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# Differential effects of elevated extracellular calcium concentrations on field potentials in dentate gyrus and $CA_1$ of the rat hippocampal slice preparation

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The effect of a short-lasting elevation of the extracellular $Ca^{2+}$ concentration (from 2 to 6 mM) on the field potentials in $CA_1$ of the rat hippocampal slice was an increase of both the $CA_1$ population spike (PS) and the excitatory postsynaptic potential (EPSP); this effect persisted after returning to 2 mM $Ca^{2+}$ Ringer, and thus can be considered as a $Ca^{2+}$-induced long-term potentiation (LTP). In the dentate gyrus (DG) a quite different effect was encountered; here the PS decreased and the EPSP increased only slightly during the perfusion with 6 mM $Ca^{2+}$, and no reproducible long-term effect was induced. The results indicate that substantial differences exist in the balance between inhibitory and excitatory processes in the neural networks of the two hippocampal subregions; these differences are enhanced during perfusion with high $Ca^{2+}$, which induces LTP in $CA_1$ but not in the DG.

The crucial role of $Ca^{2+}$ in neurotransmitter release and in paired-pulse facilitation, especially at the neuromuscular junction [6, 8] but also in the squid giant synapse [10], is well recognized. The importance of $Ca^{2+}$ has been shown also in long-term potentiation (LTP), a relatively long-lasting form of synaptic plasticity (for a review see ref. 4). LTP is typically elicited by tetanic stimulation of afferents and is characterized by a facilitation of synaptic transmission that may last for several hours in e.g. hippocampal slices. The importance of $Ca^{2+}$ in the induction of LTP has been shown in a number of studies: (i) LTP cannot be elicited at low extracellular $Ca^{2+}$-concentrations [3]; (ii) the injection of a $Ca^{2+}$-chelating agent prevents LTP induction in the postsynaptic neuron [7]; (iii) an increased uptake and retention of $Ca^{2+}$ in hippocampal slices has been reported following LTP induction [2]; and (iv) Agoston and Kuhnt [1] have recently demonstrated an increase in $Ca^{2+}$ accumulation in synaptosomes prepared from slices in which LTP was elicited.

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In fact, LTP can be induced in the pyramidal cells of area $CA_1$ in the hippocampal slice simply by an elevation of the extracellular $Ca^{2+}$-concentration for some minutes [13]. It was to be expected that the effects of an elevated $Ca^{2+}$-concentration on neurons are general phenomena. However, pilot studies suggested that the effects of elevated $Ca^{2+}$ on the granule cells of the dentate gyrus (DG) were quite different from those reported for the $CA_1$ area [13]. To investigate this in more detail we have tested simultaneously, and in the same hippocampal slice, the effects of a rise in extracellular $Ca^{2+}$ on two subsystems, the pyramidal cells of area $CA_1$ and the granule cells of the DG. In this way we could show an additional physiological difference between the $CA_1$ area and the DG to add to previously reported ones [9, 11, 12]. Furthermore, it is demonstrated that the effects of an elevated $Ca^{2+}$-concentration on synaptic transmission have to be interpreted taking into consideration the particular network involved.

Hippocampal slices were made following standard procedures: rats (160–200 g) were decapitated after ether anesthesia, the brain was removed, rinsed immediately with ice-cold (0–4°C) Ringer solution (in mM: NaCl 124, KCl 5, $CaCl_2$ 2, $MgSO_4$ 2, $NaH_2PO_4$ 1.25, $NaHCO_3$ 26, glucose 10) and the hippocampi were prepared free. Transverse slices of 500 $\mu$m were cut manually with razor blades and stored at room temperature until used. One hour before the start of an experiment one slice was transferred to the recording chamber which was continuously perfused with Ringer, saturated with 95% $O_2$–5% $CO_2$ (2–3 ml/min) at 34–35°C. Stimulation electrodes (two trimel-insulated stainless-steel wires of 60 $\mu$m diameter) were placed under visual guidance both in the stratum moleculare of the DG, in order to stimulate the perforant path, and in the stratum radiatum of $CA_1$, in order to stimulate the Schaffer collaterals. For recording evoked field potentials in DG and $CA_1$, glass microelectrodes, filled with 3 M NaCl (resistance 3–5 M$\Omega$) were positioned in the stratum granulosum and stratum pyramidale. Every 15 s paired test stimuli (interval 20 ms) consisting of bipolar, biphasic current pulses of 0.2 ms duration were delivered via one of the stimulation electrodes. The current intensity was adjusted to 50% of the intensity that elicited a saturated response, and ranged between 50 and 150 $\mu$A. The field potentials were digitized at 4 kHz using a Motorola Exorset microcomputer. Series of 4 test-stimulus pairs were applied alternatively to the DG and $CA_1$; the corresponding field potentials were recorded, averaged and stored on a diskette. The 6 mM $Ca^{2+}$ Ringer was made by adding extra $CaCl_2$ to the perfusion Ringer solution and was perfused for a period of 20 min.

The observed changes in *$CA_1$* field potentials *during* the perfusion with 6 mM $Ca^{2+}$ Ringer are in good agreement with the report of Turner et al. [13]. The amplitude of both the population spike (PS) and the slope of the field excitatory postsynaptic potential (EPSP) in response to the first stimulus of each pair ($R_1$) increased gradually in 10 out of 11 slices. On average, the maximal rising slope of the EPSP increased up to $175 \pm 17\%$ (S.E.M.) of control level ($P < 0.01$, Wilcoxon's matched-pairs signed-rank test, two-tailed) and the PS up to $198 \pm 31\%$ of control level ($P < 0.01$). The perfusion with 6 mM $Ca^{2+}$ Ringer induced a clear LTP-like effect in $CA_1$: the average response amplitudes decreased only slightly after returning to

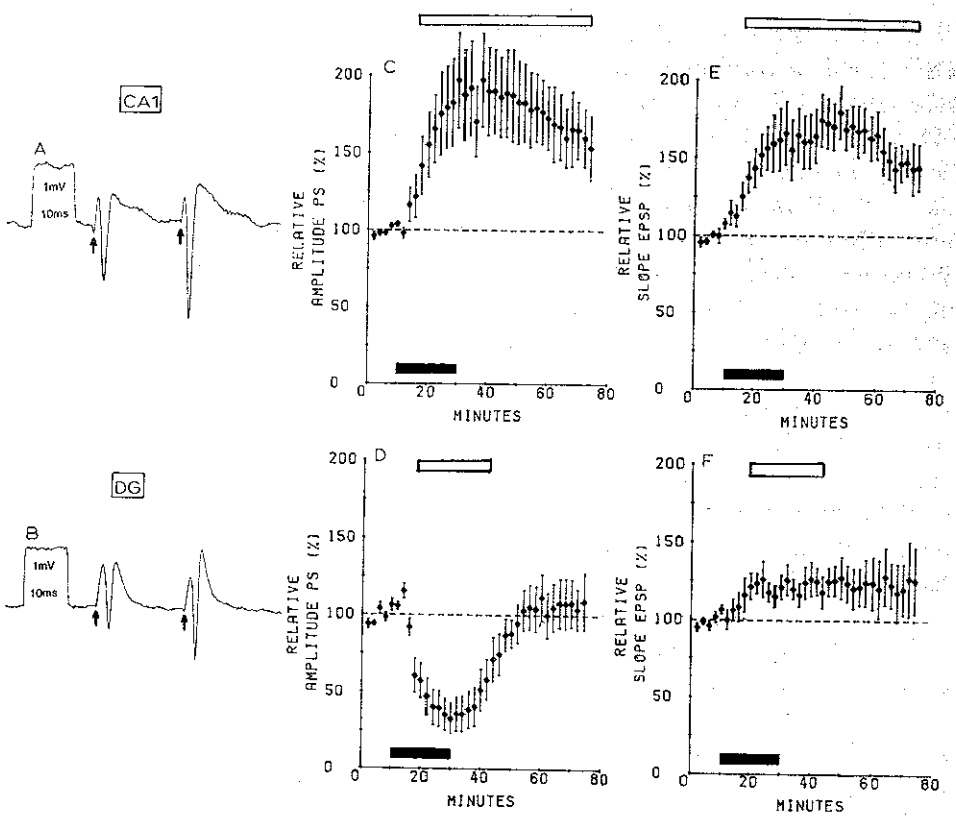


Fig. 1. Field potentials obtained in the stratum radiatum of $CA_1$ (A) and the stratum granulosum of the DG (B), during the control period. The arrows indicate the time points at which the paired stimuli were given to either the Schaffer collaterals or the perforant path. The PPF of the PS is clearly demonstrated. C–F: average of all slices ($n=11$) of the relative amplitude of the PS (C) and rising slope of the EPSP (E) of the $R_1$ in $CA_1$ and in the DG, respectively D and F. The black bar indicates the time during which 6 mM $Ca^{2+}$ Ringer was perfused. The white bar indicates the time range in which significant differences (Wilcoxon's matched-pairs signed-rank test, $P<0.05$) with respect to the control value (set at 100%) were found. The number of slices per point varied, due to the fact that not all slices were monitored during the whole period, but it was always at least 6. Note the increase in the amplitude of both PS and rising slope of the EPSP in $CA_1$ during and for a long period after the perfusion with 6 mM $Ca^{2+}$, whereas in the DG a decrease in PS was found and just a slight increase in EPSP.

2 mM $Ca^{2+}$, staying well above control level for at least 45 min (Fig. 1C, E). In 8 slices (two were only monitored before and during perfusion with 6 mM $Ca^{2+}$) the responses remained elevated during the whole recording period, which ranged from 15 to 180 min after returning to 2 mM $Ca^{2+}$. In 4 *additional* slices $CA_1$ field potentials were elicited only before and 25 min after the perfusion with 6 mM $Ca^{2+}$ Ringer, in order to check whether the series of test stimuli delivered throughout the experiment (as described above) had any effect on the $Ca^{2+}$-induced LTP. In all 4 slices a clear potentiation of both the EPSP and the PS was encountered, indicating that repeated afferent stimulation is not necessary for eliciting $Ca^{2+}$-induced LTP.

In the *DG* a clearly different effect was found. During the perfusion with 6 mM $Ca^{2+}$ Ringer a gradual *decrease* was found in the amplitude of the $R_1$ PS in all 11 slices reaching an average value as low as $33 \pm 10\%$ of control level ($P < 0.01$; Fig. 1D). After returning to 2 mM $Ca^{2+}$ the $R_1$ PS returned to a level which was not significantly different from control. The rising slope of the EPSP, in contrast, showed an increase – although less pronounced than in $CA_1$ – in 7 out of 11 slices (on average up to $127 \pm 10\%$ of control value ($P < 0.05$; Fig. 1F)). After returning to 2 mM $Ca^{2+}$ Ringer, the rising slope of the EPSP in $R_1$ remained elevated in 5 slices; in the remaining two slices the response returned to the control level or even lower. From 30 min after returning to 2 mM $Ca^{2+}$ the overall increase was no longer significant. In DG the long-term effects of the elevation of the extracellular $Ca^{2+}$ concentration were variable among different slices. In 4 slices both the PS and the EPSP showed a potentiation after returning to 2 mM $Ca^{2+}$, but in the remaining slices either no long-term effect or even a long-lasting decrease in the PS was found.

It should be noted that an increase in extracellular calcium can exert effects on both excitatory and inhibitory synapses. We therefore tested whether paired-pulse facilitation (PPF) of the PS, which depends at least partly on the relative strength of inhibitory processes, was altered during and/or after the perfusion with 6 mM $Ca^{2+}$. In the neuromuscular junction a decrease in PPF of the EPSP during elevated extracellular $Ca^{2+}$ levels has been reported [8]. In most slices during the control period, both in DG and $CA_1$, a PPF of the PS and of the EPSP in the response to the second stimulus of a pair ($R_2$) was encountered at the intensity and inter-stimulus interval (20 ms) used. During perfusion with 6 mM $Ca^{2+}$ Ringer the amount of PPF of the PS decreased in all 11 slices, both in the $CA_1$ and DG. This decrease was larger in DG than in $CA_1$ (the difference was significant at $P < 0.01$, Wilcoxon). After returning to 2 mM $Ca^{2+}$, the amount of PS PPF in $CA_1$ remained below control level in most slices; in the DG the PPF of the PS recovered to approximately control levels. The PPF of the EPSP also showed a decrease during perfusion with 6 mM $Ca^{2+}$ Ringer, both in the $CA_1$ and DG, and returned to the control level after switching to 2 mM $Ca^{2+}$ Ringer.

In discussing these findings the short- and the long-term (LTP) effects will be considered separately. The *short term* effects (i.e. *during* elevated $Ca^{2+}$) in $CA_1$ can be explained by taking into consideration that an increased extracellular $Ca^{2+}$ concentration is known to lead to an increase in transmitter release both at the neuromuscular junction [8] and in the squid giant synapse [10]. Our results would be explained if we assume that the same process takes place at the excitatory synapses of the Schaffer collaterals with the $CA_1$ pyramidal cells. The increase in $R_1$ PS indicates an enhancement of synchronous firing of these cells. In this way more interneurons, responsible for recurrent inhibition, may be recruited, which would account for the relative decrease of the $R_2$ PS (decrease in PPF) observed in $CA_1$. In the DG, however, different processes are apparently at work, since here the EPSP slope increases while the PS decreases. The former effect is of the same type as seen in $CA_1$ and may be explained in a similar way. The decrease in PS indicates a decrease in firing probability of the granule cells in contrast to that of the pyramidal cells. Two explanations

may be advanced for this effect of the elevated extracellular $Ca^{2+}$ concentration: (i) it could exert a stabilizing effect on cell membranes [5] and thus elevate the firing threshold, but it is unlikely that this effect would appear only in the DG and not in $CA_1$; (ii) it could elicit an increase of hyperpolarizing potentials to a larger degree in the DG than in $CA_1$. The latter possibility is more plausible since a number of experimental findings suggest that $CA_1$ and DG differ in excitatory/inhibitory circuits. Tielen et al. [12] have shown that Met-enkephalin can cause an increase in PS amplitude in $CA_1$ and at the same time, a decrease in PS in the DG. Furthermore, the DG may possess a larger amount of inhibitory control than $CA_1$, as suggested by GABA-receptor binding studies [9]. In addition LTP appears to be more readily elicited (after a tetanus) in $CA_1$ than in the DG; this difference can be neutralized by a blockade of the $Cl^-$-mediated inhibition [14]. An increase in inhibitory processes in the DG by an elevated $Ca^{2+}$ concentration would also explain the decrease in PPF in the DG during elevated $Ca^{2+}$, in spite of the decrease in the $R_1$ PS.

Therefore, the *short-term* effects can be accounted for by postulating that the DG has a larger inhibitory 'reserve' than $CA_1$. We assume that elevated extracellular $Ca^{2+}$ would lead to an enhancement of both excitatory and inhibitory synaptic activity. The net effect would depend on the dynamic equilibrium between inhibitory and excitatory processes within the subregion. The *long-term* effect seems to be in agreement with these considerations. The difference between the $CA_1$ and DG in this respect can be explained by a disproportionate potentiation of inhibitory processes versus excitatory processes within the DG. This leads even to an apparent decrease in the DG field potentials in some slices. In $CA_1$, however, a reproducible LTP was induced, even if the slices were not stimulated during the period of elevated $Ca^{2+}$.

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