Plasticity in Synaptic Transmission and Changes of Membrane-Bound Protein Phosphorylation

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SYNAPTIC PLASTICITY: LONG-TERM POTENTIATION (LTP)

Neural plasticity is the ability of the nervous system of changing its activity as a result of previous experience or of making adaptive changes after destruction of certain of its parts. Learning processes which become apparent as adaptive changes in behavior are the most essential manifestations of the plasticity of the nervous system. A question of great interest for neurobiologists is which are the plastic changes in the nervous system responsible for learning. An important line of research in this sense is represented by the studies of changes in neural activity in relatively simple nervous systems at both the behavioral and the physiological level, as reviewed by Kandel (1977). Here we will discuss some aspects of another research line within the same field, namely the investigation of the process of *long-term potentiation* (LTP) which may be considered to constitute a model of a learning process at the synaptic level. This phenomenon consists of the enhancement of synaptic activity that occurs for a long period of time, i.e. for some hours, after a period of electrical stimulation at relatively high frequencies or tetanization. The term *post-tetanic potentiation* (PTP) is usually reserved for the increase in excitability directly following the stimulation. The fact that synapses in the central nervous system may present the type of plastic changes characteristics of PTP was shown by Lloyd (1949) in the monosynaptic reflex pathway of the cat spinal cord. Even before this discovery the same type of phenomenon had been shown in the autonomic nervous system, viz. in the stellate ganglion of the cat, by Larrabee and Bronk (1947). PTP and LTP have been studied since in a number of neural systems among others by Curtis and Eccles (1960), Beswick and Conroy (1965) as reviewed by Kandel and Spencer (1968) and Spencer and April (1970). A new dimension was added to this phenomenon when it was shown (Goddard, 1967; Goddard et al., 1969) that the repeated application of a short tetanus to a certain pathway in the central system at regular intervals lasting, for example, 24 h, could lead to afterdischarges of progressively longer duration and result, after some days, in a convulsive seizure; this phenomenon has been called kindling and forms an interesting experimental model of epilepsy.

Even a simple account of synaptic plasticity as the present one must be complemented by a reference to another aspect of plasticity which forms a counterpart of PTP: *post-tetanic depression*. This phenomenon was first described by Evarts and Hughes (1957) in the lateral geniculate nucleus of the cat. The occurrence of post-tetanic depression depends on the duration of the tetanus.

Here we cannot discuss other interesting aspects of synaptic plasticity such as the phenomena of *habituation* and *hetero-synaptic* interactions. A useful reference to the classic literature on the subject is that of Kandel (1977).

HYPOTHESES CONCERNING THE MECHANISM OF LTP

A precise explanation of LTP is not yet available. In principle the enhancement of synaptic efficiency which is characteristic of this phenomenon may be due to changes in presynaptic and/or postsynaptic processes (summarized in Table I).

(1) At the *presynaptic level* LTP may be caused by a number of processes.

(a) An increase in the amount of transmitter released per action potential; this may be due to an increase in the concentration of Ca^{2+} ions at the presynaptic terminal, and/or to an increase in the number of vesicles available.

(b) A modification of the local turnover of transmitter at the synapse may also lead to an effective increase of the amount of transmitter.

(2) At the *postsynaptic level* LTP may be determined by a number of alternative or complementary phenomena.

(a) A modification of receptor properties, which could take place by way of several processes: an increase in the spatial extent of the chemosensitive area and/or in the density of receptor sites, an increase in chemical sensitivity of receptor sites, and an increase in the number and properties of ionic channels.

(b) A change in the spike-generating capacity of postsynaptic neurons owing to a change in the number of trigger zones, and/or in the extent of the excitable membrane, and/or in the threshold.

(c) A change in input resistance of the dendrites of the postsynaptic neuron.

When analyzing PTP in the rat neuro-muscular synapse Liley (1956) found that the amplitude of the miniature end-plate potentials (m.e.p.ps) was not altered, indicating that the sensitivity of acetylcholine receptors was not changed. He found that, rather, the number of m.e.p.ps. released per unit of time increased. Rosenthal observed (1969) in the same synaptic system but studied in the frog, that the probability of transmitter release increased during PTP and that this was dependent on the concentration of extracellular Ca^{2+}. The importance of changes in the process of transmitter release in PTP and LTP have been put in evidence in a clear way by the demonstration by Martin and Pilar (1964) that in the chick ciliary ganglion which possesses a dual chemical–electrical synapse only the former was facilitated by a tetanus.

TABLE I

SUMMARY OF HYPOTHESES CONCERNING THE MECHANISM OF LONG-TERM POTENTIATION

1. Presynaptic level

1.1. Increase in amount of transmitter released per action potential.

1.2. Modification of local turn-over of transmitter substance.

2. Postsynaptic level

2.1. Modification of receptor properties (number, availability, chemical sensitivity).

2.2. Change in spike-generating capacity (trigger zone, threshold).

2.3. Change in input resistance of dendrites.

The main current hypothesis is that plastic changes at the synapse such as LTP are due to alterations of processes responsible for presynaptic transmitter release. Nevertheless post-synaptic processes appear also to play a role in this phenomenon. One argument to support this point is the fact that in LTP in hippocampus slices there is a much more marked increase in the population spike, corresponding to an increase in the number of synchronously firing neurons, than on the size of the extracellularly recorded excitatory postsynaptic potential; this was found by Andersen et al. (1980) in LTP of the synapses of the Schaffer collaterals on CA1 pyramidal cells. Also Tielen et al. (1982) found that in LTP of the synapses of the perforant path on granule cells of the dentate area, the potentiation of the population spike lasted longer than that of the EPSP. Therefore most probably the processes responsible for LTP involve both pre- and post-synaptic processes.

STRUCTURAL CHANGES AT THE SYNAPSES AFTER LTP

The long-lasting character of potentiation suggests that the changes in neuronal excitability must be accounted for by structural changes. It is a long established idea that learning processes may be mediated by morphological changes in the connections between neurons (Lugaro, 1899) similar to those occurring during development. Some reports have indeed indicated that LTP may be associated with ultrastructural changes which can be seen with the electronmicroscope. Van Harreveld and Fifková (1975) have indicated that swelling of dendritic spines in the dentate area of the hippocampus after electrical stimulation of the perforant path fibers may be a mechanism underlying post-tetanic potentiation. Fifková and Van Harreveld (1977) interpreted the initial increase in the area of dendritic spines of the granular cells in the dentate area of the hippocampus as being caused by a glutamate-induced increase in the influx of Na^+, Cl^- and water owing to an increased permeability of the spine membrane for Na^+ ions; the long-lasting enlargement of the spines would be caused by an enhancement of the synthesis of structural proteins. Fifková and Van Harreveld advanced the hypothesis that along with an increase of protein synthesis in the perikaryon of the neuron the changed dimensions of the spines would lead to the distribution of the newly formed protein molecules into the stimulated spines. The protein molecules would move to the spines by way of a dendritic transport mechanism which has been shown to be able to transport radioactive amino acids at a rate of 3 mm/h. There are, however, still serious doubts as to whether these findings may be accepted as a valid hypothesis for explaining the processes underlying LTP.

Using a different preparation — the hippocampal slice in vitro — Lee et al. (1980) have also shown that LTP in the CA1 field of the hippocampus elicited by tetani of 100/s for 1 s was accompanied by a significant increase in the number of synaptic contacts on dendritic shafts as well as an increase in the ratio shaft/spine synapses; furthermore a reduction in variability in the postsynaptic densities, spine necks and spine areas was also found. These authors do not report, however, changes in the area of dendritic spines as reported by Fifková and Van Harreveld in the study mentioned above. Lee et al. (1980) concluded that the decrease in variability in the measures of spine densities could be due to a change in shape of the spines from an oval to a spherical shape. These changes could have influence on the area of apposition between pre- and postsynaptic elements. However, the interpretation of these results is still rather uncertain. At present we have to conclude that there is as yet no generally acceptable theory on which types of ultrastructural changes may be related to LTP.

BIOCHEMICAL PROCESSES RELATED TO LTP

There have been reports that neural activity may lead to a change in RNA synthesis; Peterson and Kernell (1970) concluded that the electrical stimulation of peripheral nerves in *Aplysia* caused an increase in the incorporation of [^{3}H]uridine into both nuclear and cytoplasmic RNA, depending on the synaptic activation. However, also in *Aplysia*, the synthesis of new proteins is not necessary for PTP since Schwartz et al. (1971) showed that blocking protein synthesis with anisomycin for periods up to 31 h did not alter PTP. This does not imply, however, that changes in synaptic excitability of a more prolonged character may not depend upon protein synthesis. Another possibility to explain potentiation which has been recently advanced by Baudry and Lynch (1980) is that the influx of Ca^{2+} into the dendritic regions induced by the tetanus would activate a membrane- bound protease which would lead to an increase in the density of available glutamate receptors. This would result in an increase in synaptic efficiency. It should be mentioned in this connection, that at the end-plate of the neuromuscular synapse the number of acetylcholine receptors is modifiable. Their number increases after denervation and it was suggested that this would reflect an increase in receptor synthesis; this process could be prevented by the administration of both RNA and protein synthesis inhibitors (see for review: Zigmond and Bowers, 1981). Considering that LTP is not blocked by these types of inhibitors (see above) any possible changes in the available number of receptors occurring during the LTP must have another explanation than in the case of denervation of the neuromuscular junctions. Therefore the hypothesis advanced by Baudry and Lynch (1980) is certainly of interest and awaits further experimental support.

There are, however, other biochemical processes at the level of the synaptic region which may underlie plastic changes. One of these processes has been investigated in recent years in some detail: the enzyme induction in catecholaminergic cells in sympathetic ganglion and adrenal medulla (Zigmond and Bowers, 1981). Electrical stimulation of the preganglionic input to the rat superior cervical ganglion lasting 10–90 min increases tyrosine hydroxylase (TH) activity in the ganglion; TH increase is maximal in 3 days after the stimulation. TH is the enzyme which synthetizes DOPA from tyrosine. Increases in the mount of TH may result in increases in the rate of catecholamine synthesis. In the pineal gland Bowers and Zigmond (1980) have shown that bilateral stimulation of the cervical sympathetic trunk produces an increase of the enzyme serotonin N-acetyl-transferase which is involved in the synthesis of melatonin from serotonin. The parameters of electrical stimulation were such that they mimic the physiological range.

One of the biochemical processes by means of which membrane properties such as permeability to different ions and state of membrane-bound receptors, can be changed is the *phosphorylation of proteins*. These processes are particularly attractive in relation to LTP because they take place at very fast rates. Phosphorylation is a process with the following characteristics: (1) it is fast since it takes place within seconds, (2) most membrane kinases use ATP as phosphate donor, (3) in the membrane it is cyclic, i.e. in the vicinity of the substrate a dephosphorylating enzyme is present, and (4) it changes the overall electrical charge of the substrate by adding two negative units of charge.

Changes in the state of membrane-bound proteins may determine the permeability of the neuronal membrane for different ions as suggested by Heald (1962) and thus they may be of crucial importance in determining the functional state of neurons (Greengard, 1976, 1978). Furthermore phosphorylation of membrane enzymes may affect their activity and thus have consequences for the neuronal metabolism.

The importance of phosphorylation of synaptic plasma membrane (SPM) fragments for the

efficiency of synaptic transmission has been put in evidence (Greengard, 1976; Rodnight, 1980). Since it is known that electrical stimulation of brain cortex slices leads to changes in protein phosphorylation (Heald, 1957; Heald, 1962), we investigated whether LTP was related to changes in phosphorylation of both proteins and phospholipids bound to the membrane.

CHANGES IN SPM PROTEIN PHOSPHORYLATION AFTER A TETANUS

In a previous study we showed (Bär et al., 1980; Tielen et al., 1982) that a tetanus applied to the perforant path fibers by way of a pulse train of 15/s for 15 s, produces an initial depression of both the extracellular EPSP and population spike measured at the level of the stratum moleculare or granulosum of the dentate; this is followed about 10 min after the tetanus by potentiation of EPSP and population spike, as shown in Fig. 1. The effect of such a tetanus on the degree of endogenous protein phosphorylation was measured in a crude mitochondrial fraction containing synaptosomal plasma membranes (SPM) in a post-hoc assay as described by Bär et al. (1980). It should be noted that in a post-hoc assay it is assumed that what is measured, is the capacity of a protein to accept phosphate radicals; if, in vivo, the protein has many unoccupied sites, it will incorporate more phosphate in the optimal in vitro assay (post-hoc) than when the protein is saturated at the onset of the incubation. This way of analyzing the capacity of phosphate incorporation in a protein has been used by a number of authors (Routtenberg et al., 1975; Zwiers et al., 1977; Holmes et al., 1977; Browning et al., 1979). In their original study Bär et al. (1980) found that hippocampal slices taken for biochemical analysis 15 min after the tetanus, showed an enhancement of the degree of phosphorylation in the post-hoc assay of a protein band of a crude mitochondrial fraction with an estimated molecular weight of 50.000 daltons (50 kdaltons). This change was not present when the slices were stimulated at low frequencies, which also did not produce LTP. Recently, Bär et al (1982) using more refined techniques, have shown that the 50 kdalton protein band

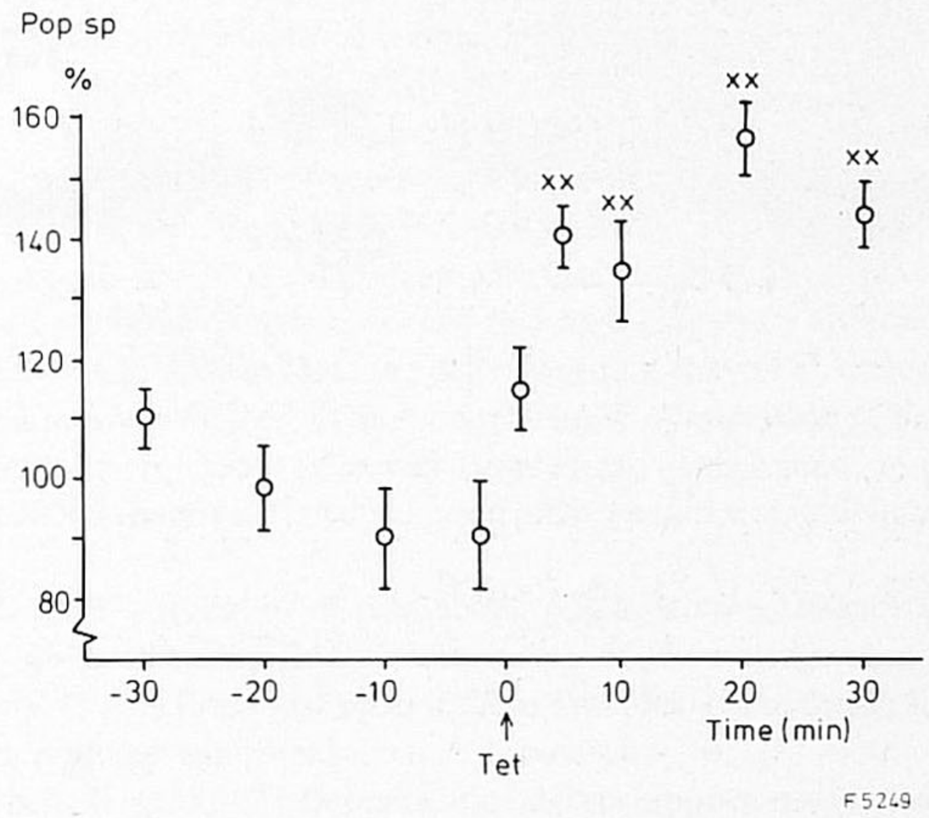

Fig. 1. Plot of the amplitude of the population spike recorded from the fascia dentata in hippocampal slices and evoked by stimulation of the perforant path. At t = 0 s a tetanus of 15 pulses/s with a duration of 15 s has been applied. Means and S.E.M. are plotted. The 100 % level corresponds to the average of means obtained in the control period, i.e. before application of the tetanus. Note the increase in amplitude of the mean population spike; the values indicated as xx differ significantly ($P < 0.05$) from control values).

consists of two proteins: a 50-kdalton and a 52-kdalton phosphoprotein with different characteristics (Fig. 2). The 50-kdalton protein has an isoelectric point (IEP) of 2.5–4.3 and it is most likely of mitochondrial origin; the phosphorylation degree of this protein is not changed when measured post-hoc in slices taken for analysis 15 min after the tetanus. The 52-kdalton protein has a less acidic IEP (5.3), it is found predominantly in plasma membrane containing fractions (SPM) and its phosphorylation is strongly affected by the tetanus under the conditions described above.

Browning et al. (1979) have analyzed protein phosphorylation related to LTP in another hippocampal sub-system (the Schaffer collaterals), viz. CA1 pyramidal cells. They analyzed their slices, however, 2 min after the tetanus. Their main finding was a decrease of phosphate incorporation (post-hoc assay) of a protein with molecular weight of about 40 kdaltons. Furthermore they found also smaller and less consistent increases in the post-hoc phosphate incorporation in protein bands of 27 kdaltons and 53-kdaltons. Recently, Finn et al. (1980) found that trifluoperazine which interferes with the induction of LRP also blocks the endogenous phosphorylation of the 40 kdalton protein. Browning et al. (1982) have further shown that the 40-kdalton phosphoprotein is enriched in the mitochondrial fraction and they identified it as the α-sub-unit of pyruvate dehydrogenase (α-PDH).

It should be noted that the discrepancy between the results presented by Bär et al. (1980) and by Browning et al. (1979) can probably by accounted for the fact that in the former study the post-hoc assay was initiated 15 min after the tetanus whereas in the latter it was done at 2 min. It appears that the degree of post-hoc phosphorylation of the 40-kdalton protein is most marked

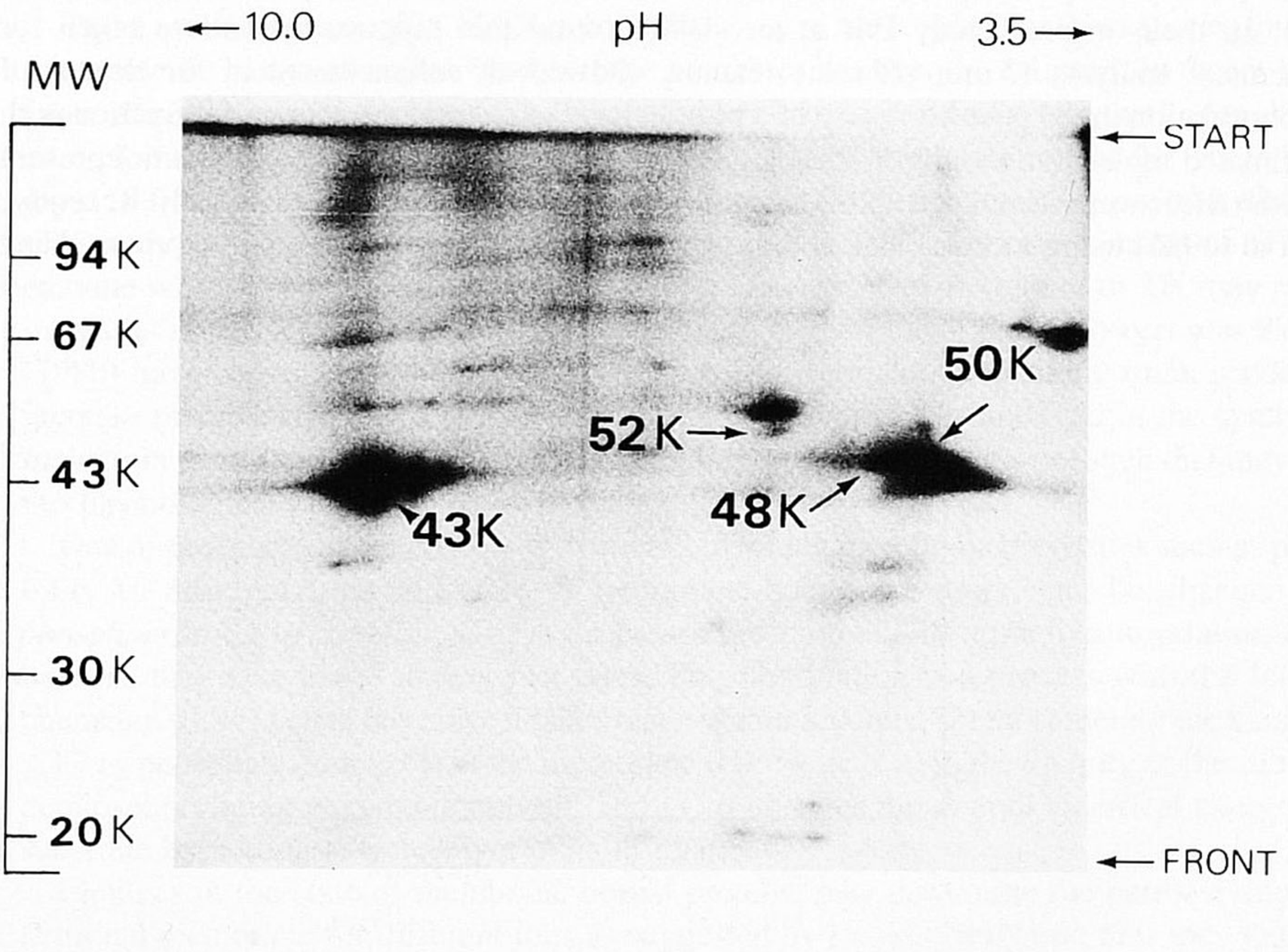

Fig. 2. Autoradiogram of two-dimensional separated phosphoproteins in a crude mitochondrial/synaptosomal cell fraction (P2) from rat hippocampus. First dimension: isoelectric focussing (IEF), pH range 3.5–9.0. Second dimension: SDS-polyacrylamide gel electrophoresis. At the left part, the position of molecular weight (MW) markers is given. Arrows indicate position of the various phosphoproteins being discussed in the text. For details of procedures see Bär et al. (1982).

immediately after the tetanus and decreases progressively attaining levels not significantly different from controls at about 10 min (Browning, personal communication); this could explain why Bär et al. (1980) found at 15 min no significant changes in the phosphorylation of the 40-kdalton band although the tendency was for this protein band to show a decrease in their post-hoc phosphorylation assay. Furthermore, Browning et al. (1979) noted also a small change in the degree of phosphorylation of a 53-kdalton protein band which may be the same which in the study of Bär et al. (1980) changed in the most marked way.

Browning et al. (1982) put forward the hypothesis that after a tetanus there is an early and transient endogenous phosphorylation of the 40-kdalton protein (α-PDH) which leads to blocking Ca^{2+} buffering by the mitochondria. This would lead to an accumulation of Ca^{2+} in the synaptosomal plasma which would influence several Ca^{2+} sensitive processes in the membrane (e.g. transmitter release) leading to an increase in synaptic efficiency.

The role played in this process by the change in phosphorylation of the 52-kdalton protein is not yet clear. This relatively late change of 52-kdalton phosphorylation may be related to conformational changes within the membrane since this protein appears to be membrane-bound. At the moment it can only be speculated whether these changes form the necessary basis for the long-lasting increase in synaptic efficiency characteristic of LTP.

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DISCUSSION

L.L. BUTCHER: Could the results you observed on phosphorylation and PTP be due to pathological or proto-pathological effects of intense, prolonged, electrical stimulation?

F.H. LOPES DA SILVA: I believe we have to distinguish between PTP or LTP on the one hand, and the phenomenon of kindling on the other. PTP or LTP can be elicited both in vitro in hippocampal slices and in vivo as I presented in my talk. In vivo it is clear, viz. from our own experiments in the hippocampus, and also from those of other laboratories, that LTP, e.g. obtained by stimulation of a tetanus applied to the Schaffer collaterals, can be elicited in freely moving rats which do not show any behavioral disturbance; furthermore, the process is reversible. Therefore, we do not think that this represents a pathological process. However, if the tetanus is applied daily, it is seen that after a number of stimuli afterdischarges occur, which tend to become progressively longer in duration. Also the afterdischarges tend to spread from the area that is stimulated to other brain areas. At a later stage behavioral convulsions may occur. This constitutes the phenomenon of kindling, which is a model of epileptogenesis. In this sense we may say that there is an evolution from LTP to epileptic activity, but it appears that this transformation involves not only quantitative, but also qualitative changes.

A. DOLPHIN: How long do the changes in extracellular K^+ and Ca^{2+} last measured after the tetanic pulse i.e., are they associated with long-term or post-tetanic potentiation?

F.H. LOPES DA SILVA: After a tetanus there are indeed changes in extracellular K^+ and also Ca^{2+}, but as far as I know there are as yet no data on whether those ionic changes are directly correlated in vivo with the synaptic electrical changes characteristic of PTP or LTP. This is a matter of current investigation in a number of labs.

D.F. SWAAB: You started off with defining plasticity. Then you gave a few examples, like recovery after brain lesion and learning, and you said you used post-tetanic potentiation as a *model* for such phenomena. In concluding you did not refer to learning, however. How then should we pass from phosphorylation to learning?

F.H. LOPES DA SILVA: I tried to avoid terms like memory and learning in order not to confuse the issue I was dealing with. What I stated is that long-term potentiation can be seen as a model of plasticity at the synaptic level, i.e., it is an adaptive change of synapses. Learning processes which become apparent as adaptive changes in *behavior*, are, of course, the most essential manifestation of plasticity of the nervous system. Of course, a change in synaptic transmission such as occurs in LTP is not equivalent to learning in behavioral terms, but the latter may depend on a series of synaptic changes which taken individually, may be analogous to LTP. Your question of how we go from phosphorylation of membrane-bound proteins to learning processes in behavioral terms, cannot be answered at the present moment. We believe a better knowledge of such membrane phenomena may give essential clues to unravel why and how changes in electrical responses of synapses such as LTP do occur. This, we hope, will give us a better insight into what we should be looking for when investigating the brain changes characteristic of the acquisition of a learning task.

G.A. FOSTER: Bearing in mind the sequential effects of glutamate on Na^+ and Ca^{2+} fluxes, is it possible to obtain long-term potentiation with glutamate itself or kainic acid?

F.H. LOPES DA SILVA: I do not know of studies showing LTP using glutamate or kainic acid, but there are investigations which show the possibility of chemical kindling by applying daily sub-convulsive doses of convulsants such as pentyltetrazol. More interesting is the possibility of obtaining chemical kindling by administrating carbachol in the amygdala (Wasterlain, personal communication). This form of chemical kindling is dose-dependent, it is blocked by atropine but not by D-tubocurarine.