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Quantitative relationship between post-tetanic biochemical and electrophysiological changes in rat hippocampal slices

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Slices of rat brain hippocampus were tetanized in the perforant path fibers. In individual slices long-term changes of the electrophysiological parameters were determined simultaneously with post hoc endogeneous phosphorylation of proteins in a lyzed crude mitochondrial/synaptosomal fraction.

A quantitative relation between the electrophysiological parameters and the degree of phosphorylation of a 52K protein was found to follow a non-linear function.

The application of one or more tetani to an afferent fiber group may cause electrophysiological and biochemical changes in the target neurons[3,10,14,22]. The electrophysiological changes may consist of post-tetanic potentiation (PTP) and/or long-term potentiation (LTP) of the evoked responses upon single test stimuli. These two types of changes have been found in many neuronal systems including the hippocampus, both in vivo[6,7,17] and in vitro[1,15,21]. However, besides potentiation also depression of the evoked response may occur[1,15,16,18].

In addition, tetanization of afferent fibers induces biochemical changes at the synaptic level[9,19]. These changes include: (i) a decrease in the activity of α-pyruvate dehydrogenase by an increased degree of phosphorylation, possibly leading to an elevation of the presynaptic calcium concentration[9]; (ii) an enhanced influx of calcium into pre- and/or postsynaptic elements[2,25]; (iii) an enhanced release of neurotransmitter[14,22]; and (iv) a calcium-induced activation of a postsynaptic protease, presumably unmasking glutamate receptors[5]. Our own research has shown that tetanization of the perforant path fibers results in an enhanced post hoc phosphorylation of a protein (M_r 52K) present in hippocampal synaptosomal plasma membranes[3,4,19,23].

The in vitro endogeneous phosphorylation of the 52K protein was not sensitive to calcium, calmodulin or cAMP[4].

In the present paper we report for the first time a quantitative correlation between the changes in amplitude of the postsynaptic potential and population spike measured extracellularly, and the degree of phosphorylation of the 52K protein occurring after tetanic stimulation of the perforant path fibers in rat hippocampal slices.

Hippocampal slices (300–400 μm; n = 30) were cut from the brains of 4 male rats (inbred Wistar TNO, Zeist; body weight 140–150 g)[24]. Immediately after cutting, the slices were placed in perfusion cups of a multicup perfusion system and preincubated as described previously[20]. After a preincubation time of 45–60 min, two stainless steel electrodes (40 μm in diameter; at 40–60 μm distance) were placed in the stratum moleculare.

Glass micropipettes filled with 3 M NaCl (2–10 MΩ) were placed in the stratum granulosum of the fascia dentata[23]. The electrophysiological parameters of interest are the population spike (Popsp) and the field postsynaptic potential (PSP). As a measure

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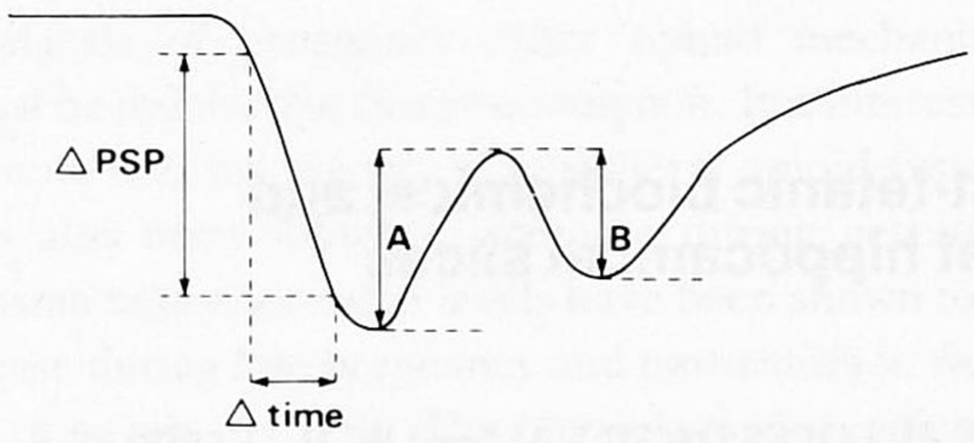

Fig. 1. A: determination of electrophysiological parameters. As a measure of the postsynaptic potential the ΔPSP/Δtime was calculated. The amplitude of the Popsp was calculated as follows: Popsp = (A + B)/2. B: relationship between the postsynaptic potential and the endogeneous phosphorylation (post hoc) of the 52K protein (in a crude mitochondrial/synaptosomal fraction) 15 min after tetanization of the dentate perforant path fibers. Vertical: the percentual change of the incorporation of radiolabelled phosphate into the 52K protein relative to the corresponding control slices. Horizontal: logarithm of the percentual change of ΔPSP/Δtime 15 min after tetanization relative to the ΔPSP/Δtime 1 min before tetanization. C: relationship between population spike and the endogeneous phosphorylation. Details as in Fig. 1B except horizontal: logarithm of the percentual change of the Popsp 15 min after tetanization with respect to the Popsp 1 min before tetanization.

of the postsynaptic potential the slope of the rising phase of the PSP was calculated giving ΔPSP/Δtime (Fig. 1A). The amplitude of the Popsp was determined by calculating the mean of the rising and falling phase of the Popsp (Fig. 1A). After clear responses to test stimuli were obtained, the stimulus–response relationship of the PSP was made and the Popsp-threshold was determined per slice. The experimental design was as follows: after a pretetanus period of 10 min 2 high frequency stimulations 5 min apart (150 pulses/s for 2 s) were given; thereafter measurements were taken over a posttetanus period of 10 min. Throughout the whole 25 min period, continuous recordings of the field PSPs and Popsp were made. At the end of the 25 min period, the slices were individually homogenized and processed for the measurement of the post hoc endogeneous protein phosphorylation in the lyzed crude mitochondrial/synaptosomal subcellular fraction (20 min × 10,000 *g* pellet, lyzed in water; see for details refs. 3, 4). Briefly, the endogeneous phosphorylation conditions of the present experiments were: 10 μg protein, 15 s incubation in the presence of 7.5 μM [γ-^{32}P]-ATP (3 μCi); 10 mM sodium acetate, 10 mM magnesium acetate, 0.1 mM calcium acetate, pH 6.5. Protein separation by PAAGE using slab gels, gel fixation, protein staining, autoradiography, densitometric scanning and density quantification were performed as described elsewhere[4,26]. The correlation between optical density and incorporated [^{32}P]phosphate was checked by counting the radioactivity in excised gel pieces. Comparisons were made only between samples on one gel. Radiolabelled membrane samples were included as a reference on each gel and the exposure time allowed a linear relationship between radioactivity and grain density per band[3,26]. Thirty hippocampal slices were randomized; 19 were subjected to both the electrophysiological and biochemical procedure, whereas 11 slices (controls) were not tetanized but otherwise treated similarly. The biochemical and electrophysiological measurements were independently carried out by different experimenters. The neurochemistry was performed by one experimenter who did not know whether the slices had been

tetanized or not. The incorporation of phosphate into the 52K protein was measured as peak height above background and expressed as percentage of the corresponding control slice[3,4].

The ΔPSPs/Δtime and the Popsp amplitudes at all measuring time points were expressed in percentages of the values obtained 1 min prior to the first tetanus per slice; a response of 100% means no change relative to the pretetanus periods. The values of ΔPSP/Δtime and of the Popsp 15 min after the first tetanus are plotted in Fig. 1B and C.

The mean post hoc endogeneous phosphorylation of the 52K protein band was significantly increased in the treated group (n = 19) as compared to the non-tetanized control slices (+24%; $P < 0.05$). Furthermore, the changes in other major phosphoprotein bands in the profile (e.g. M_r 43K, 48K) were small and did not reach significance. These data are in accordance with previous studies from our laboratory[3,4,19]. As Browning et al.[8,11] reported rapid effects of tetanic stimulation on the post hoc phosphorylation of the α-subunit of pyruvate dehydrogenase (40–43K) which had disappeared 15 min after the tetanus[9], it is not surprising that under the present conditions (10–15 min after tetanus) no effect of tetanic stimulation could be detected in the 43K band.

In Fig. 1B the percentual change in 52K phosphorylation is related to the ΔPSP/Δtime per individual slice. This data, in a semi-logarithmic plot fits a straight line with a correlation coefficient of 0.71 ($P < 0.005$). Similar results have been found for the Popsp as shown in Fig. 1C. The correlation coefficient of the straight line relating the percentual change in 52K protein phosphorylation and the log Popsp was 0.55 ($P < 0.05$). Hence, we suggest that the present data represent a quantitative correlation between electrophysiological synaptic changes and synaptic membrane protein phosphorylation. However, as in all correlative studies of this kind, the causality of the relationship remains to be determined.

As regards the subcellular localization of the 52K protein we should note that, based on two dimensional identification[4], the protein appears to be localized in light SPM and/or vesicle membranes. With respect to the nature of the 52K protein, it was considered that it could be similar if not identical to the β-subunit of tubulin based on its mobility in the two-dimensional separation system and hence that the changes after tetanic stimulation would be in line with the hypothesis of DeLorenzo et al.[13] on the crucial role of tubulin phosphorylation in synaptic exocytosis. However, extensive characterization using several 2D-separation methods, peptide mapping and immunocytochemical identification revealed that the 52K protein is not the β-subunit of tubulin[12]. Whatever the nature of the 52K protein, in view of the present quantitative correlation, it is clear that the function of this protein is related to the electrical responses of the neurons.

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