Biochimica et Biophysica Acta 854 (1986) 124–132
Elsevier

BBA 72860

Stimulation of protein methylase II from *Torpedo marmorata* by cholinergic effectors

Joachim H. Nuske

Institut für Biochemie der Freien Universität Berlin, Arbeitsgruppe Neurochemie, Thielallee 63, D-1000 Berlin 33 (Germany)

(Received May 14th, 1985)

Key words: Acetylcholine receptor; Protein methylase II; Stimulus-dependent transmethylation; (*T. marmorata*)

The enzymatic transfer of methyl groups mediated by protein methylase II onto proteins of the electroplaque tissue of *Torpedo marmorata* is described. The protein methylase II resides to the extent of 80% in the cytosol and 20% in the acetylcholine receptor-rich membrane. The kinetics of the methyl-group transfer are characteristically different in the cytosol and membrane fractions. The reaction is inhibited by phosphate with $IC_{50} = 450$ μM. The cholinergic effectors carbamoylcholine, flaxedil and α-bungarotoxin applied to the outside of the acetylcholine receptor-rich membrane vesicles stimulated the protein methylase II which is exclusively located inside the vesicles. The stimulation is biphasic and transient, yielding an increased initial velocity and a peak of activity at 2 min after the addition of the effector. The stimulation by carbamoylcholine is qualitatively similar to that elicited by the antagonist. In addition, the protein methylase II is stimulated transiently by phospholipase A_2 with a time-course clearly different from that of the cholinergic effectors. We conclude that the conformational change in the receptor-protein elicited by cholinergic effectors is efficiently transduced to the cytoplasmic methylation sites.

Introduction

Protein methylase II (*S*-adenosyl-L-methionine : protein-L-glutamate *O*-methyltransferase, EC 2.1.1.24), also called protein carboxylmethylase, extensively reviewed by Paik and Kim [1] and Kim [2], is an enzyme ubiquitous in mammalian tissue. Its presence was also shown in the Chondrichthyes (*Torpedo*) [3–5], Crustaceae (*Astacus*) (Nuske, unpublished results), Acrasiales (*Dictyostelium*) [6–8], Angiospermae (*Triticum*) [9] and Archaebacteriae (*Caldariella*) [10]. In some tissues or organisms, respectively, it was merely demonstrated or characterized, while in others the relationship between methylation of cell proteins and adequate stimuli to the cell was established. In *Dictyostelium* [8] and human neutrophils [11] one response to chemotactic stimuli was shown to be this protein methylation. The specific ligands of the serotonin receptors of the cells of developing palate tissue [12] and those of the presynaptic dopamin autoreceptors [13,14] elicited an increase in the protein methylation levels.

Since the product of the eucaryotic enzyme is intrinsically unstable under physiological conditions – the half-life of the methyl-groups may be less than 5 min [15] – the identification of the modified proteins is severely impeded. So far calmodulin is assumed to be one of the best substrate proteins [16,17], but as many as 30 different proteins were reported to have been methylated

Abbreviations: AdoMet, *S*-adenosylmethionine; [^{3}H]AdoMet, *S*-[*methyl*-^{3}H]adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; PMSF, phenylmethylsulfonyl fluoride; TPMP, triphenylmethylphosphonium.

upon an adequate stimulus [18]. In red blood cells integral membrane proteins and membrane-cytoskeleton linking proteins were demonstrated to be methylated after incubation under appropriate conditions for a prolonged time [19–22].

The electroplaque tissue of the electric ray *Torpedo californica* was reported to contain the protein methylase II [3] and the acetylcholine receptor was shown to be methylated also from the extracellular side by an enzyme from mammalian brain [4]. A transfer of methyl-groups onto the cholinergic binding site was ruled out and an enhencement of transmethylation by α-bungarotoxin was not apparent. Since the protein methylase II is known not to be very selective for substrate proteins that are not in their native environment [1], the functional interdependence of receptor stimulation and enzyme activation has to be established separately and stringently.

Here we report details of the activity of the protein methylase II present in *Torpedo marmorata* electroplaque tissue, its inhibition by sodium phosphate and the transient increase in methylation levels of proteins of an acetylcholine receptor-rich membrane preparation upon application of cholinergic effectors. We will discuss the possible involvement of methylation in the response of the acetylcholine receptor to an artificially prolonged stimulus.

Material and Methods

Material

S-[*methyl*-^{3}H]Adenosylmethionine ([^{3}H]Ado-Met) (12–14 Ci/mmol) was purchased from New England Nuclear, Dreieich, F.R.G; α-bungarotoxin was obtained from Rohm & Haas, Philadelphia, U.S.A.; Flaxedil from Boehringer-Ingelheim, F.R.G., and carbamoylcholine from E. Merck, Darmstadt, F.R.G. Ultramembranes were obtained from Amicon Corp., Witten, F.R.G., and nitrocellulose filters, BA 85, from Schleicher and Schüll, Dassel, F.R.G.

All other chemicals were from common sources and of reagent grade. *T. marmorata* was obtained live and killed by mechanical impact. The removed electroplaque tissue was frozen immediately in liquid nitrogen and stored at −70°C.

Preparation of the acetylcholine receptor-rich membranes

Membranes were prepared as described in detail by Schiebler and Hucho [23]. In short, the electroplaque tissue was homogenized with two volumes of 20 mM sodium phosphate buffer, pH 7.4, containing 400 mM NaCl, 2 mM EDTA and 10^{-4} M PMSF. Soluble proteins were removed by sedimenting all particulate material at $27000 \times g$ for 9 min in a refrigerated centrifuge. The pellet was homogenized and extracted in the same buffer without NaCl and sedimented at $39000 \times g$ for 90 min. This extraction cycle is repeated twice. The upper layer of the last pellet was resuspended in a small volume of the same buffer, layered on top of a sucrose gradient (25–50%, w/v), and subjected to isopycnic centrifugation. The isolated acetylcholine receptor-rich membranes were washed free of sucrose and resuspended to a concentration of approx. 3 mg protein/ml. These isolated membranes were subjected to the assay without further treatment.

The first supernatant was extensively dialysed against 5 mM sodium borate buffer, pH 9.2, containing 5 mM EDTA and 2.4 mM β-mercaptoethanol. The resulting turbidity was removed by centrifugation at $100000 \times g$ for 30 min. This preparation is called the soluble fraction of electroplaque tissue proteins. The supernatant was fractionated by sequential ultrafiltration over Amicon PM-30 and PM-10 membranes. The fraction retained by the PM-10 membrane yielded a 5-fold-enriched enzyme fraction, which contained considerably less endogenous substrate protein than the original supernatant.

Incorporation of the enzyme-enriched fraction into membrane vesicles

Acetylcholine receptor-rich membrane vesicles were sedimented and resuspended to 10-times their volume with the enzyme-enriched fraction. The suspension was sonicated three times for 15 s in a cleansing bath-type sonifier with 30-s intervals, passed rapidly through a syringe (20 strokes, 22 gauge needle) and sonicated again. The membranes were diluted 50-fold and sedimented. The washing was repeated, the vesicles resuspended with 50 mM Hepes-buffer, pH 6.2, and subjected to the enzyme assay.

Assay of the protein methylase II

The protein methylase II is identified by the intrinsic instability of the transferred methyl-groups at mildly alkaline pH values. Aliquots of the soluble fraction, the acetylcholine receptor-rich membrane fraction or the membranes into which the enzyme-enriched fraction was incorporated were adjusted to 0.1 mg protein/ml and incubated with 50 mM Hepes, pH 6.2, 20 μM [^{3}H]AdoMet (750 mCi/mmol) and the indicated additions at 37°C. At the indicated times, the reaction was stopped by addition to the same volume of ice-cold 20% trichloroacetic acid. The proteins were allowed to precipitate for 30 min on ice. The sample was divided and transferred to pre-wetted nitrocellulose membrane filters. The bound proteins were washed three times with 5 ml of 7% perchloric acid to remove the unreacted [^{3}H]AdoMet. The first filter was subjected to scintillation counting immediately. The second filter was buffered to pH 11 with 100 mM sodium borate buffer and incubated for 1 h at room temperature in a wet chamber to hydrolyze the unstable methyl-groups. After acidification and renewed washing to remove the generated methanol the filter was subjected to scintillation counting, too. The difference in the radioactivity between the two filters was taken as a measure of the activity of the protein methylase II, expressed as pmoles of methyl-groups incorporated into protein. Samples to which the trichloroacetic acid was added prior to the [^{3}H]AdoMet were used as controls.

Assay of AdoMet uptake

The method to determine the uptake rates of AdoMet into acetylcholine receptor-rich membrane vesicles was essentially the same as that used for the trasmethylation assay. At the end of the incubation the vesicles were not precipitated, but rapidly ultrafiltrated over pre-wetted nitrocellulose filters and immediately washed three times with 3 ml of ice-cold buffer (50 mM Hepes, pH 6.2). The filters were subjected to scintillation counting. Filters to which 100 μl of incubation buffer had been added served as control for unspecific binding of [^{3}H]AdoMet to the nitrocellulose membranes.

Protein determination

Protein was determined according to the methods of Bradford [24] or Lowry et al. [25] using bovine serum albumin as a standard.

Results

Uptake of AdoMet into acetylcholine receptor-rich membrane vesicles

The rate of the methyl-group transfer is dependent on the concentration of the co-substrate AdoMet inside the vesicles. So it is necessary to know its ambient concentrations during the time of the experiment (usually less than 10 min after the start of the reaction). As is shown in Table I, the AdoMet associated rapidly with the membrane vesicles, with a half-time of about 20 s and to a maximum of about 3 pmol. The total vesicle volume as estimated in this work is in good agreement with that determined by Changeux and coworkers [26] in spite of a different preparation protocol. From these data the internal AdoMet concentration at equilibrium was calculated to be 17.8 μM, deviating by only 9% from the maximally attainable concentration of 20 μM. Thus it seems reasonable to think of the vesicle-associated AdoMet as taken up and not as merely bound to some surface or transport protein. It should be noted that these membrane preparations are tot-

TABLE I

PARAMETERS OF *S*-ADENOSYLMETHIONINE UPTAKE INTO ACETYLCHOLINE RECEPTOR-RICH MEMBRANE VESICLES OF *TORPEDO MARMORATA* ELECTROPLAQUE TISSUE

Freshly prepared acetylcholine receptor-rich membrane vesicles at a concentration of 0.1 mg/ml protein were incubated in 50 mM Hepes, pH 6.2, containing 20 μM [^{3}H]AdoMet at 37°C. The time for incubation was varied between 10 s and 10 min. The uptake was stopped by rapidly removing the incubation buffer by filtration. Where indicated the data represent the means of five determinations ± S.E.

$t_{1/2}$ (min)	AdoMet incorporated (pmol)	Total [a] vesicle volume (μl)	Final [b] AdoMet concentration (μM)
0.36 ± 0.02	3.2 ± 0.21	0.18	17.8 ± 1.2

[a] Estimated from electronmicrographs and protein content.
[b] Inside the membrane vesicles.

ally de-energized, i.e. they contain no ATP and have no ionic gradient across the membrane. So the uptake of AdoMet is not facilitated by transport and the AdoMet cannot accumulate to concentrations exceeding that of the incubation buffer.

Activity of the protein methylase II in the soluble and the acetylcholine receptor-rich membrane fraction

Since the membrane preparation and the soluble fraction contain sufficient endogenous substrate proteins which are as yet unidentified, no external substrate protein was added to the assays.

The protein methylase II resides to the extent of about 20% in the acetylcholine receptor-rich membrane and about 80% in the soluble fraction, when prepared as described (Table II). Despite the uncertainty of the extent of the losses during the preparation, that ratio seems to be a fair estimate.

There are at least two distinct groups of substrate proteins as judged by the hydrolysis kinetics of the methyl-groups incorporated. The times of half-maximal hydrolysis are 1 min and 27 min, respectively, at pH 7.4 (data not shown).

The time course of the methyl-group transfer shows the enzyme activity of the soluble fraction to be biphasic (Fig. 1). It is fitted best by two curves of apparently hyperbolic character. The initial velocities of the two apparent transfer reactions were determined as 4.4 pmol/mg per min and 1.7 pmol/mg per min, respectively. The calculated parameters of the first transfer reaction indicate a steady state at 15.5 pmol/mg protein with a $t_{1/2}$ of 4 min. Before this reaction is completed it is superseded by the second that has a steady-state concentration of incorporated methyl-groups at 70 pmol/mg protein and a $t_{1/2}$ of 41 min. The parameters of these two curves are summarized in Table II.

The transmethylation in the acetylcholine receptor-rich membrane fraction can be fitted to a hyperbola, too. But in the membrane preparation the reaction proceeds faster and to a higher level (Fig. 1). The steady state is reached with an initial velocity of 220 pmol/mg per min ($t_{1/2} = 0.5$ min). Detailed analysis showed this hyperbola to be an approximation only and the transmethylation to be a rather complicated series of distinct enzyme kinetics which will be discussed elsewhere. The parameters of the approximated hyperbola are shown in Table II.

TABLE II

KINETIC PARAMETERS OF THE TRANSMETHYLATION REACTION IN THE SOLUBLE AND ACETYLCHOLINE RECEPTOR-RICH MEMBRANE VESICLE FRACTION OF *T. MARMORATA* ELECTROPLAQUE TISSUE

Freshly prepared acetylcholine receptor-rich membrane vesicles at a concentration of 0.1 mg/ml protein were incubated in 50 mM Hepes, pH 6.2, containing 20 μM [^{3}H]AdoMet at 37°C for 10 min. The reaction was stopped by addition of trichloroacetic acid. The filters onto which the precipitates were recovered were handled as detailed in the Methods section. For the estimation of total activity the amount of methyl-groups transferred in 10 min was used.

	Initial velocity (pmol/mg per min)	Degree of transmethylation at the steady state (pmol/mg)	$t_{1/2}$ [a] (min)	Total [b] activity (pmol)	Final total activity (%)
Soluble fraction [c]	4.4	15.5	4	5250	84
	1.7	70.0	41		
Acetylcholine receptor-rich membrane vesicles	221	118	0.5	1020 (979) [d]	16

[a] Time required to achieve the half-maximal degree of transmethylation.

[b] Typical values for the preparation used are: soluble fraction, 375 mg protein; acetylcholine receptor rich membrane vesicles, 10 mg protein.

[c] Parameters of the two best-fitting curves.

[d] Total activity determined by incorporating the [^{3}H]AdoMet into the membrane vesicles by osmotic shock.

Inhibition by phosphate

In the course of our investigations into the ideal buffering conditions for the protein methylase II we became aware that phosphate buffers inhibited the incorporation of methyl-groups into proteins. This was a bewildering finding, since a survey of the published literature showed that most authors used at least 5 mM sodium phosphate buffers. When assaying the protein methylase II from electroplaque tissue with varying concentrations of phosphate, we found the inhibition dose-dependent and half-maximal at about 450 μM (Fig. 2). This represents an inhibition of 85% at a phosphate concentration of 5 mM.

Inhibition of the protein methylase II by sodium ion was ruled out. Since the intrinsic instability of the transferred methyl-groups is independent of the presence or absence of phosphate, but is only dependent on the ambient pH, the phosphate is assumed to be an inhibitor of the methyl-group transfer reaction.

Response to cholinergic effectors

To investigate the activation of the protein methylase II by effectors of the nicotinic acetylcholine receptor of the motor endplate we incorporated the partially purified enzyme into the acetylcholine receptor-rich membrane vesicles.

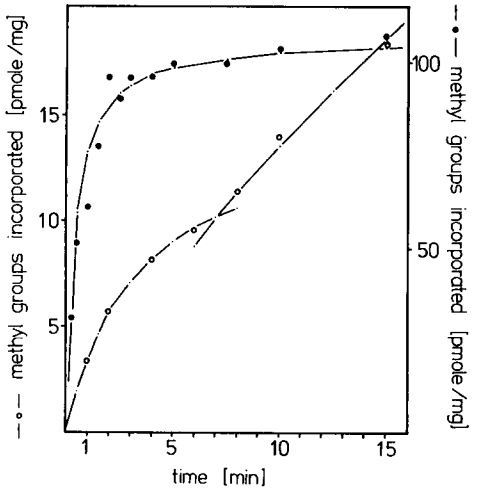

Fig. 1. Unstimulated activity of the protein methylase II of acetylcholine receptor-rich membranes (●) or the cytosolic fraction (○), incubated with 20 μM [^{3}H]AdoMet at 37°C. ·–·–·, the best-fitting curves calculated by a least-squares algorithm.

Control experiments without cholinergic effectors showed a degree of methyl-group transfer controlled by the soluble fraction of the enzyme, i.e. about 10 pmol/mg methyl-groups incorporated in 3 min (see Figs. 1 and 3). We calculated a standard control curve to compare the transmethylations in the presence of effectors. The regular deviations of this standard curve from the displayed hyperbolic behavior are not shown and will be discussed in a separate paper. In this study we tested the agonist carbamoylcholine (carbachol, 10^{-4} M), the competitive antagonist flaxedil (10^{-4} M), the neurotoxin α-bungarotoxin (10^{-6} M), and the phospholipase A_2 (10^{-6} M). The latter was necessary to prove the results with α-bungarotoxin not to be artifacts due to the phospholipase, which is a common contaminant of neurotoxin preparations from snake venoms.

The experiment was started by the simultaneous addition of the effector and [^{3}H]AdoMet to the assay to avoid both desensitization of the receptor and inhibition or abortion of the response by the formation of *S*-adenosylhomocysteine (AdoHcy), the co-product of the transmethylation and a potent inhibitor of the protein methylase II, inside the membrane vesicles. Since the effectors were added to the outside of the vesicles and the enzyme was located exclusively inside the vesicles, any specific stimulation of the protein methylase II activity by the cholinergic effectors had to be mediated by the acetylcholine receptor. The cholinergic effectors elicited two apparently tran-

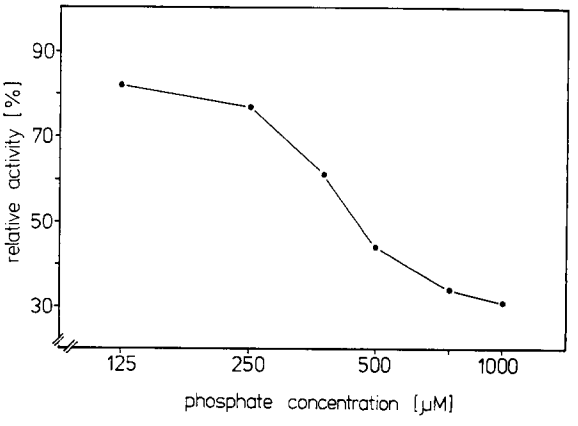

Fig. 2. Inhibition of the protein methylase II activity of the cytosolic fraction by various concentrations of sodium phosphate.

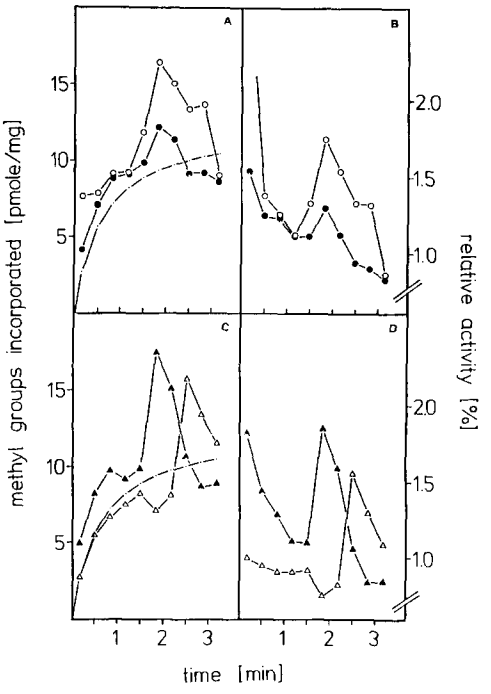

Fig. 3. Stimulation of the protein methylase II by cholinergic effectors and phospholipase A_2. Enzyme-enriched acetylcholine receptor-rich membrane vesicles were incubated with 20 μM [^{3}H]AdoMet at 37°C in the presence of either 10^{-4} M carbamoylcholine (●) or 10^{-4} M flaxedil (○), 10^{-6} M α-bungarotoxin (▲) or 10^{-6} M phospholipase A_2 (△). The reaction was started by the simultaneous addition of the respective effector and [^{3}H]AdoMet. ·–·–·, control curve, calculated as described in the text. A, C, absolute amounts of methyl-groups incorporated; B, D, the respective relative amounts.

sient changes in the enzyme activity (Fig. 3). They induced an increased initial velocity and – most prominent – a peak of enzyme activity at about 2 min after the start of the reaction. The competitive antagonist and the neurotoxin elicited a stronger response than the agonist. The time course of the transmethylation evoked by the phospholipase A_2 was obviously different in both aspects. The initial velocity was similar to that of the control, the transient increase in activity was markedly delayed and preceded by a slight decrease in the degree of methylation. Thus a contamination of the α-bungarotoxin with phospholipase A_2 can be ruled out.

The increased initial velocity of the methyl-group transfer might be induced by an increased influx of AdoMet due to stimulation by the effectors. This can be ruled out for the later transient peak of activity, because at 2 min the AdoMet has equilibrated into the vesicles.

Discussion

Since the properties of protein methylase II were extensively reviewed by Paik and Kim [1] and more recently by Kim [2], and its role in mammalian tissues was discussed by O'Dea et al. [27], it may be sufficient to summarize the major fields of research in this enzyme. The current investigations focus on three main areas: (i) the involvement in signal transduction over an excitable membrane [4,8,12,13,28,29], (ii) the modulation of enzyme activities located in the cytoplasm [16,30], and (iii) the identity of the substrate proteins and their modified amino acid moiety [19,31,32]. With this work we would like to contribute to the growing knowledge of the series of events involved in the transfer of signals over the plasma membranes of cells of excitable tissues as exemplified by the acetylcholine receptor-rich membrane of the electric ray *Torpedo mormorata.*

In the course of this study using the *T. marmorata* electroplaque tissue we became aware of some points of disagreement with the published literature. Firstly, the enzyme kinetics are curvilinear and in most cases at least biphasic. The nonlinearity is readily explained by the crude enzyme preparation used, since the apparent levels of methyl-groups incorporated depend on several factors: (i) the activity of a methyltransferase, (ii) the activity of a methylesterase, as suggested by Gagnon [33], (iii) the species of the protein methylated, since the half-life of the methyl-groups is protein-specific, and (iv) the presence of inhibitors. Activators are not known. Especially in membrane preparations the level of the steady state is strongly influenced by *S*-adenosylhomocysteine (AdoHcy), the co-product of the transmethylase reaction and a potent inhibitor of the protein methylase II. The AdoHcy is unable to pass the membrane [34] and so it is enriched inside the vesicles during the assay and progressively inhibits the enzyme activity. On the other hand, we showed AdoMet to equilibrate freely into the vesicles, as is proved, too, by the absence of any increase in transmethylation when equilibrating the lumen of the vesicles with AdoMet by osmotic shock (Table

II). A characteristic steady state of methyl-group transfer will be the result of several or all of these degrading and inhibiting activities acting on the protein methylase II and its products. The details of such a complex situation are dependent on the tissue investigated and the reproducibility of the preparation method used, i.e. of the complement of enzymes, endogenous substrate proteins and inhibitors present. The preparation of acetylcholine receptor-rich membranes as described here is a routine method of this laboratory and was shown by neurochemical experiments to be highly reproducible, free of artifacts and the vesicles to be functionally active and responding to cholinergic effectors efficiently [23].

Furthermore, the protein methylase II seems to use more than one protein as substrate. Though calmodulin was shown to be preferred [16,17], as many as 30 methylated proteins may be detected by suitable methods [28]. Therefore multiple kinetics may reflect different rates of methylation of distinct substrate proteins.

Moreover, our experiments did show an inhibition of the transmethylase reaction by phosphate. A half-maximal inhibition was achieved with a concentration of about 450 μM phosphate. In our attempts to purify the protein methylase II by affinity chromatography on AdoHcy-Sepharose exactly as described by Kim et al. [35], we found phosphate both essential to bind the enzyme to the affinity matrix and, when in excess, to elute the enzyme even in the absence of the soluble ligand AdoMet (data not shown). Electrophoretic analysis of the eluted fractions revealed them to be contaminated with proteins, including those able to serve as substrate proteins. Thus an interaction may be postulated between phosphate, substrate proteins and the immobilized AdoHcy. Furthermore, assays performed in the presence of phosphate may reflect the methylation of a subpopulation of the substrate proteins only and so generate the more or less linear and monophasic kinetics reported by others.

Previously an enhancement of the methyl-group transfer onto acetylcholine receptors of *Torpedo californica* in the presence of α-bungarotoxin was not found [3]. But this observation was made by determining the amount of incorporation of methyl-groups after a prolonged time of incubation and using a detergent-solubilized receptor preparation. So the transient changes occurring in the very first minutes after the start of the reaction were obviously missed. Only the detailed analysis of the early time courses will yield information on the effects of stimulation on the methyl-group transfer onto proteins of the acetylcholine receptor-rich membrane.

If the protein methylated is the acetylcholine receptor the transmethylation reaction would be highly substoichiometric with an estimated molar ratio of about 1%. But the identity of the methylated protein(s) has yet to be established.

The agonist carbamoylcholine causes the same transient increase as the competitive antagonist flaxedil and the neurotoxin α-bungarotoxin, albeit less pronounced. This appears at first paradoxical, since the effects of agonists and antagonists are usually presumed to be opposite. But the acetylcholine receptor is believed to be slowly desensitized by prolonged exposure to agonists — as is the case under the conditions of this assay. Desensitization is characterized by a decrease in the dissociation constant, this leading to extended occupation of the binding sites, and by holding the ion channel in a closed state. As the receptor is methylated while being in this desensitized state its conformation will be similar to that induced by the competitive antagonist and the neurotoxin and it is the change in conformation that renders the receptor or an associated protein susceptible to the modification by the protein methylase II.

This interpretation is supported by the carbamoylcholine- and flaxedil-induced incorporation of the photoaffinity label triphenylmethylphosphonium (TPMP) into the acetylcholine receptor [36] when determined under conditions similar to those used in the transmethylation assay. Evidently the conformational state of the receptor being subjected to slowly desensitizing-conditions and the state induced by competitive antagonists are comparable or even identical with respect to the binding of the photoaffinity ligand. Since the TPMP is acting from the outside and the protein methylase II from the inner side of the vesicles these conformational changes are apparently transmitted through the membrane. It will be important to learn whether the occupation of the binding sites or the closed state of the ion

channel is the ultimate reason for the enhanced susceptibility to the protein methylase II.

Unexpectedly the phospholipase A_2 elicited a methyl-group transfer, too. The time course of that transfer was clearly different from those of the cholinergic effectors, bearing only a resemblance to the last part of the response to the flaxedil stimulus. There might be several explanations for this finding: either the phospholipase may change the conformation of the receptor via a modification of the lipid composition of the membrane or the action of the phospholipase may by itself be stimulating to the protein methylase II or, whatever the mode of activation, the proteins methylated are different. Likewise the prolonged stimulation of the protein methylase II by flaxedil may be elicited by the tendency of this effector to partition into the lipid phase of the membrane.

The protein methylase II is strongly connected to the series of events involved in the transduction of adequate stimuli over the membrane and eliciting a specific response. Stimulation of the dopamin receptor [13], the serotonin receptor [12], all the chemotactic receptors so far investigated [6,11,31,38], led to an activation of the protein methylase II or to an increase in its activity. But only the acetylcholine receptor-rich membrane and the *Dictyostelium* amoebae have been investigated in more detail. In *Dictyostelium* the methylation response to a chemotactic stimulus is biphasic, too [7]. The fast transient increase in methylation levels at about 10 s after application of the stimulus seems to be related to the initiation of cytotaxis, the slower response, at about 3 min after application of the stimulus, to an adaptation to the stimulus. The former coincided with transient increases in Ca^{2+} and cGMP levels, while the second is considerably prolonged upon application of a non-removable analogue of the chemoattractant [39,40]. Likewise, the mesenchymal cells of the developing palate respond to serotonin with cytotaxis, activation of protein methylase II and a transient increase in cGMP levels [12].

By its biphasic and transient activity the acetylcholine receptor-dependent protein methylase II is comparable to that of *Dictyostelium* amoebae responding to their chemotactic agent. The acetylcholine receptor is different insofar as the enzyme is activated by both the agonist and the antagonists. In *Dictyostelium* it was shown that a defined sequence of distinct proteins served as substrates [8]. It will be interesting to learn what proteins are methylated in the acetylcholine receptor-rich membranes of *Torpedo marmorata.*

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Grant Hu 146/8-3 and Nu 25/4-1). The careful and absolutely reproducible preparation of the acetylcholine receptor-rich membranes by H. Bayer and G. Bandini was an essential prerequisite of this work and is gratefully acknowledged. I am especially grateful for the continuous support by Dr. F. Hucho.

References

1 Paik, W.R. and Kim, S. (1980) Protein Methylation; Biochemistry, A series of monographs, Vol. 1, John Wiley & Sons, New York
2 Kim, S. (1984) Methods Enzymol. 106, 295–309
3 Kloog, Y., Flynn, D., Hoffman, A.R. and Axelrod, J. (1980) Biochem. Biophys. Res. Commun. 97, 1474–1480
4 Flynn, D., Kloog, Y., Potter, L.T. and Axelrod, J. (1982) J. Biol. Chem. 257, 9513–9517
5 Nuske, J.H. (1985) Biol. Chem. Hoppe Seylers 366, 140
6 Mato, J.M. and Marin-Cao, D. (1979) Proc. Natl. Acad. Sci. USA 76, 6106–6109
7 Nuske, J.H. (1980) Eur. J. Cell Biol. 22, 161
8 Van Waarde, A. (1982) FEBS Lett. 149, 266–270
9 Trivedi, L., Gupta, A., Paik, K.W. and Kim, S. (1982) Eur. J. Biochem. 128, 349–354
10 Galetti, P., DeRosa, M., Gambacorta, A., Manna, C., Festinese, R. and Zappia, V. (1981) FEBS Lett. 124, 62–66
11 O'Dea, R.F., Viveros, O.H. and Axelrod, J. (1978) Nature 272, 462–464
12 Zimmerman, L.F., Clark, R.L. and Gamgali, S. (1983) J. Craniofacial Genet. Dev. Biol. 3, 371–386
13 Billingsley, M.L. and Roth, R.H. (1982) J. Pharmacol. Exp. Ther. 223, 681–688
14 Wolf, M.E. and Roth, R.H. (1985) J. Neurochem. 44, 291–298
15 Terwilliger, T.C. and Clarke, S. (1981) J. Biol. Chem. 256, 3067–3076
16 Gagnon, C., Kelly, S., Manganiello, V., Vaughan, M., Odya, C., Strittmatter, W., Hoffman, A. and Hirata, F. (1981) Nature 291, 515–516
17 Runte, L., Jürgensmeier, H.-L., Unger, C. and Söling, H.D. (1982) FEBS Lett. 147, 125–130
18 MacFarlane, D.E. (1984) J. Biol. Chem. 259, 1357–1362
19 Ro, J.J., DiMaria, P. and Kim, S. (1984) Biochem. J. 219, 743–749

20 Ro, J.J., Neilan, B., Magee, P.N., Paik, W.K. and Kim, S. (1981) J. Biol. Chem. 256, 10572–10576
21 Green, G.A. and Kalra, V.K. (1981) J. Biol. Chem. 256, 10565–10571
22 Galetti, P., Ingrosso, D., Nappi, A., Ioloscon, A. and Pinto, L. (1983) Eur. J. Biochem. 135, 25–31
23 Schiebler, W. and Hucho, F. (1978) Eur. J. Biochem. 85, 55–63
24 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
25 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
26 Popot, J.-L., Sagiyama, H. and Changeux, J.-P. (1976) J. Mol. Biol. 106, 469–483
27 O'Dea, R.F., Viveros, O.H. and Diliberto, E.J., Jr. (1981) Biochem. Pharmacol. 30, 1163–1168
28 Gregor, P. and Sellinger, O.Z. (1983) Biochem. Biophys. Res. Commun. 116, 1056–1063
29 Campillo, J.E. and Ashcroft, S.J.H. (1982) FEBS Lett. 138, 71–75
30 Billingsley, M.L., Velletri, P.A., Roth, R.H. and DeLorenzo, R.J. (1983) J. Biol. Chem. 258, 5352–5357
31 Aswad, D.J. (1984) J. Biol. Chem. 259, 10714–10721
32 Quick, D.P., Orchard, P.J. and Duerre, J.A. (1981) Biochemistry 20, 4724–4729
33 Gagnon, C. (1979) Biochem. Biophys. Res. Commun. 88, 847–853
34 Pike, M.C., Kredich, N.M. and Snyderman, R. (1978) Proc. Natl. Acad. Sci. USA 75, 3928–3932
35 Kim, S., Nochumson, S., Chin, W. and Paik, W.K. (1978) Anal. Biochem. 84, 415–422
36 Fahr, A., Lauffer, L., Schmidt, D., Heyn, M.P. and Hucho, F. (1985) Eur. J. Biochem. 147, 483–487
37 Sorgente, N. and Bowersox, J.C. (1984) Biochim. Biophys. Acta 805, 306–311
38 Bareis, D.L., Hirata, F., Schiffmann, E. and Axelrod, J. (1982) J. Cell Biol. 93, 690–697
39 Wurster, B., Schubiger, K., Wick, U. and Gerisch, G. (1977) FEBS Lett. 76, 141–144
40 Wick, U., Malchow, D. and Gerisch, G. (1978) Cell Biol. Int. Rep. 2, 71–79