Brain Research, 112 (1976) 183–187

© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

The effect of synaptic activation on the extracellular potassium concentration in the hippocampal dentate area, in vitro

L. C. FRITZ* and A. R. GARDNER-MEDWIN

Department of Physiology, University College London, London WC1E 6BT (Great Britain)

(Accepted April 28th, 1976)

Excitatory synaptic inputs to the granule cells of the dentate area elicit first of all EPSPs and then action potentials in the cells, giving rise to the characteristic extracellular field potentials referred to as the synaptic wave and population spike[13]. The excitability of the cell population is easily altered by prior conditioning shocks or trains of shocks[1–4,9,13], though the mechanisms of these alterations are not fully understood. It is possible that potassium (K^+) efflux from the granule cells might account for some of the alterations in excitability. It is already known that large increases in extracellular potassium concentration can accompany states of abnormal cortical function such as spreading depression[7,10,17] and epilepsy[6]. In this study we have used K^+-sensitive microelectrodes[18] to show that there are detectable changes in the K^+ concentration around the dentate granule cells of the guinea pig hippocampus after synaptic activation of a well defined afferent pathway. We are not at present able to say whether such changes in fact account for changes in excitability of the cell population.

Slices from the ventral surface of the hippocampus of guinea-pig brains were cut approximately 500 μm thick and maintained at 20–23 °C in vitro, cut surface uppermost, at the surface of an oxygenated saline solution (NaCl 124 m*M*, $NaHCO_3$ 24 m*M*, NaH_2PO_4 1.25 m*M*, KCl 6 m*M*, $CaCl_2$ 4 m*M*, $MgSO_4$ 2 m*M*, glucose 10 m*M*) under an atmosphere of moist 95% O_2–5% CO_2. The bath K^+ concentration is approximately twice that of brain extracellular fluid[5,6]; this high level has been employed by previous workers using similar techniques[4,15,19], and in our hands it favours the occurrence of synaptic activation comparable to that seen in the intact hippocampus. Some nerve fibres do, however, behave abnormally in 6 m*M* potassium in vitro and normally in 3 m*M* potassium[8]. In the present experiments the pO_2 is higher than in vivo and the temperature lower: both these conditions favour the survival of relatively thick slices with the least damage to cells. In view of the various

* Supported by a Fellowship from the U.S. National Science Foundation. Present address: The Rockefeller University, New York, N.Y. 10021, U.S.A.

abnormal conditions, further study will be necessary before the quantitative conclusions from this work can be applied confidently in vivo.

K^+-sensitive microelectrodes were made from single-barrelled micropipettes (1–4 μm diameter) whose tips were filled with a 200–500 μm column of liquid ion exchange resin (Corning Glass: 477317)[14]. The remainder of the electrode shaft was filled with 0.5 *M* KCl, with connection via a chlorided silver wire to a negative input capacitance FET preamplifier with 10^{12} Ω input resistance. Measurements of voltage were made relative to a chlorided silver wire in the slice bath and were compared with records taken either at the same time or separately, with conventional NaCl filled microelectrodes. All records were DC-coupled. The voltage recorded with the K^+-sensitive electrodes is the sum of (a) the electrical potential within the tissue, and (b) the electrode tip potential which is dependent on the external potassium concentration[18]. Double-barrelled electrodes can be used to derive the difference between the K^+-sensitive and conventional recordings at a single site[14,16]. But since slow potential changes were never observed with conventional NaCl electrodes in the present work at any of the sites studied, the interpretation of the slow potential changes seen with K^+-sensitive electrodes did not require either double-barrelled electrodes or critical positioning of a reference electrode.

The K^+-sensitive electrodes were calibrated (with reference to an NaCl pipette) in solutions of the same composition as the bath solution with added or subtracted KCl. The recorded voltage varies approximately linearly with the logarithm of the K^+ concentration over a wide range[14], and with our electrodes gave shifts in the range of 30–50 mV for a change in K^+ concentration from 1 m*M* to 10 m*M*. A figure of 40 mV per $\log_{10}$ unit is used for conversion of the observed shifts of voltage in the tissue to approximate values for increases of the K^+ concentration. The estimates for absolute increases in K^+ concentration can only be approximate, since in our experiments we were not able to obtain reliable estimates of the normal baseline K^+ concentration within the tissue; this was due to fluctuations in voltage which sometimes occurred for several minutes after an electrode was inserted. The normal K^+ concentration in the tissue is assumed to be the same as in the bath (6 m*M*), giving a calibration factor of 0.4 m*M* K^+/mV shift for small changes of $[K^+]$.

Examples of the records obtained with K^+-sensitive electrodes (K) and with conventional NaCl electrodes (V) are shown in Fig. 1B–D. The two electrodes were positioned within 100 μm of each other near the cell body layer of the dentate granule cells. With a stimulus (0.05 msec duration) delivered at a site (S1, Fig. 1A) appropriate for activating the cells via the excitatory perforant path fibres, both electrodes picked up the evoked potentials which are characteristic of this preparation when there is good activation of the granule cells[4] (upper frame, Fig. 1B). When the recorded potentials were examined with repetitive stimulation at 2/sec, slow positive deflections of the potential were seen with the K^+-sensitive electrode though not with the NaCl electrode (lower frame, Fig. 1B). Since these slow deflections were not seen in the conventional records at any sites within the tissue, they were taken to be attributable to increases of the K^+ concentration in the neighbourhood of the K^+-sensitive electrode tips. Various pieces of evidence supported this conclusion against the alternative

possibility of some kind of artefact. When the stimulation site was shifted back and forth between positions which did and which did not yield effective activation of the cells, the field potentials and the slow positive deflections came and went together (Fig. 1B–D). Both types of record were abolished when synaptic activation was blocked with a high Mg^{2+} concentration (25 mM) in the bath. And on occasions when field potentials but no slow positive deflections were recorded with high impedance resin-filled electrodes, it was generally found that the resin had withdrawn some distance up the electrode shank. Thus the recording of slow deflections appeared to require satisfactory activation of the cells and a properly functioning K^+-sensitive electrode.

Larger positive deflections were seen when an electrode was first lowered through the tissue than during withdrawal. This would be expected if the damaged tissue spaces around the electrode tip were larger on withdrawal. A study was made in three preparations of the way in which the potassium records depended on the depth of the recording site within the laminated structure, as the electrode was lowered into the slice perpendicular to the cell layers. Trains of stimulation at 2/sec were given and recordings were made at a series of increasing depths; the rises in the recorded voltage after 2 sec and 15 sec of continuous stimulation were plotted against depth (Fig. 2A, lower graphs). Sample records are shown in Fig. 2C. The cellular structures corres-

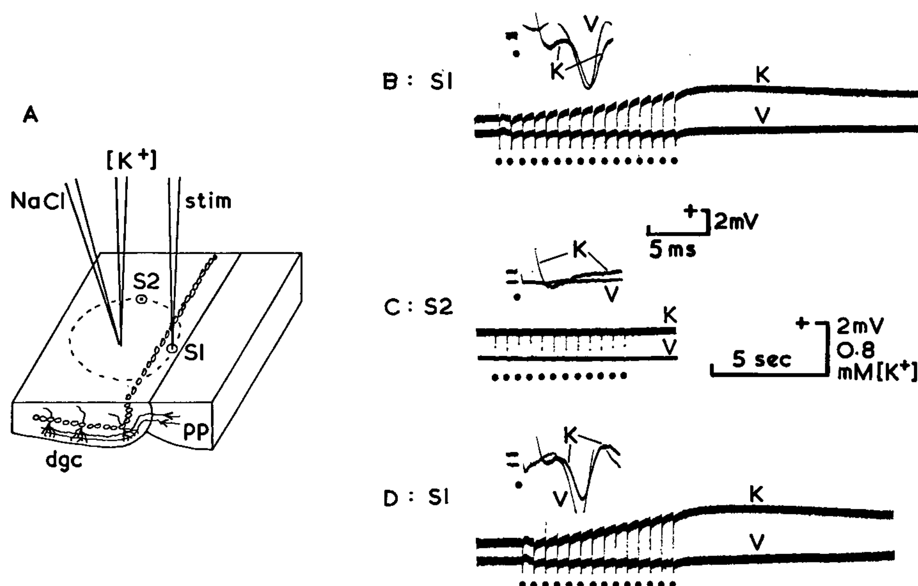

Fig. 1. Stimulation in effective and ineffective sites. A: diagram showing the positions of recording and stimulating sites on a slice. dgc, dentate granule cells; pp, perforant path fibres; S1, stimulation site for synaptic activation; S2, ineffective stimulation site for control records. B–D: records taken with stimulation at S1 (B), then at S2 (C), then again at S1 (D). Records from both K^+-sensitive electrodes (K) and NaCl-filled pipettes (V) are shown at a fast sweep speed following a single stimulus (upper traces) and at a slow sweep speed during a train of stimuli at 2/sec (lower traces). Calibrations for upper traces in C and lower traces in C also apply to upper and lower traces respectively in B and D. Dots indicate instants of stimulation.

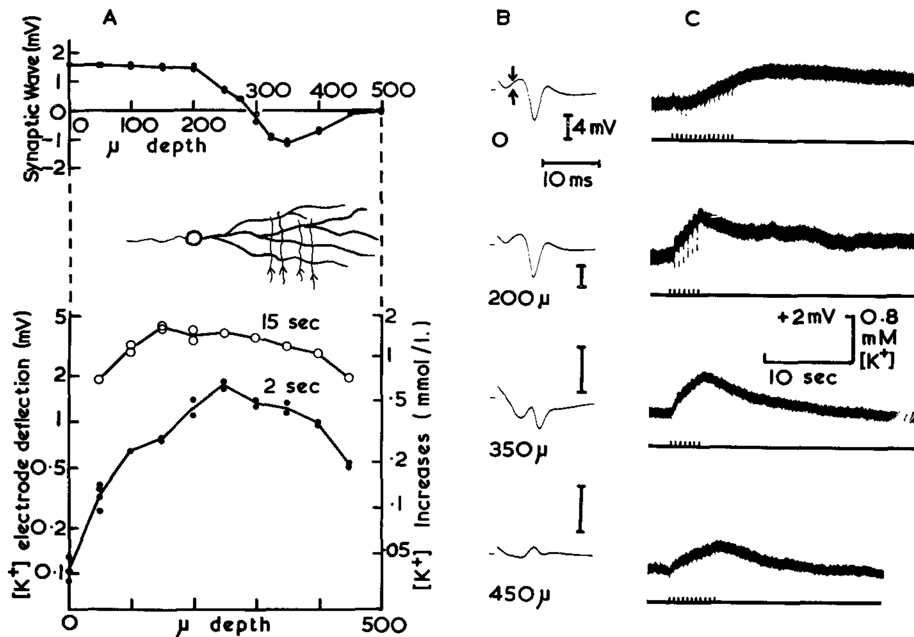

Fig. 2. Depth dependence of recordings. A: field potentials and K^+-sensitive deflections plotted against the depth of the recording electrode tips below the cut surface of a slice (Fig. 1A). Records were made during separate vertical penetrations at the same position. The synaptic wave field potentials are the voltage relative to the baseline recorded with an NaCl pipette 2.8 msec after stimulation (arrows in Fig. 2B). The K^+-sensitive traces were measured 2 sec and 15 sec after the first stimulus during a train of stimulation at 2/sec. The sketch shows the relation of the granule cell structures to the depths, inferred from the depth profile of the synaptic wave. B: records of the field potentials recorded at the specified depths. Vertical bars: 4 mV. C: K^+-sensitive records obtained at the specified depths with short stimulus trains. Calibration applies to all 4 records.

ponding to the different depths were inferred from the depth profile of the synaptic wave recorded on a subsequent penetration with an NaCl electrode in the same position[4,13] (Fig. 2A, top graph). The evoked potentials recorded with the NaCl pipettes returned to the baseline within 10–20 msec after each stimulus (Fig. 2B). The K^+-sensitive deflections recorded after only 2 sec of stimulation were seen to be largest (more than half their maximum size) over a range of about 300 μm depth, corresponding approximately to the total extent of the granule cells from the layer of cell bodies right through the molecular layer. After 15 sec of stimulation the K^+-sensitive deflections were larger and mor ewidespread (Fig. 2A). This may be attributable to diffusion.

The maximum values of the K^+-sensitive deflections seen after 15 sec stimulation at 2/sec were about 5 mV, corresponding to an increase of approximately 2 mM in K^+ concentration (from 6 to 8 mM). The maximum initial rates of rise of the K^+ deflections corresponded to about 0.5 mV per stimulus, or to rises of about 0.2 mM per stimulus. The return of the K^+ traces to baseline levels occurred slowly over a period of 10–30 sec after the cessation of stimulation. This recovery had a similar time course to the post-tetanic potentiation of the population spike seen in this preparation.

It is inferred from these results that significant changes in extracellular K^+ concentration do occur around the dentate granule cells after synaptic activation. The changes occur throughout the cell body and molecular layers of the dentate granule cells. There is evidence that in these cells action potentials may spread throughout the dendrites after synaptic activation[1,11,12]. Thus it is possible that both action potentials and the synaptic activity itself may contribute to the K^+ changes within the molecular layer.

The measured changes probably underestimate the true changes in K^+ concentration in undamaged interstitial spaces. Thus it can be inferred that changes of the order of several m*M* K^+ concentration may accompany repetitive activation. These changes may contribute to some of the after effects of repetitive stimulation with a time course of several seconds.

1 Andersen, P., Holmquist, B. and Voorhoeve, P. E., Entorhinal activation of dentate granule cells, *Acta physiol. scand.*, 66 (1966) 448–460.

2 Bliss, T. V. P. and Gardner-Medwin, A. R., Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetised rabbit following stimulation of the perforant path, *J. Physiol. (Lond.)*, 232 (1973) 357–374.

3 Bliss, T. V. P. and Lømo, T., Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path, *J. Physiol. (Lond.)*, 232 (1973) 331–356.

4 Bliss, T. V. P. and Richards, C. D., Some experiments with in vitro hippocampal slices, *J. Physiol. (Lond.)*, 214 (1971) 7P–9*P*.

5 Davson, H., *A Textbook of General Physiology*, Churchill, London, 1970.

6 Fisher, R. S., Pedley, T. A., Moody, W. J. and Prince, D. A., The role of extracellular potassium in hippocampal epilepsy, *Arch. Neurol. (Chic.)*, 33 (1976) 76–83.

7 Futamachi, K. J., Mutani, R. and Prince, D. A., Potassium activity in rabbit cortex, *Brain Research*, 75 (1974) 5–25.

8 Gardner-Medwin, A. R., Supernormality of cerebellar parallel fibres: the effects of changes in potassium concentration in vitro, *Acta physiol. scand.*, 84 (1972) 38A–39A.

9 Gardner-Medwin, A. R., The initiation of action potentials in hippocampal granule cells, *Proc. 7th Int. Meeting of Neurobiologists*, (1976) In press.

10 Grafstein, B., Mechanism of spreading cortical depression, *J. Neurophysiol.*, 19 (1956) 154–171.

11 Jefferys, J. G. R., Propagation of action potentials into the dendrites of hippocampal granule cells in vitro, *J. Physiol. (Lond.)*, 249 (1975) 16P–18P.

12 Jefferys, J. G. R., Dendritic conduction in hippocampal granule cells, *Proc. 7th Int. Meeting of Neurobiologists*, (1976) In press.

13 Lømo, T., Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation, *Exp. Brain Res.*, 12 (1971) 18–45.

14 Lux, H. D. and Neher, E., The equilibration time course of $[K^+]_o$ in cat cortex, *Exp. Brain Res.*, 17 (1973) 190–205.

15 Richards, C. D. and Sercombe, R., Electrical activity observed in guinea pig olfactory cortex maintained in vitro, *J. Physiol. (Lond.)*, 197 (1968) 667–683.

16 Vyskočil, F. and Kríž, N., Modifications of single and double-barrel potassium specific microelectrodes for physiological experiments, *Pflügers Arch. ges. Physiol.*, 337 (1972) 265–276.

17 Vyskočil, F., Kríž, N. and Bureš, J., Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats, *Brain Research*, 39 (1972) 255–259.

18 Walker, J. L., Ion specific liquid ion exchanger microelectrodes, *Analyt. Chem.*, 43 (1971) 89A–93A.

19 Yamamoto, C. and Kawai, N., Generation of the seizure discharge in thin sections from the guinea pig brain in chloride-free medium in vitro, *Jap. J. Physiol.*, 18 (1968) 620–631.