Postsynaptic long-term potentiation follows coupling of dendritic glutamate application and synaptic activation

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Summary. Dendritic depolarization, which seems to be involved in the induction of long-term potentiation (LTP), was elicited by localized glutamate application. When paired to low frequency synaptic activation in the same area, the subsequent changes had features in common with LTP, expressed as an increased probability of firing and shorter spike latency. The EPSP was not significantly increased.

Key words. Hippocampal slices; glutamate; dendritic depolarization; conjunction; LTP.

Long-term potentiation (LTP) of synaptic transmission serves as a model for activity-dependent synaptic plasticity[2-6]. Interest in the phenomenon rests upon its rapid induction and long duration, and the fact that it exists in many neuronal systems. An increased transmitter release has been demonstrated[7,8]. In addition, postsynaptic excitability modulation appears in LTP-changed pathways, since a given EPSP induces action potentials with increased probability and shorter latency[3,9,10]. For LTP induction, synaptic activation is necessary[11]. The tetanization must be above a certain strength[12], and only previously tetanized synapses show later improvement[13-15].

Dendritic depolarization has been proposed as a mediating signal[10,16,17]. By recording simultaneously with one intracellular electrode in the cell body of hippocampal CA1 pyramidal cells and a second electrode placed extracellularly in the layer of the activated dendritic synapses, tetanization (100 Hz, 16 shocks) caused a depolarizing plateau consisting of summated EPSPs and a sustained negative DC-shift at the synaptic level. With repeated trains of tetani (5-s interval), the sustained depolarization showed a cumulative growth which seemed to be correlated to subsequent development of LTP[16]. We wanted to create dendritic depolarization without tetanization of the input and couple it to synaptic activation to see whether LTP could be induced.

Materials and methods. Rat hippocampal slices were prepared and maintained in vitro in the interface between gas and perfusion fluid which contained (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, glucose 10, and was bubbled with 5% CO_2/95% O_2 to give a pH of 7.4.

CA1 pyramidal cells were impaled at the soma (fig. 1E). Responses to orthodromic synaptic stimulation of str. radiatum fibers (stim 1, 35–900 µA, with duration changed in 10-µs step from 10 to 90 µs) was recorded (fig. 1A, F) and input/output curves constructed. The EPSP amplitude was measured at a fixed latency in the middle third of its rising phase. L-glutamate (glu, 1.0 M, pH = 8.0) was applied iontophoretically (10–100 nA, 700–1200 ms) to the same dendritic region as that activated synaptically (fig. 1B, E). A sensitive, highly localized spot in the apical dendritic tree was first found (100–300 µm from the pyramidal layer, vertical extension less than 30 µm, fig. 1E). The glutamate-induced response was recorded in the soma as a depolarization with short latency (20–200 ms) and extracellularly in the dendritic area as a negative DC-shift. To simplify the interpretation, the induced depolarization was kept subthreshold (3–15 mV) for cell discharge. Responses to depolarizing current injected through the soma recording electrode (0.1–2.5 nA, 50–300 ms) and/or orthodromic activation of str. oriens (stim 2, 20–450 µA, 10–90 µs) as well as series of unpaired glu responses served as additional controls.

After at least 15 min with stable recording, conjunction was performed by superimposing a single subthreshold radiatum fiber EPSP near the end of each glu pulse (0.2–0.5 Hz for 1–5 min) (fig. 1C, D). In order to compare with LTP, only cells which could be followed for at least 15 min were accepted. Criteria for cell selection was a membrane potential more negative than −60 mV (range −60 to −82 mV, mean ± SD: −66 ± 7 mV) and spike amplitude above 85 mV (range 85 to 111 mV, mean: 98 ± 9 mV).

Results. Of 69 cells in this series, 23 cells satisfied both the electrophysiological and temporal criteria. Conjunction caused two main results: a statistically significant increase in the probability of discharge and a reduction of the spike latency. For a given input strength, a firing ratio (FR) was calculated for each cell (table). The mean FR for all 23 cells taken together was significantly increased 5 and 15 min after coupling and also for 17 cells which could be followed for 30 min (table, A). In contrast, 1 min after conjunction the FR was significantly decreased for all cells.

Analyzing individual cells, 16 of 23 had their FR increased (> 15 min) in the paired pathway (fig. 2A, table, B). In the remaining 7 cells there was a reduced firing probability with no or only partial recovery with time (fig. 2B). Figure 2C gives the mean values of the two groups. All cells with an increased FR also had a shorter latency to the spike ($p < 0.06$). The reduction of the spike latency was found at all supraliminal stimulation strengths, and at 5, 15 and 30 min after the conjunction. At these times the mean reduced latencies for moderately strong stimuli

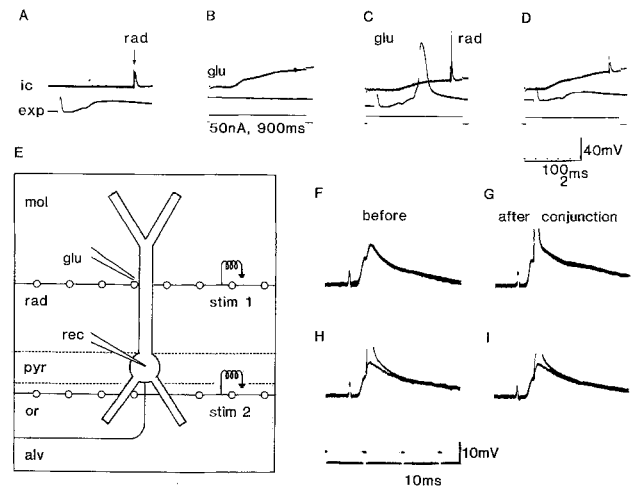

Figure 1. Cellular responses before and after conjunction. *A* Upper trace shows an intracellularly recorded response (ic) to orthodromic synaptic activation of radiatum fibers (rad, 70 µA, 50 µs). Lower trace (exp) is an expansion of the same response. *B* Response to application of glu delivered iontophoretically (50 nA, 900 ms) from a microelectrode placed at a sensitive, highly localized spot in the same apical dendritic region as the activated radiatum synapses. The first (*C*) and the last response (*D*), produced by a series of 12 glu pulses delivered every 5 seconds. An orthodromic synaptic response (rad) is delivered near the end of each glutamate-induced depolarization. *E* Diagram of the preparation and electrode arrangement. Low frequency stimulation of the fibers in str. radiatum (stim 1) was coupled to glutamate application (glu) at the same dendritic level. Responses were recorded with an intracellular electrode (rec) in the soma of the cell. alv – alveus; mol – str. moleculare; or – str. oriens; pyr – str. pyramidale; rad – str. radiatum. Two superimposed responses to stim 1 (170 µA, 50 µs) before (*F*) and 15 min after conjunction (*G*). A separate, unpaired pathway (or, stim 2, 170 µA, 50 µs) was stimulated alternately. Two responses to such stimulation are seen before (*H*) and 15 min after conjunction (*I*). The notch on the upstroke of the EPSP is a field effect of the population spike, generated by the synchronous discharge of surrounding cells.

(50 μs) were, respectively: 0.06, 0.18, 0.10 ms and for stronger stimuli (90 μs): 0.18, 0.19, 0.10 ms (n = 16).

In contrast to the effect on the FR, the mean EPSP amplitude did not show significant long-lasting changes. 15 min after conjunction, 7 of the 16 cells with increased FR showed slightly increased EPSPs, 6 had depressed EPSPs and 3 were unchanged in comparison with their preconjunction values (fig. 2D). A stronger correlation was seen in the group with reduced firing probability, where 6 out of 7 cells had depressed EPSPs and one was unchanged (fig. 2E). In almost all cells (21/23) there was a reduction of the EPSP amplitude 1 min after conjunction.

The difference between the 16 cells which showed an increased FR and the 7 which failed to do so could not be explained by significant variations in resting potential, action potential, input resistance or temperature. Neither were there any systematic differences in the parameters for glu ejection or responses (current, duration, depolarizing level, number or frequency of pulses). The transient decrease in FR and EPSP amplitude seen 1 min after conjunction could be explained by a depressive effect of the glu application on the EPSP. This depression varied in degree and duration from cell to cell. Control expriments, therefore, were performed in which only glu pulses were delivered (8 cells). Long series of glu pulses (> 50 ejections delivered at 0.2 Hz and causing just subthreshold depolarization) changed neither the FR nor the EPSP to test stimuli. Only when the glu pulse lasted longer than 500 ms was there a moderate EPSP depression, both of the radiatum and of the oriens responses.

In 12 of the cells orthodromic stimulation in the str. oriens served as an unpaired control (fig. 1H, I). Six pyramids which showed increased FR to the paired input (fig. 1F, G), and suitable probability levels, allowed statistical evaluation of the data. Fifteen minutes after conjunction the mean FR was 1.41 for the paired ($p < 0.02$) and 1.12 (n.s.) for the unpaired (oriens) input. The rheobase and spike latency in response to depolarizing current pulses did not change in any of the cells (14/14).

We also depolarized CA1 cells by two other means: 1) injection of depolarizing current (0.5–1.5 nA, 500–1200 ms) through the soma recording electrode (10 cells), 2) passage of longitudinal current (1–10 μA, 500–1500 ms) between nonpolarizing electrodes situated at the apical dendritic (cathode) and axonal (anode) cell poles (6 extracellular and 2 intracellular experiments). Neither procedure led to any change in synaptic transmission when coupled to low frequency synaptic activation, although the depolarization level and the number of soma pulses and intervals were the same as in the glu conjunction trials. The longitudinal current probably gave a local dendritic depolarization of at least 10–15 mV since reversal of the polarizing current gave a soma depolarization of this magnitude.

Discussion. The present conjunction-induced enhancement of synaptic transmission showed a short induction and long duration similar to those of LTP. Increased synaptic activation was observed after only 10 pairings and could last for more than 1 h. The effect is expressed as an enhanced probability of discharge, but in contrast to tetanus-induced LTP, conjunction did not augment the EPSP. Further, there was not any change in the responses to an unpaired synaptic input or in soma rheobasic current. Glu/synaptic conjunction, therefore, appears to produce improvement of local postsynaptic processes only. Enhancement of action potential generation with no or moderate increase of the EPSP is a commonly observed phenomenon in LTP experiments[3,9,18,19]. The effect may be mediated by the synaptic prepotential, an essential coupling process between the EPSP and the action potential[20,21]. Changes in the threshold or kinetics of the synaptic prepotential could possibly explain the greater effects on the spike than on EPSP during LTP and conjunction experiments.

Simultaneous pre- and postsynaptic activation was necessary for induction of synaptic enhancement since neither was sufficient by itself. The positive conjunctive effect of dendritic glu application could be due to depolarization, or Ca^{2+} entry through NMDA channels[22–24], or a mixture of both.

When coupled to synaptic activation, the effect of dendritic glu application contrasted with the inefficiency of equally large current-induced soma depolarization. However, conjunction with

Firing ratio and EPSP amplitude before and after conjunction

	Control	1 min	5 min	15 min	30 min
A					
n	23	23	23	23	17
Firing ratio	1.0	0.66***	1.82**	2.08*	1.65*
Normalized EPSP amplitude	1.0	0.83**	1.00	1.02	1.01
B					
n	16	16	16	16	11
Firing ratio	1.0	0.45***	2.60**	3.20**	3.03*
Normalized EPSP amplitude	1.0	0.91*	1.07	1.10	1.14

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's two-tailed t-test. *A* Mean firing ratio for all 23 cells. The firing ratio (FR) is defined as the probability of firing at a given stimulation strength after conjunction divided by the same function before coupling, each based upon 6–48 trials. In a given cell we aimed at a strength which gave a discharge probability between 0.1 and 0.6 before conjunction. Normalized EPSP amplitude is defined as the ratio between the EPSP amplitude after conjunction and its pre-coupling amplitude at the same stimulation strength. *B* The same parameters for a subset of 16 cells which all showed increased FR 15 min after conjunction. In order to include four cells which did not discharge before conjunction, but were well driven after coupling, the maximal FR for these were set to 5 (the same as the mean of the best 10 cells) to avoid the infinity value.

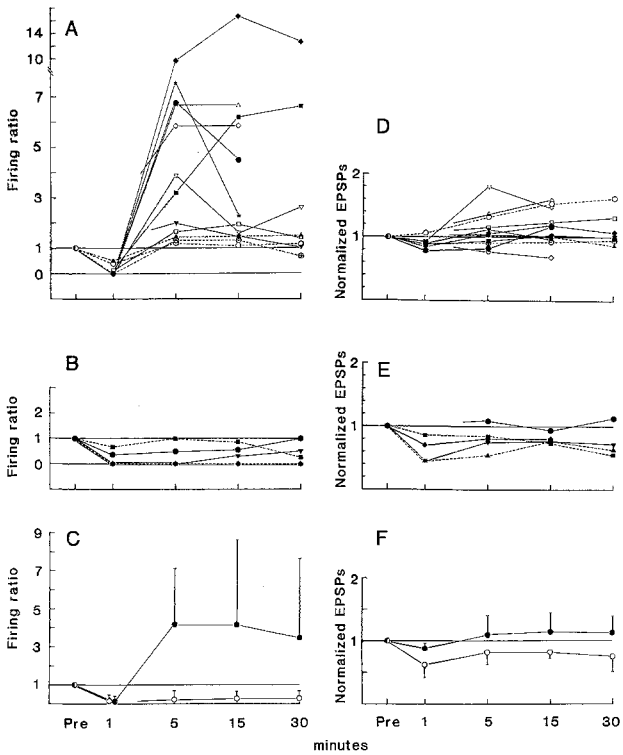

Figure 2. *A* Firing ratio (see table) of 12 cells which showed improved synaptic transmission 15 min after conjunction. Dotted lines represent cells with high probability of firing before conjunction (> 0.7). *B* Five cells with decreased FR 15 min after conjunction. Of the total material four cells are not included in the sample shown in A and two in the sample in B because the absence of preconjunctional discharges prevented FR calculation. *C* The mean of the responses in A (closed circles) and B (open circles). *D* Normalized EPSPs, same cells as in A. *E* As D, but for the cells in B. *F* The mean of the normalized EPSPs in D (closed circles) and E (open circles).

considerably stronger soma depolarizing currents (3–8 nA, 3–10 nA) has been reported to give long-lasting synaptic enhancement, expressed as an increased EPSP[25,26]. These larger soma currents probably depolarized the dendritic tree more efficiently than in our current injection experiments. The conjunction paradigm used by Kelso et al.[27], which gave EPSP increase, also elicited a strong depolarization as judged from the continuous synaptically induced discharge at 75–100 Hz. Taken together, these results suggest that the conjunction effect is not generated at the soma, but more distally, possibly at, or close to, the dendritic synapses.

Dendritic depolarization coupled to synaptic activation seem to be a common element for development of conjunction-induced changes in hippocampal and cerebellar synapses, although the direction of the change differs[28].

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Phospholipase C activation via a GTP-binding protein in tumoral islet cells stimulated by carbamylcholine[1]

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Summary. Carbamylcholine and GTP act synergistically in stimulating the production of [^{3}H]inositol-1-phosphate by digitonized tumoral islet cells (RINm5F line) prelabeled with myo-[2-^{3}H(N)]inositol. The response to these two agents is similar to that evoked by GTPγS. These findings suggest that a GTP-binding regulatory protein couples the occupancy of muscarinic receptors to activation of phospholipase C in pancreatic islet cells.
Key words. Phospholipase C; GTP-binding protein; carbamylcholine.

Cholinergic neurotransmitters stimulate phospholipase C in pancreatic islet cells, leading to the hydrolysis of phosphoinositides, formation of inositol 1,4,5-trisphosphate and diacylglycerol, redistribution of intracellular Ca^{2+}, activation of protein kinase C, and eventual stimulation of insulin release (see Malaisse[2] for review). The results of the present study suggest that a GTP-binding regulatory protein couples, in tumoral islet cells, the occupancy of muscarinic receptors to the activation of phospholipase C.

Tumoral pancreatic islet cells (RINm5F line) were cultured, harvested and counted as previously described[3]. They were preincubated for 120 min at 37 °C in a bicarbonate-buffered medium[4] containing D-glucose (2.8 mM), bovine albumin (2.5 mg/ml), K_2HPO_4 (0.1 mM) and myo-[2-^{3}H(H)]myoinositol (4.7 μM). The cells were then washed thrice, resuspended in the same buffer except for the absence of tritiated inositol and presence of 1.0 mM unlabeled myoinositol ($16 \cdot 10^6$ cells/180 μl), and mixed with 0.6 ml of an imidazole buffer (20 mM; pH 7.0) containing 0.5 mg/ml digitonin. 20 s later, the cells were centrifuged for 20 s (Beckman Microfuge) and, after removal of the supernatant, resuspended in 0.5 ml of the same medium as that used for the determination of inositol phosphate metabolism in lysed 7315c tumor cells[5]. Aliquots of the digitonized cell suspension (30 μl) were mixed with an equal volume of the same medium containing, as required, carbamylcholine, GTP or GTPγS. After 10-min incubation at 37 °C, the reaction was halted by adding 1.5 ml of a mixture of $CHCl_3/CH_3OH/HCl$ (12 N) (2/1/0.01, v/v/v) and 0.44 ml of KCl (57 mM). After centrifugation (1 min, 800 × g) an aliquot (0.6 ml) of the upper aqueous phase was mixed with 2.0 ml H_2O, neutralized with 0.17 ml of Tris (0.3 M), and applied to a Dowex AG1X8 column (1.0 ml, 200–400, formate form) for separation of inositol phosphates[6]. The radioactive content of the lipids extracted from the pellet of digitonized cells amounted to 5.17 ± 0.24 fmol/10^3 cells and, over 10 min incubation, the basal production of [^{3}H]-inositol 1-phosphate averaged 15.6 ± 1.7 amol/10^3 cells (n = 15 in both cases), these results being expressed by refer-