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Cellular mechanisms of potassium homeostasis in the mammalian nervous system

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Double-barrelled ion-sensitive microelectrodes were used to measure changes in the intracellular activities of K⁺, Na⁺, and Cl⁻ (aKᵢ, aNaᵢ, aCl⁻) 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 aKᵢ. The decrease of aKᵢ during carbachol was accompanied by a rise of aNaᵢ, whereas in the presence of GABA decreases of aKᵢ and aCl⁻ were seen. The reuptake of K⁺ released during the action of carbachol was completely blocked by ouabain, whereas furosemide inhibited the aKᵢ recovery after the action of GABA. In glial cells, in contrast to the observations in the sympathetic neurones, aKᵢ and aCl⁻ increased, whereas aNaᵢ 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 aKᵢ 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 Ba²⁺ are useful pharmacological tools to separate these mechanisms.

Introduction

Enhanced neuronal activity in the central nervous system is accompanied by transient elevations in the extracellular K⁺ activity (aKₑ), (Somjen 1979; Nicholson 1981; Sykova 1983). The rise of aKₑ is the consequence of K⁺ release from neurones via voltage- or Ca²⁺-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 aKₑ (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 Ba²⁺-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⁻ (aKᵢ, aNaᵢ, aCl⁻, 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 Ba²⁺ 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; NaHCO₃, 25; NaH₂PO₄, 1.2; MgSO₄, 1.2; CaCl₂, 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 μm 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; NaHCO₃, 25; NaH₂PO₄, 1.2; MgCl₂, 1.0; CaCl₂, 1.5; and glucose, 10 (equilibrated with 95% O₂ - 5% CO₂; pH 7.4). Drugs were added to the superfusion fluid. Cortical neurones

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Ion-sensitive microelectrodes were activated by electrical stimulation of the lateral olfactory tract with platinum wire electrodes.

The properties and the methods used for the construction and calibration of double-barrelled ion-sensitive microelectrodes are described in detail elsewhere (Gräfe 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.

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 \(aK_0\) and a decrease of \(aK_t\). The post-carbachol K\(^+\) reuptake is accompanied by an undershoot of \(aK_t\) and membrane hyperpolarization. Neither phenomenon occurs after the end of the GABA application. This indicates that two different mechanisms might contribute to the \(aK_t\) recovery after carbachol and GABA. This conclusion was confirmed by pharmacological observations: ouabain blocked completely the post-carbachol \(aK_t\) 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 mM during an increase of \(aK_t\) of about 4 mM (Ballanyi et al. 1987). During the depolarization \(aK_t\) increased by about 10 mM.

**Activity-related changes of intraglial ion activities**

The base-line level of \(aK_t\) as measured in 12 cells with a mean membrane potential \((E_m)\) of \(-84.4 \pm 3.3\) mV was \(65.9 \pm 6.6\) mM (mean \pm SD). Therefore a close correspondence between the calculated \(E_K\) of \(-85.6\) mV (\(aK_t\) at rest = 2.2 mM) and the glial \(E_m\) is revealed. Intraglial \(aNa\) had a resting level of \(25.2 \pm 3.0\) mM. All glial \(aNa\) activities increased by about 6.0 \pm 1.9 mM (n = 12), and \(aCl\) decreased by about 4.0 \pm 1.9 mM (n = 12), and \(aCl\) decreased by about 4.0 \pm 1.9 mM (n = 12). In conclusion, these observations demonstrate that activity-related glial K\(^+\) accumulation is accompanied by a rise of intraglial \(aCl\) and by a fall of \(aNa\).

**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 (Sjödin 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. 1.** An overview of changes in membrane potential \((E_m)\) and in intra- and extra-cellular K\(^+\) activity \((aK_0, aK_t, \text{ respectively})\) 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.
depolarization into a K⁺-induced hyperpolarization, (ii) Ba²⁺ raised the intragal aKᵢ, base-line level and partially blocked the K⁺ uptake, and (iii) Ba²⁺ completely blocked the rise of intragal aClᵢ usually seen during a stimulus-induced rise of aKₑ. 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 aKᵢ occur during repetitive stimulation of the lateral olfactory tract. The center column shows a stimulation period with the same stimulus parameters 8 min after Ba²⁺ (0.5 mM) was added to the bathing solution. The glial cell is now depolarized and responds with a hyperpolarization to the rise of aKₑ. Now, only a small elevation of aKᵢ is seen during the stimulation period. The right column is from a period 12 min after Ba²⁺ was removed by the normal bathing solution and shows that the effect of Ba²⁺ 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 aKᵢ in the normal bathing solution and 12 min after Ba²⁺ was added to this solution (see Fig. 3). The membrane depolarization during the application of Ba²⁺ 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 aKᵢ (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 Ba²⁺-containing bathing solution. Ouabain blocked both the stimulus-related remaining rise of aKᵢ and the membrane hyperpolarization. This indicates that an electrogenic Na⁺–K⁺ pump underlies the membrane hyperpolarization and also the Ba²⁺-resistant K⁺ uptake. (Ouabain did not block the stimulus-induced rise in aKₑ; on the contrary, the stimulus-related rise of aKₑ was slightly bigger than usual; see Fig. 6 in Ballanyi et al. 1987.)

The behaviour of intragal aClᵢ, in the presence of Ba²⁺ indicates a high Cl⁻ conductance of the glial membrane. In the experiment illustrated in Fig. 5, a Cl⁻-sensitive microelectrode was used to record aClᵢ and Eₘ of a glial cell (Fig. 5A), whereas a K⁺-sensitive microelectrode was positioned extracellularly to measure aKₑ (Fig. 5B). Two stimulus trains in normal solution
neurone e. s. glia

\[ \begin{array}{c}
\text{Na}^+ & \rightarrow & \text{K}^+ \\
\text{K}^+ & \rightarrow & \text{Na}^+ \\
\text{Cl}^- & \rightarrow & \text{Cl}^- \\
\text{Ba}^{2+} & \rightarrow & \text{Ba}^{2+} \\
\text{GABA}_A & \rightarrow & \text{GABA}_A \\
\text{furosemide} & \rightarrow & \text{furosemide} \\
\text{ouabain} & \rightarrow & \text{ouabain} \\
\end{array} \]

**Fig. 6.** Summary of K\(^+\) uptake mechanisms in mammalian neurones and glia. After a rise of neuronal aNa\(_0\) 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\(^+\)]-dependent K\(^+\)–Cl\(^-\) cotransport is found to maintain the normal aK\(_e\), 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 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 motoneurones (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 intraglial 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 Horowicz (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\(_{Cl}\) 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; Dietzgel 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 intraglial 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).

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