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Research Note

Long-term depression at synapses in slices of rat hippocampus can be induced by bursts of postsynaptic activity

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Summary. In slices of rat hippocampus, a train of conditioning pulses that would produce long-term potentiation (LTP) if applied to afferent inputs was found to produce a long-lasting depression of Schaffer collateral/commissural synapses on CA1 cells when instead it was applied to the CA1 axons. The depression lasted undiminished for up to 2 h (the maximum duration of recording). Intracellular recording showed that long-term depression (LTD) of e.p.s.p. amplitude occurred in 66% of cells when this antidromic conditioning stimulation was delivered in normal medium, and in 100% of cells when the antidromic stimulation was delivered in medium containing sufficient Mg^{++} to block all synaptic transmission. We infer that the difference is because conditioning stimuli sometimes activated test synapses in normal Mg^{++} but could not in high Mg^{-+}. The fact that LTD could be induced in high Mg^{++} eliminates enhanced inhibitory feedback as a possible mechanism of the long lasting synaptic depression and demonstrates that the mechanism is probably postsynaptic. Resting membrane potential and cell input resistance were the same before and after conditioning, so persisting changes in these postsynaptic parameters can not be the explanation for LTD. LTD of the sort described in this paper could have significant implications for models of learning and memory.

Key words: Long-term depression – Longterm potentiation – Hippocampus – Rat

Introduction

Long-term potentiation (Bliss and Lømo 1973) is a long-lasting increase in the efficacy of synapses caused by a brief burst of high-frequency activity through these synapses. The potentiation is confined to the synapses activated and other synapses on the same cell are subject to a long-lasting heterosynaptic depression (Lynch et al. 1977; Bliss et al. 1983; King and Levy 1986). Abraham and Goddard (1983) suggest that this heterosynaptic depression may reflect a generalized activity-dependent reduction in synaptic efficacy, with potentiation overpowering depression only for the tetanized inputs. The idea of a generalized postsynaptically mediated depression is suggested by the experiments of Pockett and Lippold (1986, 1988) who showed that high frequency antidromic stimulation of CA1 cells, in hippocampal slices bathed in a high concentration of magnesium to block synaptic transmission during the tetanus, caused a long-lasting depression of synaptic potentials. In experiments described in this paper we used intracellular recording techniques to extend the initial extracellular observations and hence are able to eliminate some possible mechanisms for the effect.

Methods

Transverse slices approximately 400 μm thick from adult rat hippocampus were prepared and maintained in a submerged-slice recording chamber at 34 °C. The slices were superfused with artificial cerebrospinal fluid of composition: NaCl, 124 mM; KCl, 4.5 mM; NaH_2PO_4, 1.25 mM; $MgCl_2$, 1 mM; $NaHCO_3$, 20 mM; glucose, 10 mM; $CaCl_2$, 2.5 mM; bubbled with 95% O_2 and 5% CO_2 (Andersen et al. 1987). In high magnesium solutions the composition was the same except that $MgCl_2$ was increased to 25 mM and NaCl was reduced to 88 mM to maintain osmolarity. In a few experiments, 100 μM bicuculline methiodide was added to the artificial cerebrospinal fluid.

CA1 neurons were penetrated with glass microelectrodes filled with 3 M KCl or 4 M KAcetate and having resistances of 60 to 160MΩ. On penetration, neurons were hyperpolarised with 0.5 to 1.0 nA of current for 5 to 10 min until the resting potential had stabilised. Excitatory postsynaptic potentials (referred to as "test" e.p.s.p.s) were recorded in response to stimulation of the Schaffer collateral/commissural pathway with a monopolar tungsten electrode once every 20 s. In order to obtain e.p.s.p.s of 6–10 mV with-

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out evoking action potentials in the postsynaptic cell, 0.1 to 0.4 nA of hyperpolarising current was often passed into the cell for the duration of the experiment, resulting in measured resting membrane potentials of −75 to −90 mV. Bridge balance was monitored using 100 ms pulses of hyperpolarising current delivered through the recording electrode. Apparent input resistance was monitored at intervals throughout the recording period, also using 100 ms pulses of current. Excitability of neurons was measured by the number of action potentials firing in response to a 100 ms injection of 0.2 nA depolarising current into the neuron. Antidromic spikes and sometimes postsynaptic potentials were evoked by bipolar constant current stimuli delivered though a tungsten electrode positioned at the alveus/oriens border, on the opposite side of the recording electrode from the Schaffer collateral/commissural stimulating electrode. In some experiments conditioning trains of 6 bursts of 50 0.1 ms bipolar pulses at 100 Hz, delivered at 10 s intervals, were applied through this electrode. An antidromic action potential followed each of these pulses in most cases.

Data was collected on FM tape, then digitised and analysed off-line on an IBM XT computer, with software developed from the commercial ASYST package (Macmillan Software Company). A frequency analysis performed on the data indicated that filtering at 500 Hz was appropriate to remove noise without affecting the signal, so the data from tape was filtered with a low pass Low Q filter at 500 Hz before digitisation. Amplitudes of single e.p.s.p.s and the voltage responses to applied hyperpolarising current pulses were measured at predetermined latencies and plotted against time using LOTUS 1-2-3 software, then saved in ASCII format and sent via MACLINK to an Apple Macintosh for statistical analysis with the STATVIEW package. Simple linear regressions were performed on all control data, to test for the stability of e.p.s.p. amplitude over time. The regression lines were *t*-tested for zero slope and data from any experiment where the e.p.s.p. declined or increased in amplitude during the initial control period of 10–15 min were disregarded. The result of any experimental manoeuvre (e.g. application and washout of Mg^{++}, or application of antidromic conditioning trains) was assessed by applying a Mann-Whitney *U* test to 30 measurements of e.p.s.p. amplitude made before the manoeuvre and 30 measurements made after the manoeuvre. All tests were performed at the 95% level of significance. Given the high resistance of the recording electrodes used and the fact that tip potential sometimes changed while the electrode was in the cell, it was deemed unwise to place too much reliance on resting potential measurements. However, data from cells where the e.p.s.p. amplitude fell at the same time as a sudden, large, apparent depolarisation of the cell membrane occurred were disregarded.

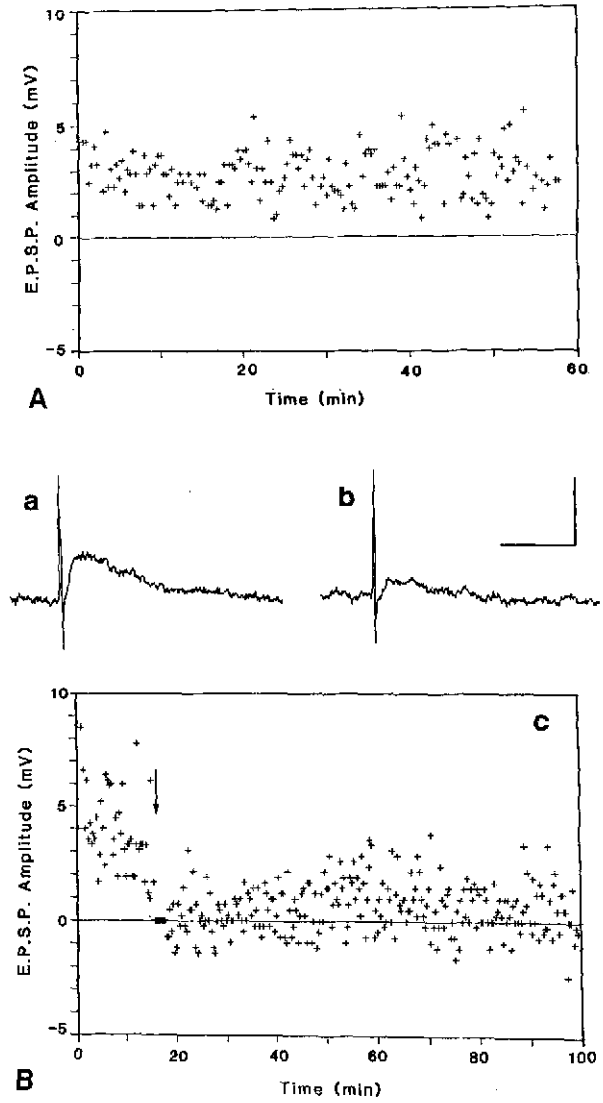

Fig. 1A, B. Effect of antidromic stimulation on e.p.s.p.s in CA1. **A** Control recording of e.p.s.p. amplitude. Each cross represents the amplitude of a single compound e.p.s.p., evoked at 0.05 Hz, recorded intracellularly, all from the same CA1 cell, to show variability over the course of 1 h. **B** Effect on e.p.s.p amplitude of antidromic conditioning stimulation in normal medium. **a** Average of 10 successive e.p.s.p.s before conditioning. **b** Average of 10 successive e.p.s.p.s after conditioning Vertical bar = 5 mV, horizontal bar = 50 ms, these apply to both **a**, and **b**. **c** Each cross represents the amplitude of one e.p.s.p. recorded intracellularly from a CA1 pyramidal cell. Arrow indicates the time of delivery of the antidromic conditioning stimuli. Some e.p.s.p.s are apparently negative because of the way they are measured: the potential at a baseline point is subtracted from the value at the peak of the e.p.s.p. for each sweep, therefore any noise or spontaneously occurring potentials at either of these points will lead to increased variance in the measurement of e.p.s.p. amplitude

Results

The experiments were carried out on a total of 33 CA1 neurons, mean resting membrane potential -71 mV $\pm$ 3.4 mV; mean input resistance 52.7 $\pm$ 2.6 MΩ, mean spike amplitude 81.4 $\pm$ 1.3 mV, ($\pm$S.E.M.) in which e.p.s.p.s satisfied the criteria for stability set out in Methods. The mean recording time in these cells was 1.7 $\pm$ 0.12 (S.E.M.) h.

Control recordings

To see whether any generalised depression of function leading to a depression of test e.p.s.p. amplitude over a lengthy recording period was a common occurrence under our recording conditions, we recorded e.p.s.p.s and input resistance from 8 CA1 neurons for periods ranging from 30 min to 120 min, without any experimental manipulation. In 5 neurons the e.p.s.p. amplitude and input resistance were stable for the duration of the recording period (example shown in Fig. 1A). In the other three neurons the resting potential stayed approximately constant and the input resistance was stable but the e.p.s.p. amplitude rose, by between 25% and 75% of the original amplitude.

Effect on test e.p.s.p. amplitude of antidromic conditioning stimulation in normal medium

When 6 bursts of 50 bipolar stimuli at 100 Hz were delivered to the alveus/oriens border on the opposite side of the CA1 cell from the orthodromic stimulating electrode, a variety of changes in e.p.s.p. amplitude resulted. In 6 of 9 cells with stable resting membrane potential and stable baseline e.p.s.p. amplitude, the e.p.s.p. amplitude was significantly depressed following the antidromic conditioning stimulation. The depression always lasted for the duration of the recording period, which ranged from 8 min after conditioning to 85 min (mean 32 ± 8 min). The depression varied in magnitude, resulting in e.p.s.p.s that were between 47% and 14% of the amplitude of test e.p.s.p.s during the control period. An example is shown in Fig. 1B. In one of the nine cells conditioning stimuli had no effect on e.p.s.p. amplitude. In the remaining two cells, e.p.s.p. amplitude was potentiated by the conditioning. In one case there was potentiation to 135% of control e.p.s.p. amplitude, which declined to control levels after 20 min and in the other there was potentiation to 161% of control amplitude after 40 min.

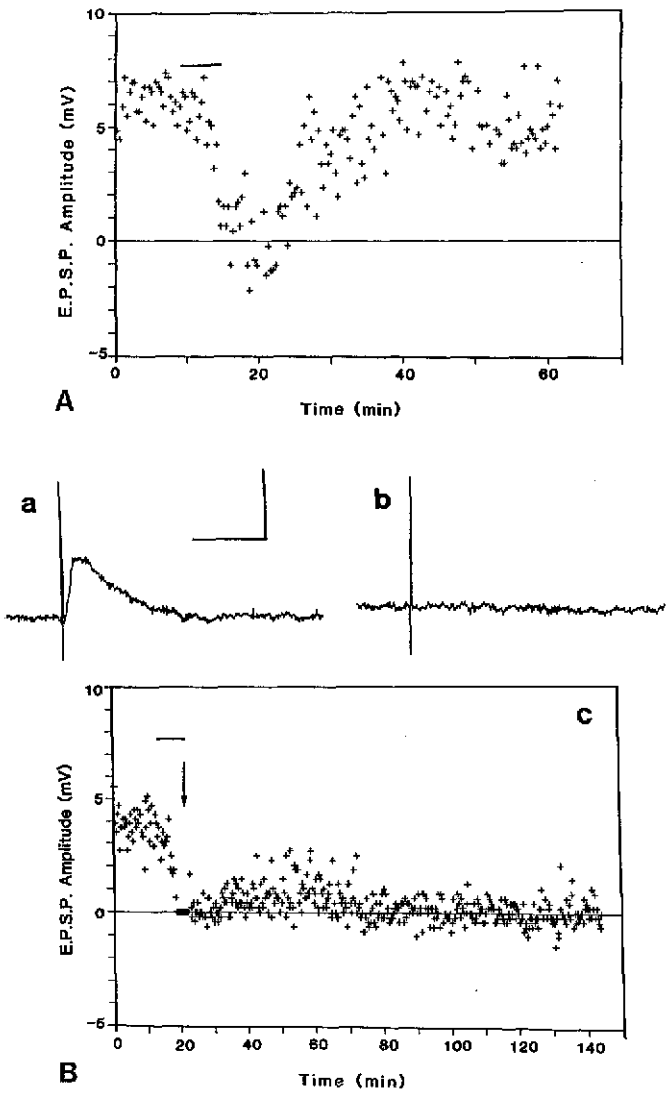

Fig. 2A, B. Effect on e.p.s.p. amplitude of antidromic stimulation carried out in the presence of synaptic block. **A** Effect of application and washout of 25 mM Mg^{++} on e.p.s.p. amplitude. Each cross represents the amplitude of one e.p.s.p. recorded intracellularly from a CA1 pyramidal cell. The horizontal bar indicates the period that the slice was bathed in high Mg^{++} artificial cerebrospinal fluid. **B** Effect of antidromic conditioning stimuli applied in the presence of 25 mM Mg^{++} on e.p.s.p. amplitude. **a** Average of 10 successive e.p.s.p.s recorded before conditioning. **b** Average of 10 successive e.p.s.p.s recorded after conditioning. Vertical bar = 5 mV Horizontal bar = 50 ms. Both bars apply to both **a**, and **b**. **c** Each cross represents the amplitude of one e.p.s.p. recorded intracellularly from a CA1 pyramidal cell. Arrow indicates the time of delivery of the antidromic conditioning stimuli. Horizontal bar indicates the period the slice is bathed in high Mg^{++} artificial cerebrospinal fluid

Effect on test e.p.s.p. amplitude of antidromic conditioning stimulation in high magnesium medium

The aim of this set of experiments was to examine the effects of antidromic conditioning stimulation delivered in the absence of any synaptic transmission. Reasons for delivering the antidromic tetanus in conditions which prevented synaptic transmission were a) to prevent antidromic stimuli from activating the test synapses (which would presumably induce LTP), by back conduction up axons which had dropped en passage synapses on the CA1 cell recorded from; and b) to eliminate activation of a long lasting form of feedback inhibition as a possible mechanism of depression.

The experiments were conducted in two parts. First, controls were done to see how consistently it was possible to superfuse the preparation with a high enough concentration of Mg^{++} to eliminate all synaptic transmission and then wash it off and regain an undiminished e.p.s.p. amplitude on restoration of normal Mg^{++} concentration. These controls also showed how long it took to completely wash out the Mg^{++} effect. Secondly the same experiment was performed and in addition antidromic conditioning stimuli were applied when the high Mg^{++} had eliminated synaptic transmission. The Mg^{++} was then washed out and the amplitude of test e.p.s.p.s measured.

Individual antidromic stimuli often elicited a synaptic response as well as an antidromic action potential. This synaptic event was always depolarising when the CA1 cells had been penetrated with microelectrodes filled with KCl. It could be distinguished from a depolarizing after potential because it had a different threshold from the spike. To ascertain whether it was an e.p.s.p. or a Cl^- mediated i.p.s.p., we carried out two tests: 1) In 5 cells tested with 100 μM bicuculline methiodide the synaptic potential was unaffected once, obliterated three times and halved in amplitude once. 2) With 3 M potassium acetate in the microelectrode, this synaptic event was observed as an i.p.s.p. with reversal potential at about −70 mV. Thus conditioning stimuli could evoke Cl^- mediated i.p.s.p.s and/or other synaptic events in addition to antidromic action potentials. The

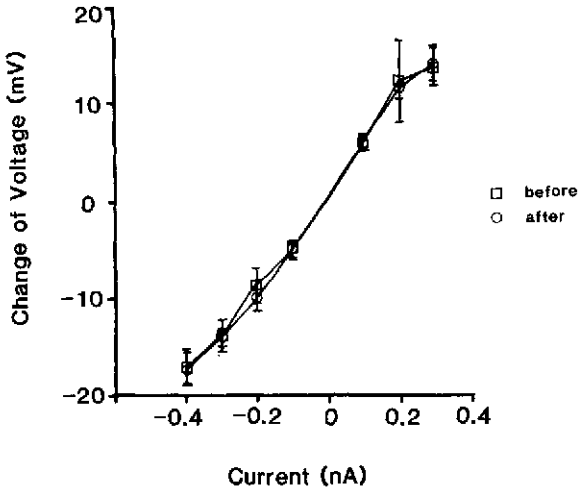

Fig. 3. Lack of effect of conditioning on input resistance of CA1 cell. 100 ms pulses of current were injected into cells before application of 25 mM Mg^{++} and conditioning stimuli and after a time allowing complete washout of 25 mM Mg^{++}, and the voltage responses recorded. Points show mean ± 1SD for 7 cells in which conditioning caused depression of e.p.s.p. amplitude. Open squares -before conditioning. Open diamonds – after conditioning

synaptic response evoked by alveus/oriens stimulation always took longer to disappear in 25 mM Mg^{++} than the Schaffer collateral/commissural e.p.s.p., so its disappearance was used as a criterion for elimination of synaptic activity by Mg^{++}.

In 6 control experiments where resting potential and baseline e.p.s.p. amplitude were constant, 25 mM Mg^{++} completely eliminated synaptic transmission and test e.p.s.p. amplitude was completely restored after Mg^{++} washout (no significant difference, statistics performed as in Methods) in all cases. An example is shown in Fig. 2A. In every case the test e.p.s.p. had completely recovered by 15 min after beginning of Mg^{++} washout.

In further experiments, antidromic conditioning stimuli were applied as soon as 25 mM Mg^{++} had eliminated synaptic transmission and then the Mg^{++} was washed out. In all of the 11 cells where the resting membrane potential remained stable throughout the experiment and the baseline pre-conditioning e.p.s.p. amplitude was stable for 10 min, conditioning caused a significant depression of test e.p.s.p. amplitude which persisted after the 15 min Mg^{++}-washout period. An example is shown in Fig. 2B.

In 8 of the 11 cells the depression lasted unchanged for the duration of the recording period: more than 60 min post-washout in 4 cases and 15, 30, 40 and 45 min post-washout in the other 4 cases. The average depression in these 8 cells was 66% ± 4.3 (mean ± S.E.). i.e., the post-conditioning e.p.s.p.s were 33% as big as the preconditioning e.p.s.p.s at the end of the recording period.

In 3 of the 11 cells the depression was relatively short-lasting, with e.p.s.p. amplitude returning to preconditioning values by 20, 30 and 60 min respectively after the 15 min washout period.

Effect of conditioning on input resistance and excitability

The input resistance of the CA1 cells was unaffected by conditioning stimuli. Measurements of input resistance were made in all of the three cells where conditioning in the presence of 25 mM Mg^{++} caused relatively short-term depression of e.p.s.p. amplitude and 4 of the 8 cells where it caused longer term depression. Current/voltage plots representing the mean of all data are shown in Fig. 3.

Excitability was either unaffected or slightly increased by antidromic conditioning. In 9 neurons showing tetanus-induced synaptic depression, there was no tetanus-induced change in excitability in 6 neurons and a small increase (from 2 spikes in response to injection of 100 ms 0.2 nA depolarising pulse before conditioning to 3 spikes after) in 3 neurons.

Discussion

We have shown that backfiring of a CA1 cell by stimulation of its axon with a pattern of impulses that would cause LTP if delivered through a synaptic pathway, causes a long-lasting depression of efficacy of synaptic transmission onto the CA1 cell. This work confirms and extends the findings of Pockett and Lippold (1986, 1988). When the backfiring took place in normal medium, long-term synaptic depression was observed in 66% of CA1 cells and there was no effect or long-term synaptic potentiation in the other 33%. It is probable that the LTP occurred in these cases because the conditioning stimuli activated the test synapses (by backfiring) as well as the CA1 axons, since when the conditioning stimuli were delivered in a high Mg^{++} solution, which eliminated synaptic transmission, a long-lasting depression of the test synaptic response occurred in all cases.

The synaptic depression was observed as a significant decrease in intracellularly recorded e.p.s.p. amplitude. This decrease was not likely to be due to a generalised decline in the in vitro preparation, since in 8/8 cases where no conditioning stimuli were delivered, e.p.s.p. amplitude either stayed constant or increased during the 0.5 to 1.5 h recording periods. (The increase in e.p.s.p. amplitude evoked at 0.05 Hz in 3 of the 8 control neurons is unexplained but, while interesting in itself, poses no problems of interpretation for the experiments where conditioning stimuli caused e.p.s.p. depression, since the change observed in the control would tend to counteract rather than augment the change in the experimental case.) Neither was the synaptic depression described here an artefact of incomplete replacement of high Mg^{++} solution with normal Mg^{++} concentrations, since in all of 6 control experiments where resting potential and baseline e.p.s.p. amplitude were constant, high Mg^{++} washout completely restored e.p.s.p. amplitude. Thus the synaptic depression observed appears to be genuinely a consequence of the antidromic conditioning stimuli.

The long-term depression is not secondary to a generalised decrease in input resistance of the CA1 cells, as our observations show that input resistance does not

change after conditioning. This result is as expected from evidence that LTP (which often involves firing of postsynaptic cells with a pattern of activity similar to ours as part of its induction) does not alter input resistance of postsynaptic cells in CA1 (Andersen et al. 1977, 1980; Barrionuevo and Brown 1985; Bliss et al. 1987) or CA3 (Barrionuevo et al. 1986). Nor is the synaptic depression described in this paper a particularly long-lasting form of feedback inhibition (i.e. LTP of feedback inhibitory synapses), since the conditioning works even when it is delivered in the absence of any synaptic transmission.

An obvious next step in this study is to see whether intracellular conditioning stimulation will mimic the effects of antidromic conditioning stimulation. Meanwhile, we report our observations that bursts of postsynaptic activity induce long-term depression of synaptic inputs, since the phenomenon may well be important for understanding the modulation of synaptic efficacy in the hippocampus. We speculate that LTD of the sort described in the present paper would have a number of effects. For example, it would serve to enhance the relative efficacy of synapses being potentiated during LTP, by depressing the efficacy of surrounding inactive synapses on the same cell. It would also (assuming that prior LTP does not protect against LTD) prevent repeated LTP from saturating a synapse, by ensuring that the efficacy of the synapse is turned down every time other synaptic pathways onto the same postsynaptic cell are potentiated. This latter point makes it difficult to conceive of LTP per se as being the permanent memory store.

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