Long-term Potentiation and Intrinsic Disinhibition

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ABSTRACT

Long-term modulation of neuronal excitability through regulation of inhibitory potassium channels is presented as a mechanism for at least one component of LTP in the hippocampus. Currents flowing through these channels can normally restrict depolarizations. A release from this restriction, an "intrinsic disinhibition" potentiates excitatory signals. Experimental evidence indicates that functional potassium currents are necessary for LTP induction. A reduction of the transient A-current is unlikely to be the cause of LTP. Histamine, noradrenaline and cyclic AMP (blockers of a Ca-activated K-current, IAHP) can mimic the epsp/spike potentiation which represents a postsynaptic component of LTP. An endogenous action of these amines is however not necessary for the induction of LTP.

KEYWORDS

Long-term potentiation, hippocampus, intracellular recording, intrinsic disinhibition

INTRODUCTION

Long-term potentiation (LTP) in the hippocampus has received considerable attention because of its possible involvement in learning and memory processes (Bliss and Lomo, 1973; Andersen et al, 1980; McNaughton, 1983; Krug et al, 1984; Reymann et al, 1982; Voronin, 1983). The mechanism of this synaptic plasticity is unknown. It is characterized by a long lasting (hours to days) facilitation of excitatory transmission following brief tetanic stimulation of afferent fibers. Good evidence for changes on both, pre- and postsynaptic sites has been presented. Intracellular recording with K-acetate filled electrodes usually reveals only a small epsp increase during LTP but a marked increase in the probability of firing and a decrease in spike latency (Andersen et al, 1980; Haas and Rose, 1982, 1984). With K-chloride filled electrodes, however, when the typical epsp - ipsp sequence is almost entirely depolarizing (through shift of the chloride equilibrium potential) significantly larger epsp increases were observed during LTP. This

suggests that synaptic inhibition is not reduced, but may in fact be enhanced during LTP (Haas and Rose, 1984). Apart from the synaptic recurrent and feed foreward inhibition (Alger and Nicoll, 1982; Andersen et al, 1964), intrinsic inhibitory mechanisms are also operating in pyramidal cells (see Fig. 1). Alkon (1984) and his coworkers have indeed provided a good case for a biophysical memory trace through reduction of ionic currents in the gastropod Hermissenda. Activation of voltage- and calcium dependent potassium currents normally restricts depolarizing signals, produces afterhyperpolarizations (AHPs) and an accommodation of firing in response to prolonged (0.5-1 s) depolarizations (Alger and Nicoll, 1980; Schwarzkroin and Stafstrom, 1980; Wong and Prince, 1981; Brown and Griffith, 1983; Madison and Nicoll, 1982; Haas and Konnerth, 1983; Haas 1984). Among the potassium currents described in CA 1 pyramidal neurones (Adams and Galvan, 1986) at least three (IK, IC, IA) could interfere with epsps. Tetraethylammonium (TEA), 4-aminopyridine (4-AP), caesium and lithium ions (Haas, 1982) which can block these currents enhance epsps. Noradrenaline and histamine, which block the long lasting calcium activated potassium current (IAHP) in hippocampal pyramidal cells have been shown to prolong LTP (Hopkins and Johnston, 1983; Bliss et al, 1983; histamine: own unpublished result). Although these amines are not directly increasing epsps they enhance the population spike evoked by a given epsp for prolonged periods of time and could therefore be involved in the postsynaptic epsp versus spike potentiation. A reduction in such intrinsic inhibitions could be important in the production, modulation and maintenance of LTP and the present experiments are designed to test their involvement in this phenomenon.

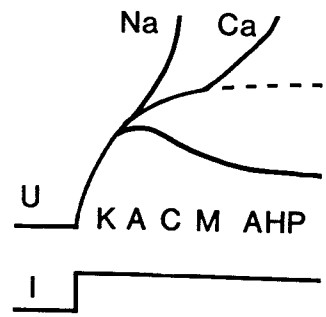

Fig. 1.

Schematic diagram depicting the voltage response (U) of a hippocampal pyramidal cell to depolarizing current injection (I). The passive reponse is indicated by a brocken line. In reality the response deviates from this theoretical curve because, depending on voltage level and intracellular calcium concentration, several inward (Na, Ca) or outward potassium currents (K, A, C, M, AHP) are activated. The latter are inhibitory and restrict normally excitatory potentials. Some of these currents are under the control of neurotransmitters or -modulators and linked to intracellular events, second messengers and energy state.

METHODS

Transverse slices were prepared from the hippocampi of rats and incubated in a perfusion chamber modified from our original design

(Haas et al. 1979) to reduce the volume to about 50 ul and allow rapid under- and superfusion with artificial cerebrospinal fluid. Drugs were added to this medium and equilibrated within less than 1 min in the chamber. For some experiments a larger double chamber which contained upto ten slices per compartment was used. A stimulating electrode was placed in the stratum radiatum of CA 1 region where the Schaffer collaterals and commissural fibers make excitatory contacts with the apical dendrites of pyramidal cells. An extracellular electrode monitored the field excitatory postsynaptic potential (epsp) in stratum radiatum or the population spike in the pyramidal layer. Intracellular recording from pyramidal cells was performed using electrodes filled with 2M KCl or CsCl. Resting potentials were more negative than 60 mV, spikes larger than 80 mV and input impedances higher than 40 megaohms.

RESULTS

In a first series of experiments KCl filled electrodes were used for recording. Accommodation of firing during a long depolarizing pulse (500 msec), and the afterhyperpolarization after a short burst (100 msec) were compared before and at least 10 min after afferent tetanization by 4 trains of 500 msec duration and 100 Hz. Although a lasting increase of the depolarizing potential was always observed (Haas and Rose, 1984) no change was found in input impedance, accommodation or the AHPs. These phenomena are measured at the cell body while the changes responsible for LTP are localized to dendritic sites at least 100 micrometers away. Similar experiments on dendrites are impracticable at present. Caesium ions from a CsCl filled electrode however are presumably diffusing out to these sites where they could block inhibitory potassium conductances.

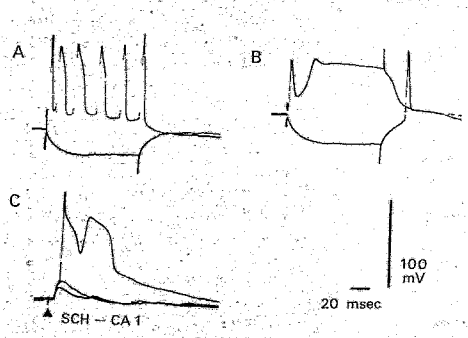

Fig. 2.

Intracellular record from a hippocampal pyramidal cell with a caesium filled electrode. A: 3 min after impalement, B: 10 min later, cell is partially depolarized. A and B are superimposed sweeps illustrating responses to ± 0.5 nA current injection. C: The cell was repolarized by continuous current injection, and the response to afferent synaptic stimulation is shown (3 superimposed sweeps). All records in this and the following figures are from the CA1 area of the rat hippocampal slice (in vitro). Negative is always downwards.

A few minutes after impalement of a pyramidal cell with a CsCl filled electrode action potentials became wider and the input impedance increased (Johnston et al, 1980) (Fig. 2A). Chloride dependent ipsps became reversed and the cells depolarized (Fig. 2B). The AHPs were replaced by an afterdepolarization. In this situation synaptic stimulation always caused the firing of action po-

tentials, obscuring the synaptic potentials. Continuous negative current injection was therefore used to keep the cells in a polarized state (Fig. 2C) and occasional depolarizing pulses were given to monitor the constancy of the caesium effects. Synaptic potentials were now evoked every 10 sec and, after at least 20 min of stable recording, 4 short tetani (500 msec, 100 Hz) were applied. In 12 cells it was possible to record a reasonably sized synaptic potential without firing for at least 15 min before and 15 min after the induction of LTP. The dendritic field always showed a lasting increase but none of the intracellularly recorded synaptic potentials was enhanced. The field epsps, measured at a fixed time after the stimulus, increased on the average (n=7) from 1.04 ± 0.18 (SD) mV to 1.46 ± 0.24 (SD) mV while the concomitant intracellular potentials (resting potential to peak) decreased from 9.16 ± 3.5 (SD) mV to 7.57 ± 4.1 (SD) mV. Fig. 3. illustrates one of these experiments. Posttetanic potentiation (PTP) and paired pulse facilitation were observed in both the intracellular and the extracellular record. The failure to elicit LTP in caesium loaded hippocampal pyramidal cells in the present experiments suggests that functioning potassium currents are necessary for the induction of LTP. One way to test this possibility further is the investigation of the interference with LTP of drugs which block various potassium currents.

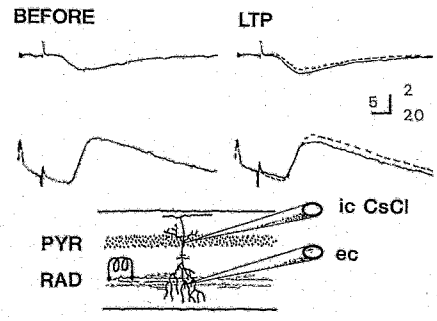

Fig. 3.

Synchronous extra- and intracellular recording from the dendritic area (upper traces, ec) and the cell body of a CA 1 pyramidal cell (lower traces, ic). Intracellular recording with a caesiumchloride (CsCl) filled electrode which blocks several potassium currents. These traces begin with a hyperpolarizing current injection to prevent the cell from firing an action potential in response to synaptic stimulation. Right traces were taken 15 min after LTP induction (broken lines: before). While the dendritic field is enhanced the intracellularly measured epsp is reduced (averaged record showed it not significantly altered). Thus LTP was evoked in the neighbouring cells but not in the one with K-channels blocked by caesium Calibration: 5 msec, 2 and 20 mV.

The A-current

A good candidate current possibly involved in long-term modulation of excitatory signals is the transient A-current (IA). It has been identified in invertebrates as an early, transient po-

tassium current distinct from both the calcium activated IC and the delayed rectifier (IK, Thompson, 1977) and is present in hippocampal pyramidal cells (Gustaffson et al, 1982). IA can be rapidly activated from a resting membrane potential of about 70 mV by less than 20 mV depolarizations. In fact, when it is blocked by 4-aminopyridine (4-AP) psps are enhanced, probably as a result of its actions on both, pre- and postsynaptic elements (Buckle and Haas, 1982; Haas et al, 1983). A long-term reduction of IA has been found associated with a learning process in Hermissenda (Acosta-Urquidi et al, 1984). We have thus examined the role of IA in hippocampal LTP.

Several slices were placed in each of the two independent compartments of the chamber. The only difference was the presence of 4-AP (50 uM) in the perfusion fluid supplying one of the compartments. A test stimulus adjusted to an intensity which evoked a dendritic field epsp of about 2 mV amplitude was given every 30 sec. It was measured at a fixed latency from the stimulus with a sample and hold amplifier and displayed on a chart recorder. When the epsp had been stable for at least 10 min, a tetanus of 500 msec duration at 100 Hz was given every 3 seconds until a total of 4 tetani had been delivered. In 3 such experiments 26 slices were tested, 13 in normal and 13 in 4-AP containing perfusion medium. The increase in epsp amplitude 1 min and 10 min after tetanization were taken as the measure of post-tetanic potentiation (PTP) and long-term potentiation (LTP). The average epsp increase for both PTP and LTP was larger in the slices exposed to 4-AP, although this difference was not significant (Haas and Greene, 1985). In a second series of experiments, tetani were given every 10 min until a saturation of LTP had been reached, Now 4-AP (50-100 uM) was added and in each of 4 experiments the epsp was further enhanced. One such experiment is illustrated in Fig. 4.

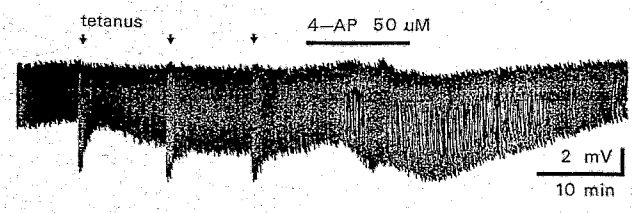

Fig. 4. Continuous display of extracellular epsp slope. Each stroke in the record represents the size of a single epsp measured between zero and the voltage at a fixed latency from the stimulus and registered with a sample and hold amplifier. Three tetani were given until LTP was maximal (saturated). In this situation, addition of 4-aminopyridine, an A-current blocker, could still further enhance the epsp. This, together with the observation that LTP was equally inducible and long lasting in the presence or absence of 4-AP makes a direct involvement of A-current modification in LTP unlikely.

Calcium activated currents

Two Ca-dependent potassium currents have been identified in CA 1 pyramidal cells. The first, IC, is rapidly activated and inactivated and strongly voltage dependent while the second, IAHP, is ac-

tive over a wide voltage range and a long time (seconds) after Ca-inflow. IAHP is not inactivated but does presumably reflect the time course of intracellular Ca sequestration. IC is sensitive to TEA while IAHP is rather specifically blocked by histamine, noradrenaline and cyclic AMP (see introduction). In the presence of 5 mM TEA it was possible to evoke LTP, and the increase in epsp amplitude was not significantly different from that obtained in an untreated slice. I have also investigated the effects of 2-5 mM TEA before and after induction (saturation) of LTP in a manner similar to the one described above for 4-AP. In the state of LTP saturation 2 mM TEA clearly further enhanced the field epsp. In the light of the only partial and unspecific blockade of several potassium currents definite conclusions cannot be drawn from these results

Noradrenaline (NA) has been shown to strengthen and prolong LTP (Hopkins and Johnston, 1984). I have found a similar effect with histamine (HA). The possibility exists that such amines (including also acetylcholine) are released together with the excitatory transmitters by tetanic but not low frequence stimulation and would, through blocking a Ca activated potassium current, potentiate signal transmission. If this were the case antagonists of these amines should prevent or at least reduce the induction of LTP. However, in the presence of propranolol, cimetidine and atropine (1-10 uM) LTP could be induced in our experiments without difficulty. The field epsp and population spike increase 10 min after tetanization was not different from normal.

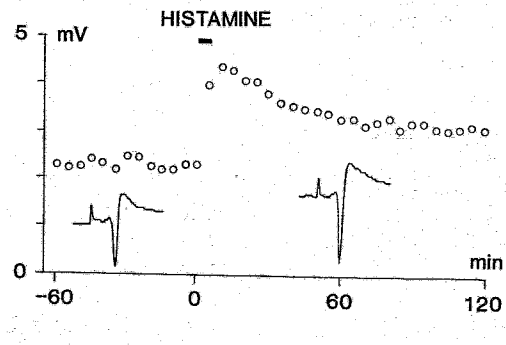

Fig. 5.

Long lasting epsp / population spike enhancement after a short perfusion with histamine. Similar effects were seen with H2-receptor agonists, noradrenaline, beta agonists and with forskolin. All these drugs enhance intracellular cyclic AMP and block the calcium dependent potassium current IAHP. Each point represents the average size taken from 9 population spikes every 5 minutes. Inserts show such averages before and during the enhanced state (10 msec sweeps).

HA and NA, at concentrations of 1-10 uM, perfused for 5-10 min evoked long lasting increases of the population spike, while the field epsp remained unchanged. This effect is probably related to the block of IAHP and mediated by cyclic AMP (Haas 1984, 1985). The duration of the epsp / spike potentiation was recently reinvestigated in a fast perfusion chamber which eliminated a significant contribution of drug equilibration in the chamber to the time course of the drug effects. HA and impromidine (H2-agonist) as well as NA and isoproterenol (beta-agonist) caused often marked increases of the population spike lasting for more than 60 minutes (Fig. 5). Occasionally, with either substance, the effect showed no tendency for recovery within 1-2 hrs. Thus a perfusion

with the amine for 5 min seemed to have switched on an enhanced state of signal transmission. While perfusion with the H2 and beta-antagonists, cimetidine and propranolol, prevented the agonist effects, these antagonists were ineffective once the enhancement had been evoked (after the agonist perfusion).

DISCUSSION

Several conclusions may be derived from these experiments: Further support is provided for our earlier conclusion that reduced synaptic inhibition is not responsible for LTP in CA 1 pyramidal cells. Since the enhancement of depolarizing potentials recorded intracellularly with KCl-filled electrodes was always greater than that recorded with K-acetate filled electrodes, it seems that the recurrent and feed foreward ipsps are also enhanced rather than reduced after an LTP-inducing stimulus (see also Buzsaki and Eidelberg, 1982).

The presence of caesium in a pyramidal cell prevents the induction of LTP in this cell, suggesting that normally functioning potassium channels in the postsynaptic membrane are a precondition for LTP. We were unable to demonstrate changes in potassium dependent potentials with intracellular recordings in the soma during LTP but a modification of K-currents might well be occurring locally at the dendritic sites contacted by the afferent fibers. It is unlikely that such events could be recorded at the cell soma. However their occurrence would be prevented by diffusion of caesium into the cell. Presynaptic forms of synaptic plasticity, paired pulse facilitation and PTP but not LTP were recorded in the caesium loaded cells. The actual reduction of postsynaptic potentials in caesium loaded cells during LTP could be explained by the increased extracellular field and perhaps by a shunting effect of increased feed foreward inhibition. These may also be reasons for the surprisingly small increases in epsps recorded with K-acetate filled electrodes (Andersen et al, 1980; Haas and Rose, 1984).

If LTP depends on a down regulation of the transient A-current then one would expect a block of this current to create a situation where LTP cannot be evoked. Furthermore, when LTP is maximal (saturated) after several tetani, one might expect the A-current antagonist 4-AP to be less effective. Both these expectations are not fulfilled. Rather, LTP seems more readily elicitable and the magnitude of the potentiation is perhaps even larger in the presence of 4-AP. Wherever the locus of 4-AP action is, it should have interfered with LTP in our experiments if IA were specifically involved. From these results we conclude that a modulation of the A-current is not involved in the production of LTP.

In partial contrast to the results obtained in the CA3 area (Hopkins and Johnston, 1984) we have not been able to prevent or block LTP with propranolol in CA1. Similarly, cimetidine, a histamine H2 antagonist and atropine as well as all three antagonists together could not prevent LTP induction. Therefore, there is little evidence that would directly link the LTP promoting effect of HA and NA to the mechanism of LTP in CA1. Nevertheless, these two amines can produce a postsynaptic epsp / spike potentiation which is presumably mediated through cyclic AMP, similar in appearance and time course to the postsynaptic component of LTP. Cyclic AMP accumulation could, like in other systems, lead through protein phosphorylation to regulation of ion channels or Ca-sequestration. It seems worthwile to consider this possibility for LTP as well.

HA and NA have been shown to block the long lasting afterhyperpolarizations due to the potassium current (IAHP) (Madison and Nicoll, 1982; Haas and Konnerth, 1983; Pellmar, 1984; Haas and Greene, 1986). This results in a preferential and profound potentiation of longer lasting excitatory signals like the one evoked by the tetanic stimulation employed for inducing LTP. Therefore, an enhanced response and Ca influx occurs if those amines are present during the tetanization. This and the lasting effect on epsp / spike potentiation of the amines can explain an increase in amplitude and duration of LTP.

The data presented here indicate a postsynaptic locus for LTP but do not exclude presynaptic components. The basic phenomena, perhaps initiated by a large Ca inflow, might in fact be similar on both pre- and postsynaptic sites. Modulation of excitability through the more economic regulation of inhibitory potassium channels (rather than the excitatory sodium and calcium channels) is an attractive hypothesis for at least one component of LTP in the hippocampus. Potassium currents can normally restrict excitatory signals and the release from this restriction, an "intrinsic disinhibition" may well be responsible for a lasting enhancement of these signals.

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