Neuroscience Letters, 38 (1983) 275–279
Elsevier Scientific Publishers Ireland Ltd.

INTRACELLULAR FREE SODIUM AND POTASSIUM, POST-CARBACHOL HYPERPOLARIZATION, AND EXTRACELLULAR POTASSIUM-UNDERSHOOT IN RAT SYMPATHETIC NEURONES

K. BALLANYI, P. GRAFE* and G. TEN BRUGGENCATE

Department of Physiology, University of Munich, Pettenkoferstr. 12, 8000 München 2 (F.R.G.)

(Received April 15th, 1983; Accepted April 25th, 1983)

Key words: ion-sensitive microelectrodes – intracellular sodium – intracellular potassium – Na^+,K^+-pump – ouabain – carbachol – rat sympathetic ganglion

Double-barrelled ion-sensitive microelectrodes were used to record the free intracellular Na^+- and K^+-concentrations ($[Na^+]_i$, $[K^+]_i$) and to determine their relation to changes in membrane potential and extracellular K^+ ($[K^+]_e$) in rat sympathetic ganglia. The application of 50 μmol/l carbachol resulted in an elevation of $[K^+]_e$ followed by a post-carbachol $[K^+]_e$-undershoot. The membrane depolarization of the sympathetic neurones was associated with an increase in $[Na^+]_i$ and a decrease in $[K^+]_i$. A membrane hyperpolarization and a recovery of $[K^+]_i$ and $[Na^+]_i$ to their baseline levels were observed during the $[K^+]_e$-undershoot. The time course of the $[K^+]_e$-undershoot correlated exactly with the duration of the rise in $[Na^+]_i$ and decrease of $[K^+]_i$. No K^+-reuptake occurred in the presence of ouabain. These data confirm, by direct measurements of intracellular ion concentration changes, the contribution of the Na^+,K^+-pump to the post-carbachol membrane hyperpolarization and $[K^+]_e$-undershoot.

Stimulus- and neurotransmitter-induced activity in the peripheral and central nervous system of mammals is accompanied by an elevation of the free extracellular K^+-concentration ($[K^+]_e$). After the end of the stimulation, there is a transient $[K^+]_e$-undershoot. Authors reporting $[K^+]_e$-undershoots in cat cerebral cortex [10, 13], cat medulla oblongata and spinal cord [11, 12], rat cerebellum [16] and rat sympathetic ganglion and vagus nerve [6], explained this phenomenon as an enhancement of active K^+-pumping, which in turn ought to be due to an accumulation of intracellular Na^+. However, direct measurements of the free intracellular Na^+- and K^+-concentrations in conjunction with neuronal membrane potential during the $[K^+]_e$-undershoot have not yet been described. We have performed such experiments in mammalian sympathetic neurones using double-barrelled ion-sensitive microelectrodes with very fine tips. Our results confirm previous suggestions about the kinetics of intracellular Na^+ and K^+ during the $[K^+]_e$-undershoot.

Experiments were performed on neurones of the superior cervical ganglion of

*Author for correspondence.

rats. Ganglia were isolated, desheathed and continuously superfused in a recording chamber with Krebs solution (30°C) containing (in mmol/l): NaCl 118; KCl 4.8; $NaHCO_3$ 25; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5 and D-glucose 10. Pre- and post-ganglionic nerve trunks were fixed with two suction electrodes, one for electrical stimulation and the other for recording the post-ganglionic compound action potential. Measurements of free intracellular Na^+- and K^+-concentrations ($[Na^+]_i$, $[K^+]_i$) and $[K^+]_e$ were made with double-barrelled ion-sensitive microelectrodes with tip-diameters less than 0.3 μm [1]. Reference barrels were filled with 1 mol/l magnesium acetate (pH adjusted to 7.4; electrode resistance about 100 MΩ). Ion-sensitive barrels were filled with K^+-exchanger (Corning 477317), valinomycin-cocktail or Na^+-ligand (ETH 227 [14]). The methods used to construct and calibrate the ion-sensitive microelectrodes have been described elsewhere [8, 9].

For data analysis, only those measurements were taken into account where both membrane potential and ion concentrations reached a steady-state after impalement. Early steady-state $[K^+]_i$-baseline level was 121.7 ± 9.7 mmol/l (mean ± S.D.; n = 30). The corresponding mean action potential amplitude was 70.7 ± 13.9 mV (n = 30) at a membrane resting potential of −45.3 ± 5.4 mV (n = 30). The

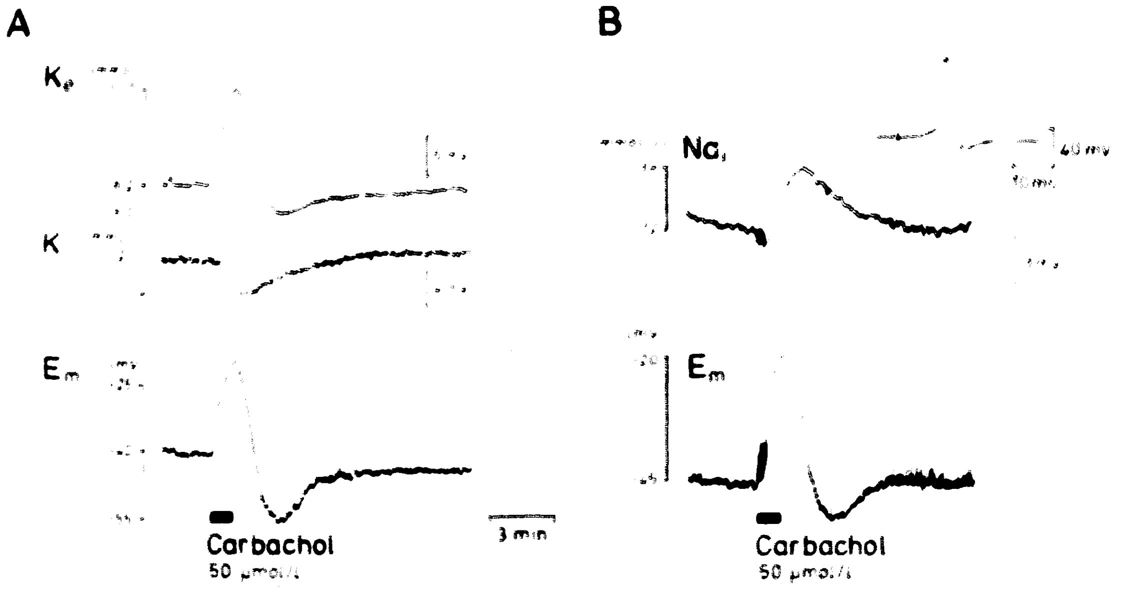

Fig. 1. Simultaneous measurements of carbachol-induced changes of intracellular and extracellular free K^+-concentrations (K_i and K_e) (A), and free intracellular Na^+-concentration (Na_i) and membrane potential (E_m) (B). Carbachol was added to the superfusion fluid for 1 min. The slow membrane potential changes were accompanied by transient increases in Na_i and K_e and a decrease in K_i. The kinetics of the recovery of Na_i and K_i to baseline levels were very similar to each other. Note the delayed onset of changes of the intracellular ion-concentrations with respect to the membrane depolarization. The increase in K_e had its maximum at the same time as K_i had its lowest level. The noise on the traces for E_m, Na_i and K_i is partly due to spontaneous neuronal activity of the cells. Inset in B shows a typical electrically elicited action potential of 75 mV amplitude (membrane resting potential was −45 mV). Two different neurones in A and B, respectively.

$[Na^+]_i$-baseline level was 11.4 ± 3.3 mmol/l) (mean ± S.D.; n = 13) at a mean membrane resting potential of −41.3 ± 5.8 mV (n = 13); action potential amplitude was 64.5 ± 7.2 mV (n = 13). In the first series of experiments the kinetics of $[Na^+]_i$ and $[K^+]_i$ were compared with changes of $[K^+]_e$ (Fig. 1). A typical increase of $[K^+]_e$ followed by a $[K^+]_e$-undershoot was induced by the application of carbachol (50 μmol/l, 1 min) via the superfusion solution (Fig. 1A; see refs. 6 and 7). The intracellular recordings made with the ion-sensitive microelectrodes revealed a simultaneous membrane depolarization of 23.3 ± 4.2 mV (mean ± S.D.; n = 9), a rise of $[Na^+]_i$ between 4 and 9 mmol/l (Fig. 1B), and a fall of $[K^+]_i$ between 8 and 20 mmol/l. Both the $[Na^+]_i$ increase and the $[K^+]_i$ decrease lagged behind the beginning of the membrane depolarization. The ionic changes reached their maximum values during the early phase of the repolarization of the membrane. During the $[K^+]_e$-undershoot the membrane hyperpolarized, and $[K^+]_i$ and $[Na^+]_i$ recovered to their baseline levels. The intracellular ion concentrations reached their resting levels at the end of the $[K^+]_e$-undershoot.

The observations concerning the kinetics of the ion concentration shifts are in

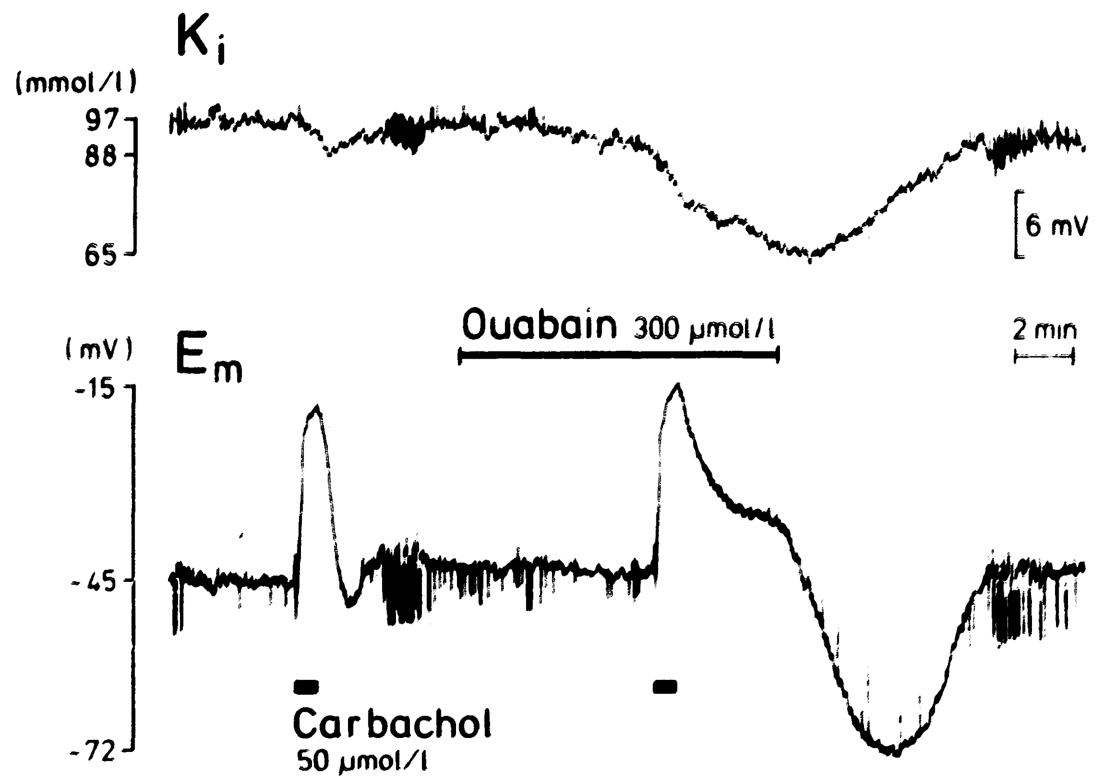

Fig. 2. Carbachol-induced changes of free intracellular K^+-concentration (K_i) in normal Krebs solution and in the presence of ouabain. In normal Krebs, carbachol typically induced a transient decrease of K_i. In the presence of 300 μmol/l ouabain, however, the initial carbachol-induced K_i decrease was followed by a further decrease of K_i. When ouabain was washed out, a rapid reuptake of K^+ accompanied by a considerable membrane hyperpolarization was observed. Deflections on both the traces at the end of the post-carbachol hyperpolarization are due to spontaneous activity of the neurone.

general accordance to the measurements of $[Na^+]_i$ and $[K^+]_i$ made by flame photometry in rat superior cervical ganglia [2]. They extend these data by a comparison of the behaviour of $[K^+]_i$ during the $[K^+]_e$-undershoot. A $[K^+]_i$-level which remains below the control value during the $[K^+]_e$-undershoot has also been observed in photoreceptors of the drone retina [4], in Retzius cells in the leech [5], and during a post-glutamate and post-stimulus membrane hyperpolarization in frog motoneurones [3, 9]. Our data also reveal that $[Na^+]_i$ remains elevated until the end of the $[K^+]_e$-undershoot. This fact supports previous, theoretical assumptions about the kinetics of intracellular Na^+ [6, 10–13, 16].

In a second series of experiments the contribution of the Na^+,K^+-pump to the $[K^+]_i$-recovery phase was investigated. A typical experiment is illustrated in Fig. 2. After a control application in normal Krebs solution, carbachol was reapplied in the presence of ouabain (300 μmol/l). The K^+ released by the neurones during the application of carbachol, did not appear to be taken up under these circumstances. However, after the end of the ouabain superfusion an increase of $[K^+]_i$ and a membrane hyperpolarization were observed. This indicates that the Na^+,K^+-pump is the main factor involved in the homeostasis of carbachol-induced ion concentration changes. This post-ouabain hyperpolarization also implies an electrogenic coupling ratio in analogy to the Na^+,K^+-pump of other neurones [15].

In conclusion, our data show, first, that double-barrelled ion-sensitive microelectrodes can be used to determine the free intracellular Na^+- and K^+-concentrations in mammalian neurones; and secondly, they confirm, by direct measurements of intracellular ion concentration changes, the contribution of an electrogenic Na^+,K^+-pump to the $[K^+]_e$-undershoot.

This research was supported by the Wilhelm Sander Stiftung. We thank Ms. I. Englmaier, Mrs. C. Müller and Ms. G. Schneider for expert technical assistance and Dr. A. Barber for correcting the English. We are grateful to Prof. Simon, ETH Zürich, for the gift of the valinomycin-cocktail and Na^+-ligand.

1 Brown, K.T. and Flaming D.G., New microelectrode techniques for intracellular work in small cells, Neuroscience, 2 (1977) 813–827.

2 Brown, D.A. and Scholfield, C.N., Changes of intracellular sodium and potassium ion concentrations in isolated rat superior cervical ganglia induced by depolarizing agents, J. Physiol. (Lond.), 242 (1974) 307–319.

3 Bührle, Ch.Ph. and Sonnhof, U., The ionic mechanism of the excitatory action of glutamate upon the membranes of motoneurones of the frog, Pflügers Arch., 396 (1983) 154–162.

4 Coles, J.A. and Tsacopoulus, M., Potassium activity in photoreceptors, glial cells and extracellular space in the drone retina: changes during photostimulation, J. Physiol. (Lond.), 290 (1979) 525–549.

5 Deitmer, J.W. and Schlue, W.R., Measurements of the intracellular potassium activity of Retzius cells in the leech central nervous system, J. exp. Biol., 91 (1981) 87–101.

6 Förstl, J., Galvan, M. and ten Bruggencate, G., Extracellular K^+-concentration during electrical stimulation of rat isolated sympathetic ganglia, vagus and optic nerves, Neuroscience, 7 (1982) 3221–3229.

7 Friedli, C., Kinetics and changes in PO_2 and extracellular potassium activity in stimulated rat ganglia. In Advances in Experimental Biology and Medicine, Plenum Press, New York, 1978, pp. 94.
8 Grafe, G., Rimpel, J., Reddy, M.M. and ten Bruggencate, G., Lithium distribution across the membrane of motoneurones in the isolated frog spinal cord, Pflügers Arch., 393 (1982) 297–301.
9 Grafe, P., Rimpel, J., Reddy, M.M. and ten Bruggencate, G., Changes of intracellular sodium and potassium ion concentrations in frog spinal motoneurones induced by repetitive synaptic stimulation, Neuroscience, 7 (1982) 3213–3220.
10 Heinemann, U. and Lux, H.D., Undershoots following stimulus-induced rises of extracellular potassium concentration in cerebral cortex of cat, Brain Res., 93 (1975) 63–76.
11 Kříž, N., Syková, E. and Vyklický, L., Extracellular potassium changes in the spinal cord of the cat and their relation to slow potentials, active transport and impulse transmission, J. Physiol. (Lond.), 249 (1975) 167–182.
12 Krnjević, K. and Morris, M.E., Factors determining the decay of K^+-potentials and focal potentials in the central nervous system, Canad. J. Physiol. Pharmacol., 53 (1975) 923–934.
13 Lothman, E., La Manna, J., Cordingley, G., Rosenthal, M. and Somjen, G., Responses of electrical potential, potassium levels, and oxidative metabolic activity of the cerebral neocortex of cats, Brain Res., 88 (1975) 15–36.
14 Steiner, R.A., Oehme, M., Ammann, D. and Simon, W., Neutral carrier sodium ion-selective microelectrode for intracellular studies, Analyt. Chem., 51 (1979) 351–353.
15 Thomas, R.C., Electrogenic sodium pump in nerve and muscle cells, Physiol. Rev., 52 (1972) 563–594.
16 Ullrich, A., Steinberg, R., Baierl, P. and ten Bruggencate, G., Changes in extracellular potassium and calcium in rat cerebellar cortex related to local inhibition of the sodium pump, Pflügers Arch., 395 (1982) 108–114.