Phosphorylation of the Presynaptic Protein B-50 (GAP-43) Is Increased during Electrically Induced Long-Term Potentiation

C. Gianotti,* M. G. Nunzi,* W. H. Gispen,[†] and R. Corradetti[‡]

*FIDIA Research Laboratories
Via Ponte della Fabbrica 3/A
35031 Abano Terme (PD)
Italy

[†]Rudolf Magnus Institute
University of Utrecht
Vondellaan 6
3521 GD Utrecht
The Netherlands

[‡]Department of Preclinical and Clinical Pharmacology
University of Florence
Viale G. B. Morgagni
50134 Firenze
Italy

Summary

The protein B-50 (F1, GAP-43) is a presynaptic-specific substrate of protein kinase C, functionally related to neurotransmitter release. An increase in phosphorylation of this protein has been proposed as a molecular mechanism underlying long-term potentiation (LTP). B-50 phosphorylation measured by quantitative immunoprecipitation in rat hippocampal slices incubated in the presence of radiolabeled inorganic phosphate was increased for at least 1 hr after the induction of LTP in the CA1 region. No significant changes in B-50 phosphorylation were observed in untetanized slices stimulated at low frequency. The direct demonstration of an increased phosphorylation of the protein B-50 during LTP is consistent with the hypothesis that presynaptic mechanisms contribute to maintenance of LTP.

Introduction

The long-term potentiation (LTP) of electrophysiological responses produced by a train of high frequency stimulation in the hippocampus (Bliss and Lømo, 1973) has been proposed as a model to study the cellular mechanisms of learning (Teyler and DiScenna, 1987). The induction of LTP in the CA1 subregion of the hippocampus involves N-methyl-D-aspartate receptor activation and consequent calcium-induced stimulation of various intracellular enzymes (Collingridge et al., 1983; Malinow et al., 1988, 1989; for a review see Bliss and Lynch, 1988). The maintenance of LTP appears in part due to an increase in glutamate release from presynaptic terminals (Bliss et al., 1986; Bekkers and Stevens, 1990; Malinow and Tsien, 1990). Among the possible molecular mechanisms underlying the increase in synaptic release, recent work focused attention on the activation of protein kinase C (PKC), a calcium/phospholipid-dependent enzyme (Nishizuka,

1984) involved in several neuronal processes, including neurotransmitter release and learning (Malenka et al., 1986; Corradetti et al., 1989; Olds et al., 1989).

In particular, several studies addressed the role of PKC in LTP using activators (Malenka et al., 1986; Colley et al., 1989) and/or inhibitors of PKC (Lovinger et al., 1987; Malinow et al., 1988, 1989) or intracellular injection of the purified enzyme (Hu et al., 1987). A correlation between changes in PKC distribution and learning has been found in the hippocampus (Olds et al., 1989). An activation of PKC, following a train of high frequency electrical stimulation able to evoke LTP in the hippocampus both in vivo and in vitro, has been demonstrated (Akers et al., 1986; Reymann et al., 1988; Malinow et al., 1988, 1989). Most of these studies did not discern between PKC in the pre- or postsynaptic compartment.

The B-50 protein (F1, GAP-43, neuromodulin) (Benowitz and Routtenberg, 1987; Andreasen et al., 1983), a nervous tissue-specific substrate of PKC (Aloyo et al., 1982), is predominantly localized in the presynaptic compartment (Gispen et al., 1985; Oestreicher and Gispen, 1986; Goslin et al., 1988; Van Lookeren Campagne et al., 1989; for review see Coggins and Zwiers, 1991), and its phosphorylation has been correlated to neurotransmitter release (Dekker et al., 1989a, 1989b). Most interestingly, in vivo LTP was demonstrated to be accompanied by an increased post hoc phosphorylation of protein B-50 (Akers and Routtenberg, 1985; Chan et al., 1986; Nelson et al., 1989), thereby suggesting a role for presynaptic PKC in LTP. To confirm and extend these observations and to circumvent problems associated with the post hoc assay (De Graan et al., 1989; Linden and Routtenberg, 1989), we studied the effect of tetanic stimulation in rat hippocampal slices on the in situ phosphorylation of B-50. At various times after induction of LTP, the extent of B-50 phosphorylation was measured following immunoprecipitation from the ³²P-prelabeled slices. Our results indicate occurrence of a relatively rapid and persistent increase in the degree of phosphorylation of the presynaptic substrate B-50 during maintenance of LTP.

Results

Extracellular Recording from Potentiated and Unpotentiated Slices

Stimulation of the stratum radiatum with a train of high frequency pulses (100 Hz, 1 s) was followed by LTP in 23 out of 27 preparations. The increase in synaptic efficacy was established immediately after the train, and within 10 min a stable potentiation lasting for longer than 1 hr was achieved (Figure 1A). At 60 min after the induction of LTP the average increase in slope of dendritic-evoked potentials was 74% ± 25%,

mean $\pm$ SEM ($n = 8$), and that in amplitude of evoked population spikes was $118\% \pm 40\%$, mean $\pm$ SEM ($n = 3$). Input-output curves obtained by gradual increases in stimulus strength, before and 60 min after induction of LTP, showed a leftward shift of the curve after LTP (Figures 1B and 1C). This indicates that the essential characteristics of LTP obtained in our experimental conditions are similar to those observed in submerged slices in the presence of phosphate (Chavez-Noriega et al., 1989).

In the four slices in which the high frequency stimulation did not induce LTP, the evoked responses showed only a posttetanic potentiation, and test re-

sponses declined within 5 min after the train (data not shown) to baseline responses (see Figure 3). Slices that did not receive a train of high frequency stimulation showed no significant changes in evoked responses throughout the experimental period (Figure 1A).

In Situ B-50 Phosphorylation during LTP

Estimation of phosphorylated B-50, expressed as a percentage of that of unstimulated slices, was carried out on potentiated slices matched with controls receiving only low frequency test pulses ($n = 6$ per each experimental group).

Figure 2 exemplifies the changes in B-50 phosphory-

Figure 2. Comparison of the Effects of Low Frequency or High Frequency Stimulation on B-50 Phosphorylation

(A) Autoradiogram of a representative experiment showing the level of phosphorylation of B-50 in one unstimulated slice (lanes 1) in comparison with that of the matched slices receiving a low frequency stimulation (lanes 2) or in which LTP was induced by a train of high frequency pulses (lanes 3). To show qualitatively the specificity of anti-B-50 antiserum, the remainder of the proteins from the three slices were pooled and either precipitated without anti-B-50 antiserum (lanes 4) or precipitated with anti-B-50 antiserum preabsorbed with purified B-50 (lanes 5). As expected in both cases, the disappearance of the B-50 band was observed. Note the presence of an unidentified protein band with an apparent molecular mass of 250 kd (lanes 1, 2, 3, and 5): the presence of this band after preabsorption with purified B-50 (lanes 5) indicates its binding to immunoglobulin G different from those raised against B-50.

(B) Densitometric scan of lanes 1-3 in (A). Scans of duplicate samples are shown superimposed. Calibration bar indicates 0.25 densitometric units.

Figure 3. The Increase in B-50 Phosphorylation Is Correlated with the Expression of LTP

(A) Stimulation of stratum radiatum at a frequency of 0.017 Hz (LFS; $n = 11$) for 70 min does not produce a significant change in B-50 phosphorylation (closed bars) in comparison with matched unstimulated slices taken as control values for biochemical estimation. During the same period the evoked potentials did not show any significant change (open bars; for the complete time course see Figure 1A). In slices where a train of high frequency stimulation (HFS) produced an LTP (HFS & LTP; $n = 11$) a significant increase in B-50 phosphorylation was observed 60 min after LTP induction ($P < 0.02$ versus LFS). In four slices in which the HFS did not induce LTP (HFS no LTP),

B-50 phosphorylation was not significantly increased in comparison with LFS ($P > 0.04$) but was significantly lower than that obtained in the HFS & LTP group ($P < 0.05$). Given are the means $\pm$ SEM. Asterisks indicate significant changes versus LFS and HFS no LTP. (B) Individual values of the increase in EPSP slope in slices receiving an HFS (HFS & LTP/HFS no LTP; [A]) plotted against the increase in B-50 phosphorylation. The slices were considered potentiated when the dendritic EPSP slope (closed circles) was increased by 30% or more 1 hr after the HFS. The three experiments in which the somatic response was recorded showed smaller increases in EPSP slope (closed squares) but were included in the potentiated group (in [A]) due to the evident increase in population spike amplitude ($+118\% \pm 40\%$; see text). A statistically significant correlation between the two parameters was found.

lation observed in a typical experiment in which LTP was followed for 60 min (lanes 1–3). The average values of B-50 phosphorylation from all the experiments ($n = 11$) in which LTP was followed for 60 min are summarized in Figure 3, together with the corresponding electrophysiological correlates. A significant increase in B-50 phosphorylation was found in the potentiated slices ($47\% \pm 8\%$, mean $\pm$ SEM; $p < 0.02$) but not in control slices ($11\% \pm 8\%$, mean $\pm$ SEM; $p > 0.1$).

Figure 3A also shows that in the four slices that received the high frequency train not followed by LTP, B-50 phosphorylation did not significantly differ from that of stimulated control slices. When the change in excitatory postsynaptic potential (EPSP) slope of responses evoked from tetanized (potentiated and unpotentiated) slices, measured 60 min after the delivery of high frequency pulses, was plotted against the increase in B-50 phosphorylation, a statistically significant ($P < 0.05$) correlation between the increases in B-50 phosphorylation and in synaptic efficacy was found (Figure 3B). In addition, when the phosphorylation of B-50 was measured 60 min after the high frequency train in slices in which LTP was prevented by the presence of $50 \mu\text{M}$ D(-)-2-amino-5-phosphonopentanoic acid (D-APV) (EPSP slope $106\% \pm 2\%$ of pretetanus value; mean $\pm$ SEM, $n = 8$), no significant change was found ($102\% \pm 10\%$ of unstimulated controls; mean $\pm$ SEM; $P > 0.2$).

Figure 4 shows the increase in B-50 phosphorylation at different intervals from the delivery of high frequency stimulation able to elicit LTP. Ten minutes after the train, B-50 phosphorylation values were not significantly different from those observed at 30 or 60 min after LTP induction.

LTP-Induced B-50 Phosphorylation Is Independent from Repeated Presynaptic Stimulation

To ensure that the B-50 phosphorylation was not a consequence of the repeated activation of the synaptic pathway, in four slices the monitoring of LTP by test pulses was interrupted 10 min after LTP induction, i.e., when a stabilization of a potentiated response could be observed. In this group of experiments the average increase in B-50 phosphorylation measured 60 min after the high frequency stimulation amounted to $41\% \pm 7\%$ (mean $\pm$ SEM), a value similar to that observed in the other potentiated slices ($47\% \pm 5\%$;

Figure 4. The Increase in B-50 Phosphorylation Occurs within 10 Min from the High Frequency Stimulation

The increase in phosphorylated B-50 (closed squares) is plotted against the time after high frequency stimulation. The time in abscissae is inclusive of the 4 min needed to remove the electrodes and to homogenize the slices. Given are the means $\pm$ SEM of four to nine experiments.

$n = 11$) and significantly ($p < 0.05$) higher than that measured in control unpotentiated slices ($7\% \pm 5\%$; $n = 11$).

Discussion

Our results demonstrate that, shortly after LTP induction by a train of high frequency stimulation in the stratum radiatum of the CA1 region in rat hippocampal slices, the phosphorylation of B-50, as measured *in situ*, is significantly increased in comparison with that of unpotentiated slices. The failure by a train of high frequency stimulation to produce LTP in some preparations is not an uncommon finding (Schwartzkroin and Wester, 1975). The reason(s) for these failures remains however elusive. A deterioration of the slices was unlikely, since the evoked potentials showed a posttetanic potentiation and no rundown during the 60 min following the high frequency train. On the other hand, a threshold for LTP induction has been repeatedly demonstrated as well as the need for cooperativity among the different fibers (for a detailed discussion see Bliss and Lynch, 1988). It is then conceivable that, in these slices, the level of activation required to induce LTP was not achieved by the afferent stimulation. Nevertheless, whatever the reason for the lack of induction of LTP in some preparations, the unchanged degree of B-50 phosphorylation in slices that failed to show LTP after the high frequency stimulation further supports that the increase in B-50 phosphorylation is strictly related to maintenance of LTP and not to the delivery of a high frequency train. This conclusion is confirmed by the lack of increase in B-50 phosphorylation in the experiments in which the induction of LTP by a high frequency train was prevented by the presence of D-APV. Finally, it has been recently shown that activation of presynaptic terminals after the train is not necessary for LTP maintenance (Pockett and Slack, 1991). Accordingly, our data show an increase in phosphorylation of B-50 in slices that received no low frequency test stimulation after induction of LTP. Taken together, the present data strongly support the suggestion that increased phosphorylation of this presynaptic protein is involved in maintenance aspects of LTP.

In transverse hippocampal sections the highest density of B-50 immunoreactivity has been observed in the CA1 region (Oestreicher and Gispén, 1986). Although the amounts of B-50 in the CA3 and dentate regions are not negligible as a whole, it is unlikely that the mossy fibers originating from the granule cells of dentate gyrus, as well as the terminals of the perforant pathway, were directly activated by our stimulation and therefore participated in the increased phosphorylation. On the other hand, since we measured changes in phosphorylated B-50 in the whole slices, this could have led to an underestimation of B-50 phosphorylation directly related to LTP in the CA1 region.

It has been repeatedly suggested (Bliss and Lynch, 1988; Linden and Routtenberg, 1989) that, following LTP induction, activation of PKC leads to an increased release of the excitatory neurotransmitter(s) (probably glutamate and/or aspartate; Nadler et al., 1976; Corradetti et al., 1983) from presynaptic nerve endings in the CA1 region of the hippocampus. The exact mechanism by which the phosphorylated B-50 might increase the electrophysiologically recorded synaptic efficacy is still a matter of investigation. Phosphorylation of B-50 by PKC in hippocampal slices and brain synaptosomes has been directly correlated with neurotransmitter release (Dekker et al., 1989a, 1989b). It is therefore conceivable that the phosphorylation of B-50 is involved in the LTP-dependent increase in the release of excitatory neurotransmitters. Although in our experimental conditions an inhibition of phosphatases cannot be disregarded, the phosphorylation of B-50 is more probably due to activation of a subtype of PKC during LTP (Akers et al., 1986; Malinow et al., 1988, 1989; Reymann et al., 1988). In line with this notion is the report by De Graan et al. (1988) who demonstrated that in hippocampal slices the PKC activator phorbol ester increased the *in situ* B-50 phosphorylation and induced LTP-like potentiation of synaptic activity. Furthermore, increasing evidence supports the notion that calcium-dependent enzymes, such as PKC and the calmodulin-dependent protein kinase II, form part of the molecular mechanisms underlying the various phases of LTP. In this context, the increased availability of calmodulin that follows the phosphorylation of B-50 (Lin and Storm, 1990; De Graan et al., 1990) could also promote the activation of the calmodulin-dependent protein kinase II in the presynaptic terminal and consequent phosphorylation of synapsin I, the degree of which has been recently demonstrated to be related to neurotransmitter release (Llinas et al., 1991).

In summary, our data provide direct evidence that, in presynaptic terminals of the pathway receiving a train of high frequency stimulation able to induce LTP, the phosphorylation of B-50 increases shortly (<10 min) after the train and persists for at least 1 hr. Such an effect may contribute to the sustained release of neurotransmitter(s) from the presynaptic terminals, necessary for the maintenance of LTP.

Experimental Procedures

Preparation of Slices, Incubation, and Electrophysiological Recording

Adult male Wistar-Kyoto rats, 200–400 g body weight, were purchased from Charles River, Italy. Hippocampal slices (400 μ m) obtained as previously described (Corradetti et al., 1989) were incubated for 1 hr, at room temperature, in an oxygenated (95% O_2 , 5% CO_2) artificial cerebrospinal fluid composed of 124 mM NaCl, 3.33 mM KCl, 1.25 mM KH_2PO_4 , 2 mM $MgSO_4$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, 10 mM D-glucose (pH 7.4). For each experimental assay, three slices were transferred at $35^\circ C \pm 1^\circ C$ in an interface-type static bath (0.7 ml) containing a virtually phosphate-free artificial cerebrospinal fluid (phosphate omitted). The slices were laid on a nylon net and were oxygenated by a humidi-

fied O₂, CO₂ mixture. The three slices were tested for electrophysiological viability by delivering test stimuli in the stratum radiatum through bipolar nichrome electrodes and extracellular recording from the dendritic or somatic CA1 pyramidal cell region. Control experiments showed that under these conditions stable recording of evoked responses could be followed for 4–6 hr, in spite of the absence of phosphates. To minimize damage of the tissue only one to three tracks were allowed in the attempt to optimize the electrophysiological recording. Then 100–120 μ Ci of [³²P]orthophosphate (and when appropriate D-APV, TOCRIS NEURAMIN; final concentration, 50 μ M) was added and allowed to equilibrate for 90 min before starting stimulation to collect baseline responses. Two slices were stimulated at 0.017 Hz (0.12 ms duration), and the third remained as the unstimulated control. Evoked responses were recorded on a digital tape recorder (48 kHz sampling rate, DTR 1200, BioLogic) for later analysis (DATA 6000, Analogic). After obtaining 10 min of control responses, using test stimuli evoking 30%–40% of maximal response in the preparation, one of the slices received either a train of high frequency stimulation (100 Hz, 1 s, near maximal stimulus strength) or a single test pulse. Then the slices were stimulated at 0.017 Hz for periods up to 1 hr. At the end of the stimulation period the electrodes were removed, and the net with the slices was rapidly transferred from the incubation bath to biochemical processing.

Estimation of Protein Phosphorylation

The quantification of the in situ phosphorylation of B-50 was carried out by adapting the method described by De Graan et al. (1989) to smaller samples. Briefly, the incubation was stopped by two quick washes in ice-cold buffer solution containing 1.2 mM NaHPO₄, 100 mM NaF, 10 mM EDTA, 5 mM EGTA (pH 7.4). Slices were homogenized in 60 μ l of the same solution in the absence of phosphates with Teflon plotter homogenizers. Part of the homogenate (40 μ l) was added to 20 μ l of stop-mix solution containing 62.5 mM (final concentration) Tris-HCl (pH 6.8), 2% SDS, 10% glycerol (v/v), 5% β -mercaptoethanol (v/v), 0.001% bromophenol blue and stored at 4°C until B-50 immunoprecipitation. The remainder of the homogenate was used to determine protein content (Bradford, 1976) and ³²P incorporation into total proteins by trichloroacetic acid precipitation: aliquots of homogenate (triplicate samples) were spotted on 3MM Whatman filter paper; the filters were washed with trichloroacetic acid, ethanol, and acetone (to remove phospholipids); and radioactivity was measured using a Packard 2000CA liquid scintillation counter.

Immunoprecipitation of ³²P-Labeled B-50

³²P labeling of B-50 was determined by quantitative immunoprecipitation of B-50 from the homogenate suspended in the stop-mix solution (De Graan et al., 1989). Overnight incubation of 15–25 μ g of total protein homogenate with the anti-B-50 antiserum (kindly provided by Dr. A. B. Oestreicher) at the final dilution of 1:200 was carried out at 4°C in 400 μ l of a solution containing 200 mM NaCl, 10 mM EDTA, 10 mM NaH₂PO₄, 0.5% Nonidet P-40 (v/v), and 0.1% SDS (pH 7.4). Specificity of the antiserum was tested by preabsorption with purified B-50 (Oestreicher et al., 1983; see also Figure 2, lanes 5). Pansorbin (Calbiochem) containing protein A was used to precipitate immunoglobulin G. After 45 min of incubation at room temperature, precipitates were collected by centrifugation, washed, resuspended in stop-mix solution, and boiled for 10 min. The suspensions were analyzed by 11% SDS-polyacrylamide gel electrophoresis in duplicate or triplicate, followed by autoradiography. Quantification of ³²P incorporation in B-50 was done using densitometric scanning of the autoradiogram.

Expression of Data

The ³²P incorporation of B-50 as measured by densitometric scanning was corrected for the values of total ³²P incorporation in the slices obtained with the trichloroacetic acid precipitation. Only the experiments showing less than 10% difference in ³²P total incorporation among slices of the same experiment were consid-

ered. Values of stimulated slices were expressed as percentage of the value of the unstimulated control in each experiment to allow statistical evaluation of results from different experiments. Statistical evaluation of the differences in B-50 phosphorylation and electrophysiological responses was carried out with the ANOVA analysis and the Student's *t* test for unpaired or paired samples as appropriate. Unless otherwise stated, values were considered significantly different for *P* < 0.05.

Acknowledgments

We are grateful to Dr. P. N. E. De Graan for helping us in setting up the biochemical assay and Dr. B. Oestreicher for supplying the antibodies. We thank Dr. A. Routtenberg and Dr. A. Leon for helpful discussion during the course of the work and reviewing the manuscript. We thank Ms. A. Porta for the skillful technical collaboration. This work was developed in collaboration with the Italian Consortium for Technologies and Drugs for the Cerebral Aging (CITFI) within the National Program of Pharmacological Research (Rif. 1139/191-078603), under the Italian Ministry of University Scientific and Technological Research (MURST). This work was partly supported by a Consiglio Nazionale delle Ricerche (CNR) grant (N. 91.00595.04) to R. C.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC Section 1734 solely to indicate this fact.

Received December 31, 1991; revised March 12, 1992.

References

- Akers, R., and Routtenberg, A. (1985). Protein kinase C phosphorylates a 47 M_r protein (F1) directly related to synaptic plasticity. *Brain Res.* 334, 147–151.
- Akers, R. F., Lovinger, D. M., Colley, P. A., Linden, D. J., and Routtenberg, A. (1986). Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. *Science* 231, 587–589.
- Aloyo, Y. J., Zwiers, H., and Gispen, W. H. (1982). B-50 protein kinase C in rat brain. *Prog. Brain Res.* 54, 303–315.
- Andreasen, T. J., Luetje, C. W., Heideman, W., and Storm, D. R. (1983). Purification of a novel calmodulin binding protein from bovine cerebral cortex membrane. *Biochemistry* 22, 4618–4624.
- Bekkers, J. M., and Stevens, C. F. (1990). Presynaptic mechanism for long-term potentiation in the hippocampus. *Nature* 346, 724–729.
- Benowitz, L. I., and Routtenberg, A. (1987). A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism, and synaptic plasticity. *Trends Neurosci.* 10, 527–532.
- Bliss, T. V. P., and Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of anaesthetized rabbit following stimulation of the perforant pathway. *J. Physiol.* 232, 331–356.
- Bliss, T. V. P., and Lynch, M. A. (1988). Long-term potentiation of synaptic transmission in the hippocampus: properties and mechanisms. In *Long-Term Potentiation: from Biophysics to Behavior*, P. W. Landfield and S. A. Deadwyler, eds. (New York: Alan R. Liss), pp. 3–72.
- Bliss, T. V. P., Douglas, R. M., Errington, M. L., and Lynch, M. A. (1986). Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats. *J. Physiol.* 377, 391–408.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of dye binding. *Anal. Biochem.* 72, 248–254.
- Chan, S. Y., Murakami, K., and Routtenberg, A. (1986). Phosphoprotein F1: purification and characterization of a brain kinase C substrate related to plasticity. *J. Neurosci.* 8, 3618–3627.

- Chavez-Noriega, L. E., Bliss, T. V. P., and Halliwell, J. V. (1989). The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. *Neurosci. Lett.* 104, 58-64.
- Coggins, P. J., and Zwiers, H. (1991). B-50 (GAP-43): biochemistry and functional neurochemistry of a neuron-specific phosphoprotein. *J. Neurochem.* 56, 1095-1106.
- Colley, P. A., Sheu, F.-S., and Routtenberg, A. (1989). Dose-dependent phorbol ester facilitation or blockade of hippocampal long-term potentiation: relation to membrane/cytosol distribution of protein kinase C activity. *Brain Res.* 495, 205-216.
- Collingridge, G. L., Kehl, S. J., and McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.* 334, 33-46.
- Corradetti, R., Moneti, G., Moroni, F., Pepeu, G., and Wieraszko, A. (1983). Electrical stimulation of the stratum radiatum increases the release and neosynthesis of aspartate, glutamate, and gamma-aminobutyric acid in rat hippocampal slices. *J. Neurochem.* 41, 1518-1525.
- Corradetti, R., Pugliese, A. M., and Ropert, N. (1989). The protein kinase C inhibitor 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7) disinhibits CA1 pyramidal cells in rat hippocampal slices. *Br. J. Pharmacol.* 98, 1376-1382.
- De Graan, P. N. E., Heemskerk, F. M. J., Dekker, L. V., Melchers, B. P. C., Gianotti, C., and Schrama, L. H. (1988). Phorbol esters induce long- and short-term enhancement of B-50/GAP43 phosphorylation in rat hippocampal slices. *Neurosci. Res. Commun.* 3, 175-183.
- De Graan, P. N. E., Dekker, L. V., Oestreicher, A. B., Van der Voorn, L., and Gispen, W. H. (1989). Determination of changes in the phosphorylation state of the neuron-specific protein kinase C substrate B-50 (GAP43) by quantitative immunoprecipitation. *J. Neurochem.* 52, 17-23.
- De Graan, P. N. E., Oestreicher, A. B., De Wit, M., Kroef, M., Schrama, L. H., and Gispen, W. H. (1990). Evidence for the binding of calmodulin to endogenous B-50 (GAP-43) in native synaptosomal plasma membranes. *J. Neurochem.* 55, 2139-2141.
- Dekker, L. V., De Graan, P. N. E., Oestreicher, A. B., Versteeg, D. H. G., and Gispen, W. H. (1989a). Inhibition of noradrenaline release by antibodies to B-50 (GAP43). *Nature* 342, 74-76.
- Dekker, L. V., De Graan, P. N. E., Versteeg, D. H. G., Oestreicher, A. B., and Gispen, W. H. (1989b). Phosphorylation of B-50 (GAP43) is correlated with neurotransmitter release in rat hippocampal slices. *J. Neurochem.* 52, 24-30.
- Gispen, W. H., Leunissen, J. L. M., Oestreicher, A. B., Verkleis, A. J., and Zwiers, H. (1985). Presynaptic localization of B-50 phosphoprotein: the (ACTH)-sensitive protein kinase substrate involved in rat brain polyphosphoinositide turnover. *Brain Res.* 328, 381-385.
- Goslin, K., Schreyer, D. J., Skene, J. H. P., and Banker, G. (1988). Development of neuronal polarity: GAP-43 distinguished axonal from dendritic growth cones. *Nature* 336, 672-674.
- Hu, G.-Y., Hvalby, O., Walaas, S. I., Albert, K. A., Skjeflo, P., Andersen, P., and Greengard, P. (1987). Protein kinase C injection into hippocampal pyramidal cell elicits features of long term potentiation. *Nature* 328, 426-429.
- Lin, Y., and Storm, D. R. (1990). Regulation of free calmodulin levels by neuromodulin: neuron growth and regeneration. *Trends Pharmacol. Sci.* 11, 107-111.
- Linden, D. J., and Routtenberg, A. (1989). The role of protein kinase C in long-term potentiation: a testable model. *Brain Res. Rev.* 14, 279-296.
- Llinas, R., Gruner, J. A., Sugimori, M., McGuinness, T. L., and Greengard, P. (1991). Regulation by synapsin I and Ca²⁺-calmodulin-dependent protein kinase II of transmitter release in squid giant synapse. *J. Physiol.* 436, 257-282.
- Lovinger, D. M., Wong, K. L., Murakami, K., and Routtenberg, A. (1987). Protein kinase C inhibitors eliminate hippocampal long-term potentiation. *Brain Res.* 436, 177-183.
- Lynch, M. A., Errington, M. L., and Bliss, T. V. P. (1989). Nordihydroguaiaretic acid blocks the synaptic component of long-term potentiation and the associated increases in release of glutamate and arachidonate: an *in vivo* study in the dentate gyrus of the rat. *Neuroscience* 30, 693-701.
- Malenka, R. C., Madison, D. V., and Nicoll, R. A. (1986). Potentiation of synaptic transmission in the hippocampus by phorbol esters. *Nature* 321, 175-177.
- Malinow, R., and Tsien, R. W. (1990). Presynaptic enhancement shown by whole-cell recording of long-term potentiation in hippocampal slices. *Nature* 346, 177-180.
- Malinow, R., Madison, D. V., and Tsien, R. W. (1988). Persistent protein kinase C activity underlying long-term potentiation. *Nature* 335, 820-824.
- Malinow, R., Schulman, H., and Tsien, R. W. (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* 245, 862-866.
- Nadler, J. V., Vaca, K. W., White, W. F., Lynch, G. S., and Cotman, C. W. (1976). Aspartate and glutamate as possible neurotransmitters of excitatory hippocampal afferents. *Nature* 260, 538-540.
- Nelson, R. B., Linden, D. J., and Routtenberg, A. (1989). Phosphoproteins localized to presynaptic terminal linked to persistence of long-term potentiation (LTP): quantitative analysis of two-dimensional gels. *Brain Res.* 497, 30-42.
- Nishizuka, Y. (1984). Studies and perspectives of protein kinase C. *Science* 233, 305-312.
- Oestreicher, A. B., and Gispen, W. H. (1986). Comparison of immunocytochemical distribution of the phosphoprotein B-50 in the cerebellum and hippocampus of immature and adult rat brain. *Brain Res.* 375, 267-279.
- Oestreicher, A. B., Van Dongen, C. J., Zwiers, H., and Gispen, W. H. (1983). Affinity-purified anti-B-50 protein antibody: interference with the function of phosphoprotein B-50 in synaptic plasma membranes. *J. Neurochem.* 41, 331-340.
- Olds, J. L., Anderson, M. L., McPhie, D. L., Staten, L. D., and Alkon, D. L. (1989). Imaging of memory-specific changes in the distribution of protein kinase C in the hippocampus. *Science* 245, 866-869.
- Pockett, S., and Slack, J. R. (1991). Presynaptic activity in the period immediately after conditioning is not necessary for long-term potentiation. *Neurosci. Lett.* 121, 81-82.
- Reymann, K. G., Brodemann, R., Kase, H., and Matthies, H. (1988). Inhibitors of calmodulin and protein kinase C block different phases of hippocampal long-term potentiation. *Brain Res.* 461, 388-392.
- Schwartzkroin, P. A., and Wester, K. (1975). Long-lasting facilitation of a synaptic potential following tetanization in the *in vitro* hippocampal slice. *Brain Res.* 89, 107-119.
- Teyler, T. J., and DiScenna, P. (1987). Long-term potentiation. *Annu. Rev. Neurosci.* 10, 131-161.
- Van Lookeren Campagne, M., Oestreicher, A. B., Van Bergen en Henegouwen, P. M. P., and Gispen, W. H. (1989). Ultrastructural immunocytochemical localization of B-50/GAP43, a protein kinase C substrate, in isolated presynaptic nerve terminals and neuronal growth cones. *J. Neurocytol.* 18, 479-489.