and partially blocked the K^+ uptake, and (iii) Ba^{2+} completely blocked the rise of intragial aCl_i usually seen during a stimulus-induced rise of aK_e . These effects are illustrated in Figs. 3, 4, and 5.

Figure 3 shows three excerpts from a continuous recording from a single cortical glial cell. The left column illustrates that in the normal bathing solution a membrane depolarization and a rise of aK_i occur during repetitive stimulation of the lateral olfactory tract. The center column shows a stimulation period with the same stimulus parameters 8 min after Ba^{2+} (0.5 mM) was added to the bathing solution. The glial cell is now depolarized and responds with a hyperpolarization to the rise of aK_e . Now, only a small elevation of aK_i is seen during the stimulation period. The right column is from a period 12 min after Ba^{2+} was replaced by the normal bathing solution and shows that the effect of Ba^{2+} is reversible.

Figure 4 is an example of experiments in which the mechanism of the stimulus-related glial hyperpolarization was explored. The left column and center column show changes in the membrane potential and aK_i in the normal bathing solution and 12 min after Ba^{2+} was added to this solution (see Fig. 3). The membrane depolarization during the application of Ba^{2+} can be explained by a reduced K^+ conductance. A reduced efflux of K^+ from the glial cell in the presence of an active Na^+-K^+ pump then results in an increase of aK_i (see also Fig. 8 in Ballanyi et al. 1987). The right column is from a period 15 min after ouabain (5 μM) was added to the Ba^{2+} -containing bathing solution. Ouabain blocked both the stimulus-related remaining rise of aK_i and the membrane hyperpolarization. This indicates that an electrogenic Na^+-K^+ pump underlies the membrane hyperpolarization and also the Ba^{2+} -resistant K^+ uptake. (Ouabain did not block the stimulus-induced rise in aK_e ; on the

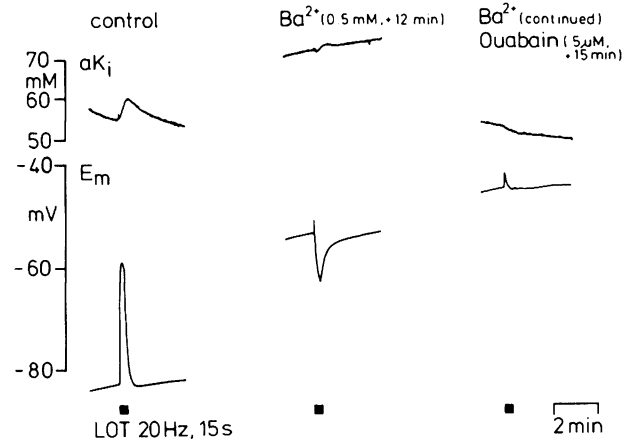


FIG. 4. Effects of barium and ouabain on stimulus-induced increases in intracellular K^+ activity (aK_i) and glial membrane potential (E_m). The left column shows the typical rise of aK_i during a stimulus-induced depolarization of the glial cell. In the presence of Ba^{2+} (0.5 mM) an elevation of the aK_i base-line level, smaller increase of aK_i , and stimulus-induced membrane hyperpolarization were seen. The Ba^{2+} -resistant rise of aK_i and the membrane hyperpolarization were completely blocked 15 min after ouabain (5 μM) was added to the Ba^{2+} -containing solution. LOT, lateral olfactory tract.

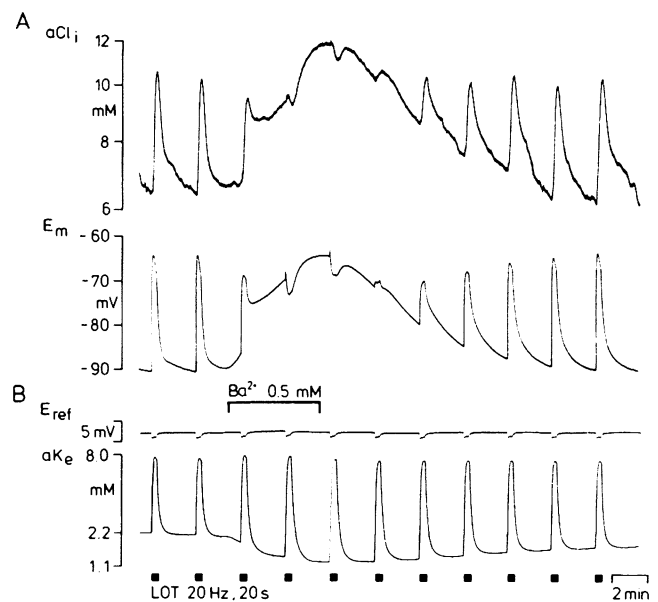


FIG. 5. Effects of barium on intracellular Cl^- activity (aCl_i) and extracellular K^+ (aK_e). After two control stimulation trains, Ba^{2+} (0.5 mM) was added to the bathing solution. The typical glial depolarization in Ba^{2+} was accompanied by an increase in aCl_i base-line. The Ba^{2+} -induced shift of stimulus-related depolarization into hyperpolarization was mirrored by a shift from an increase in aCl_i to a decrease. After the washout of Ba^{2+} , the membrane potential (E_m) as well as aCl_i recovered to base-line values. LOT, lateral olfactory tract. (From Ballanyi et al. 1987, reprinted with kind permission of The Physiological Society.)

contrary, the stimulus-related rise of aK_e was slightly bigger than usual; see Fig. 6 in Ballanyi et al. 1987.)

The behaviour of intragial aCl_i in the presence of Ba^{2+} indicates a high Cl^- conductance of the glial membrane. In the experiment illustrated in Fig. 5, a Cl^- -sensitive microelectrode was used to record aCl_i and E_m of a glial cell (Fig. 5A), whereas a K^+ -sensitive microelectrode was positioned extracellularly to measure aK_e (Fig. 5B). Two stimulus trains in normal solution

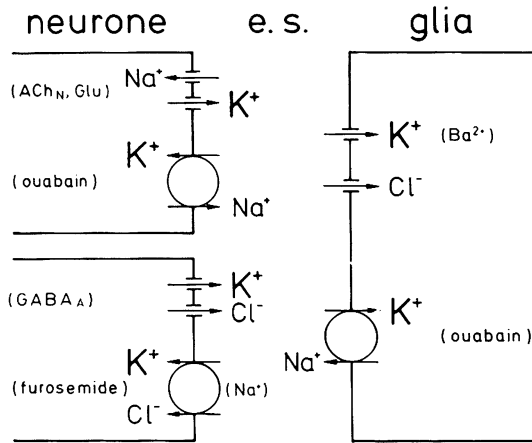


FIG. 6. Summary of K^+ uptake mechanisms in mammalian neurones and glia. After a rise of neuronal aNa_i via the opening of nicotinic cholinergic (ACh_N) or glutamate (Glu) receptors, the Na^+-K^+ pump restores the intracellular K^+ activity. If neurones release K^+ together with Cl^- (during a depolarizing action of GABA), a furosemide-sensitive, $[Na]_e$ -dependent K^+-Cl^- cotransport is found to maintain the normal aK_i resting level. Our results indicate that glial cells also possess at least two types of K^+ uptake mechanisms. There is passive K^+ uptake via Ba^{2+} -sensitive K^+ channels (together with passive uptake of Cl^-) and also the activation of a glial Na^+-K^+ pump secondary to a rise of extracellular K^+ (see also text). e. s., extracellular space.

at the beginning of this recording revealed the stimulus-related rise of aK_e , membrane depolarization and accompanying rise of aCl_i usually seen in the glial cells. Ba^{2+} was added to the bathing solution and within a few minutes the membrane depolarized by more than 20 mV and aCl_i increased to about 12 mM. Repetitive stimulation of the lateral olfactory tract during Ba^{2+} induced a small membrane hyperpolarization and a slight fall in aCl_i . Therefore, the increase in resting aCl_i , as well as the stimulus-related decrease of aCl_i , clearly indicates the close relationship between the membrane potential and aCl_i . If aCl_i were to follow changes in aK_e , then the Ba^{2+} -induced increase of the aK_e base-line and the stimulus-related aK_e increase should have altered aCl_i in the opposite direction. Previously in the literature pertaining to glia, results from two kinds of experiment led to the conclusion that glial cells do not have a significant Cl^- conductance. First, no change in glial membrane potential was seen during the transition from a high to a low extracellular Cl^- medium. This fact may be explained by a lack of Cl^- conductance. However, if intracellular Cl^- were to leave the cell as rapidly as the decrease of aCl_i , E_{Cl} would always remain close to E_m ; there would be no potential change in spite of a Cl^- conductance (see Fig. 4 in Ballanyi et al. 1987). Secondly, some authors did not observe changes in glial input resistance in Cl^- -free extracellular medium. These experiments should be repeated with the substitution of bigger anions, in view of the discovery of large anion-conducting channels in glial cells (Gray and Ritchie 1985).

Discussion

Figure 6 summarizes our data obtained with intracellular ion-sensitive microelectrodes from rat sympathetic neurones and guinea-pig cortical glial cells. Neurones, on the one hand, exchange intracellular K^+ with Na^+ if an increase in Na^+ conductance leads to a membrane depolarization. A ouabain-sensitive Na^+-K^+ pump restores both ion concentration gradients involved, and this electrogenic reuptake is accompanied

by a membrane hyperpolarization and an undershoot of aK_e . Examples of such a situation are shown by the action of glutamate on frog spinal motoneurons (Sonnhof et al. 1976) and of carbachol (via stimulation of nicotinic acetylcholine receptors) on rat sympathetic ganglia (Ballanyi et al. 1984). If, on the other hand, neurones are depolarized as a consequence of an increase in Cl^- conductance (for example by the activation of $GABA_A$ receptors), both Cl^- and K^+ are released from the cell. It was found that in such a situation an electroneutral, Na^+ -dependent, and furosemide-sensitive K^+-Cl^- carrier is involved in the neuronal K^+ homeostasis (Ballanyi and Grafe 1985).

Glial cells also have at least two mechanisms to take up K^+ (Ballanyi et al. 1987). One component is a ouabain-sensitive Na^+-K^+ pump that, in contrast to neurones, is activated by excess extracellular K^+ . The electrogenic pump current of this transport mechanism is normally short-circuited by the high resting conductance of the glial membrane. However, a ouabain-sensitive membrane hyperpolarization can be seen in the presence of Ba^{2+} (see Figs. 3 and 4). The Na^+-K^+ pump most probably contributes to the decrease of intragial aNa_i which is observed during a rise of aK_e , although other factors may contribute to this phenomenon.

The other mechanism, by which glial cells take up K^+ , is a Ba^{2+} -sensitive K^+ conductance. This finding, together with the observation of a high Cl^- conductance of the glial cells (see Fig. 5), indicates that the K^+ uptake can occur via a mechanism suggested by Boyle and Conway (1941) and Hodgkin and Horowitz (1959) to explain passive uptake of K^+ into muscle fibres. These authors point out that during an elevation of aK_e , the presence of a Cl^- conductance will prevent E_m from reaching the new E_K . Hence K^+ (as well as Cl^-) can continue to flow into the cell. Our data are compatible with this view. Another mechanism by which K^+ uptake could occur through Ba^{2+} -sensitive channels would be via spatial buffer currents (Orkand et al. 1966; Coles and Orkand 1983; Gardner-Medwin 1983; Dietzel et al. 1982). According to this view, K^+ would enter the glial syncytium through K^+ channels as a result of a spatial potential gradient along the membrane of the electrically coupled glia; the rise of intragial aCl_i would be explained by a redistribution of Cl^- ions within the linked glial cells. Our experiments do not exclude such a mechanism. However, the existence of a passive KCl uptake would allow the K^+ to be stored in the glial cell in the immediate neighbourhood of the active neurone, where it might be more readily available to the neurone during recovery from K^+ loss (Gray and Ritchie 1985).

Acknowledgements

We thank Professor G. ten Bruggencate for constant encouragement and helpful discussions during the course of this project. Miss G. Schneider and Mrs. C. Müller provided expert technical and secretarial assistance. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 220).

- BALLANYI, K., and GRAFE, P. 1985. An intracellular analysis of γ -aminobutyric-acid-associated ion movements in rat sympathetic neurones. *J. Physiol. (London)*, **365**: 41–58.
- BALLANYI, K., GRAFE, P., REDDY, M. M., and TEN BRUGGENCATE, G. 1984. Different types of potassium transport linked to carbachol and GABA actions in rat sympathetic neurones. *Neuroscience (Oxford)*, **12**: 917–927.
- BALLANYI, K., GRAFE, P., and TEN BRUGGENCATE, G. 1987. Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. *J. Physiol. (London)*, **382**: 159–174.

- BOYLE, P. J., and CONWAY, E. J. 1941. Potassium accumulation in muscle and associated changes. *J. Physiol. (London)*, **100**: 1–63.
- COLES, J. A., and ORKAND, R. K. 1983. Modification of potassium movement through the retina of the drone (*Apis mellifera*) by glial uptake. *J. Physiol. (London)*, **340**: 157–174.
- DIETZEL, I., HEINEMANN, U., HOFMEIER, G., and LUX, H. D. 1982. Stimulus-induced changes in extracellular Na^+ and Cl^- concentration in relation to changes in the size of the extracellular space. *Exp. Brain Res.* **46**: 73–84.
- GARDNER-MEDWIN, A. R. 1983. Analysis of potassium dynamics in mammalian brain tissue. *J. Physiol. (London)*, **335**: 393–426.
- GRAFE, P., BALLANYI, K., and TEN BRUGGENCATE, G. 1985. Changes of intracellular free ion concentrations, evoked by carbachol or GABA, in sympathetic neurons. *In* Ion measurements in physiology and medicine. *Edited by* Kessler, M., Harrison, D. K., and Höper, J. Springer, Berlin, Heidelberg, New York. pp. 184–188.
- GRAY, P. T. A., and RITCHIE, J. M. 1985. Ion channels in Schwann and glial cells. *Trends Neurosci.* **8**: 411–415.
- GRISAR, T., FRANCK, G., and DELGADO-ESCUETA, A. V. 1983. Glial contribution to seizure: K^+ activation of (Na^+ , K^+)-ATPase in bulk isolated glial cells and synaptosomes of epileptogenic cortex. *Brain Res.* **261**: 75–84.
- HILLE, B. 1984. Ionic channels of excitable membranes. Sinauer Associates Inc., Sunderland, MA.
- HODGKIN, A. L., and HOROWICZ, P. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (London)*, **148**: 127–160.
- KIMELBERG, H. K., and FRANGAKIS, M. V. 1985. Furosemide- and bumetanide-sensitive ion transport and volume control in primary astrocytes from rat brain. *Brain Res.* **361**: 125–134.
- KUKES, G., ELUL, R., and DE VELLIS, J. 1976. The ionic basis of the membrane potential in a rat glial cell line. *Brain Res.* **104**: 71–92.
- MEIER, P. C., AMMANN, D., MORF, W. E., and SIMON, W. 1980. Liquid-membrane ion-sensitive electrodes and their biomedical applications. *In* Medical and biological applications of electrochemical devices. *Edited by* Koryta, J. J. Wiley & Sons Ltd., New York. pp. 13–91.
- NICHOLSON, C. 1981. Brain-cell microenvironment as a communication channel. *In* The neurosciences—Fourth study program. *Edited by* Schmitt, F. O., and Worden, F. G. MIT Press, Cambridge, U.S.A. pp. 457–476.
- ORKAND, R. K., NICHOLLS, J. G., and KUFFLER, S. W. 1966. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of Amphibia. *J. Neurophysiol.* **29**: 788–806.
- SJODIN, R. A., and ORTIZ, O. 1975. Resolution of the potassium ion pump in muscle fibers using barium ions. *J. Gen. Physiol.* **66**: 269–286.
- SOMJEN, G. G. 1979. Extracellular potassium in the mammalian central nervous system. *Annu. Rev. Physiol.* **41**: 159–177.
- SONNHOF, U., GRAFE, P., and KRUMNIKL, J. 1976. Interaction of glutamate with the sodium pump in spinal motoneurons of the frog. *Pfluegers Arch.* **362**: R34.
- SYKOVA, E. 1983. Extracellular K^+ accumulation in the central nervous system. *Prog. Biophys. Mol. Biol.* **42**: 135–190.
- WALZ, W., and HERTZ, L. 1982. Ouabain-sensitive and ouabain-resistant net uptake of potassium into astrocytes and neurons in primary cultures. *J. Neurochem.* **39**: 70–77.
- 1983. Functional interactions between neurones and astrocytes. II. Potassium homeostasis at the cellular level. *Prog. Neurobiol.* **20**: 133–183.
- WALZ, W., and HINKS, E. C. 1985. Carrier-mediated KCl accumulation accompanied by water movements is involved in the control of physiological K^+ levels by astrocytes. *Brain Res.* **343**: 44–51.
- WALZ, W., SHARGOOL, M., and HERTZ, L. 1984. Barium-induced inhibition of K^+ transport mechanisms in cortical astrocytes—its possible contribution to the large Ba^{2+} evoked extracellular K^+ -signal in brain. *Neuroscience (Oxford)*, **13**: 945–949.