Biochimica et Biophysica Acta, 436 (1976) 675–685

BBA 77357

DISTRIBUTION OF PROTEIN KINASE ACTIVITIES IN SUBCELLULAR FRACTIONS OF RAT BRAIN

MALCOLM WELLER* and IAN MORGAN**

Centre de Neurochimie, 11, Rue Humann, 67085 Strasbourg (France)

(Received December 8th, 1975)

SUMMARY

The subcellular distribution of histone and phosvitin kinase activities in brain has been studied and the ability of the various fractions to catalyse the phosphorylation of their endogenous proteins (intrinsic protein kinase activity) also examined. Synaptosome membrane fragments have little or no histone or phosvitin kinase activity but contain the highest concentration of cyclic AMP-stimulated intrinsic protein kinase activity. Homogenisation of the membrane fragments in Triton X-100 increased the histone kinase activity but on centrifugation it was all recovered in the supernatant, while the insoluble material contained all the intrinsic protein kinase activity. These results indicate that the intrinsic protein kinase activity of cerebral membrane fragments is due to the presence of a kinase enzyme which is specific to certain membrane proteins. The intrinsic protein kinase activity of synaptosome membrane fragments is a rather slow reaction which takes several minutes to saturate all the acceptor proteins.

INTRODUCTION

It was first observed in 1970 by Weller and Rodnight [1] and later confirmed by other workers [2–4] that microsomal and synaptosomal membrane fragments from mammalian brain contained a cyclic AMP stimulated protein kinase which catalysed the phosphorylation of serine residues in endogenous proteins. We refer to this as the "Intrinsic Protein Kinase Activity" of the fragments. Two soluble protein kinases have also been isolated from brain one of which is stimulated by cyclic AMP and catalyses the phosphorylation of histones [5] while the other is unaffected by cyclic AMP and catalyses the phosphorylation of phosvitin [6, 1]. Both enzymes can, in addition, act on a number of other proteins but the former enzyme cannot act on phosvitin and the

* Present address: University of Stellenbosch, Department of Chemical Pathology, Tygerberg Hospital, P.O. Box 63, Parowvallei 7503, Cape, South Africa.

** Present address: Research School of Biological Sciences, The Australian National University, Department of Behavioural Biology, Box 475 P.O., Canberra City A.C.T., Australia.

latter shows only slight if any activity with histones [7, 8]. The two soluble enzymes may be easily separated by chromatography on phosphorylated cellulose [9].

It is tempting to suggest that it is one of these enzymes which, being bound to cerebral membrane fragments, catalyses the phosphorylation of certain of the membrane proteins. Both of the enzymes may be easily extracted in soluble form from brain tissue but, in an investigation of the subcellular distribution of histone kinase activity in rat brain, Maeno et al. [10] claimed that about 30 % was bound to membrane fragments and that microsomal and synaptosomal plasma membrane fragments had a similar specific activity to that of the total homogenate. This work has been widely assumed to indicate that the bound histone kinase activity is responsible for the phosphorylation of the membrane proteins. It has, however, been found that samples of synaptosome membrane fragments from ox brain which have been washed under hypotonic conditions cannot catalyse the phosphorylation of histones although they contain high intrinsic protein kinase activity [11]. Such membrane fragments can, however, catalyse the phosphorylation of phosvitin. About 20 % of the total rat brain phosvitin kinase activity is present in the microsomal fraction while 25 % is found in the crude mitochondrial fraction [6]. Since all the observations which had been made on the distribution of protein kinase activities were obtained with rather crude subcellular fractions we examined the distribution of the three types of protein kinase activity in more highly purified material. Our observations indicate quite clearly that the enzyme which catalyses the phosphorylation of endogenous proteins in cerebral membrane fragments is distinct from the soluble enzymes which catalyse the phosphorylation of histones or phosvitin (as well as certain other proteins) and it appears likely that the intrinsic protein kinase activity of the membrane fragments is due to a distinct and specific protein kinase.

MATERIALS AND METHODS

Preparation of subcellular fractions

Subcellular fractions were prepared from adult rat brain as previously described [12]. Detailed enzymic, chemical and morphological characterizations of the fractions have been reported [13].

[γ-^{32}P]ATP was prepared by the method of Glynn and Chappell [14] and purified by the method of Rodnight et al. [15].

Measurement of intrinsic protein kinase activity of subcellular fractions

Samples of subcellular fractions were incubated at a concentration of about 200 μg/ml for 0.5 min (unless otherwise stated) in the presence of 50 mM Tris · HCl (pH 7.4), 1 mM $MgCl_2$, 0.5 mM [^{32}P]ATP (specific radioactivity 1–10 · 10^6 cpm/μmol) at 37 °C in a volume of 0.5 ml. Reactions were terminated by the addition of 2 ml ice-cold 10 % trichloroacetic acid. The denatured proteins were washed twice by resuspension in, and centrifugation from, 2 ml lots of ice-cold 10 % trichloroacetic acid, M orthophosphoric acid and then suspended in 0.5 ml of 0.1 M NaOH, heated at 37 °C for 15 min, and protein reprecipitated by adding 2 ml of 10 % trichloroacetic acid. The precipitates were washed once with 1 ml of ethanol/ether (1 : 1 v/v) and dissolved at 100 °C in 2 ml of 0.1 M NaOH, the radioactivity in the solution being counted by Cerenkov radiation. Cyclic AMP stimulated activity is expressed as the

difference between the activity measured in the presence of cyclic AMP and the activity measured in its absence.

Measurement of histone kinase activity

Samples of subcellular fractions were incubated at a concentration of about 200 μg/ml with 50 mM Tris · HCl, 10 mM $MgCl_2$, 0.5 mM [γ-^{32}P]ATP, 1 mM papverine, 10 μM cyclic AMP and 1 mg histone (type IIA from calf thymus, Sigma, St Louis, U.S.A.) in a volume of 0.5 ml for 1 min at 37 °C, these being the optimal conditions for measuring histone kinase activity [7]. Reactions were stopped by the addition of trichloroacetic acid and the precipitated proteins washed and counted exactly as described for the measurement of intrinsic protein kinase activity except that trichloroacetic acid was used at a concentration of 20 % because of the solubility of certain histones at lower concentrations. In all experiments controls were measured which contained either histone but no subcellular fraction or the appropriate subcellular fraction but no histones. The sum of the two controls was always subtracted from the test result.

Measurement of phosvitin kinase activity

Samples of subcellular fractions were incubated at a concentration of about 200 μg of protein/ml with 50 mM Tris · HCl pH 7.4, 200 mM NaCl, 5 mM $MgCl_2$, 0.5 mM [γ-^{32}P]ATP and 1 mg of phosvitin (Sigma, St. Louis, U.S.A.) in a volume of 0.5 ml for 1 min at 37 °C, these being the optimal conditions for measuring the activity [6].

Reactions were terminated by the addition of 10 % trichloroacetic acid and the precipitated protein washed and counted exactly as described for measuring intrinsic protein kinase activity. In all cases two controls were measured either in the absence of phosvitin or in the absence of the appropriate subcellular fraction. The sum of the two controls was always subtracted from the test result.

RESULTS

Membrane fragments from brain contain enzymes which can catalyse the dephosphorylation as well as the phosphorylation of certain endogenous membrane proteins [17, 18]. The time-course of protein phosphorylation measured in vitro is, therefore, a function of both types of enzyme activity. Since the phosphatase is much more rapid than the kinase, however, measuring the initial rate of phosphorylation gives a close approximation to the protein kinase activity [19]. For this reason, the time-courses of phosphorylation of the fractions were first determined. Dephosphorylation of the phosphorylated proteins would be expected to occur, if protein phosphatase activity was present, when all the ATP had been hydrolysed. From Fig. 1 it may be seen that during the 3 min reaction time studied, dephosphorylation only occurred in the supernatant fraction and the original homogenate, presumably due to more rapid hydrolysis of ATP in the presence of these fractions. It is certainly well established that synaptosomal plasma membrane and microsomal fragments contain intrinsic protein phosphatase activity [17] but they would not be expected to contain sufficient ATPase activity to hydrolyse all the ATP in 3 min.

The intrinsic protein kinase activity of rat brain microsomal and synaptosome

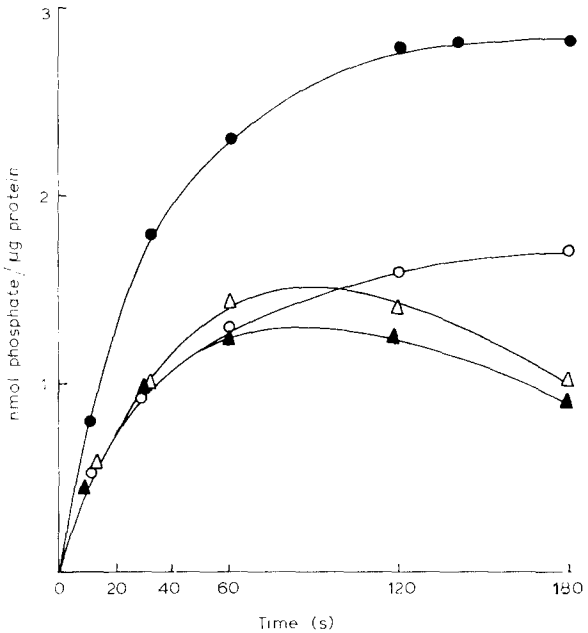

Fig. 1. Time-course of the phosphorylation of several subcellular fractions from brain. Samples of the fractions were incubated with [^{32}P]ATP in the presence of 10 μM cyclic AMP at 37 °C and the amount of phosphate transferred to the proteins determined as described in the text. (●), synaptosomal plasma membranes; (○), synaptic vesicles; (△), total homogenate; (▲), primary soluble fraction. Similar results were obtained in three separate experiments.

membrane fragments, like that of similar fragments from guinea pig brain [20], is considerably faster than that observed with preparations from ox brain [1, 11, 18].

On the basis of the time courses of phosphorylation observed with the various subcellular fraction (Fig. 1) we decided to measure the amount of phosphate incorporated after 30 s incubation as this approximates to the initial rate of protein phosphorylation and so can be taken as an estimate of the intrinsic protein kinase activity of the fractions [19].

Distribution of intrinsic protein kinase activity

From Table I it can be seen that intrinsic protein kinase activity is widely distributed among brain subcellular fractions. The soluble fraction (both from total brain and from synaptosomes), microsomes, purified synaptosome plasma membranes and synaptic vesicles are all quite active. The highest specific activity was present in the synaptosome membrane fragments which had an enrichment of 1.9-fold over that found in the original homogenate. Some enrichment was also seen in preparations of synaptic vesicles and in microsomal fragments, but not in the supernatant fractions. Cyclic AMP stimulated the phosphorylation of all the fractions except the synaptic vesicles and cyclic GMP (10 μM) was also without effect on the phosphorylation of this fraction. Cyclic AMP stimulated activity was present to the highest extent in the synaptosome membrane fragments which show an enrichment of

TABLE I

DISTRIBUTION OF INTRINSIC PROTEIN KINASE ACTIVITIES IN SUBCELLULAR FRACTIONS OF RAT BRAIN

Enzyme activities were determined as described in the text and are shown as means ±standard deviations with the number of observations in parenthesis. Cyclic AMP stimulated activity is defined as the difference between the intrinsic protein kinase activity measured in the presence or absence of cyclic AMP (10 μM).

Fraction	Intrinsic protein kinase activity (nmol phosphate transferred/mg transferred/mg protein per 30 s)			
	−Cyclic AMP		Cyclic AMP stimulated activity	
	Activity	Enrichment	Activity	Enrichment
Total homogenate	0.70±0.08 (8)	–	0.32±0.11 (8)	–
Synaptic vesicles	0.94±0.12 (6)	1.35	0.06±0.05 (4)	0.19
Synaptosomal membranes	1.34±0.15 (7)	1.91	0.52±0.1 (7)	1.63
Microsomes	0.92±0.09 (6)	1.32	0.28±0.04 (6)	0.88
Synaptosomal soluble	0.72±0.08 (9)	1.03	0.39±0.06 (6)	1.22
Primary soluble	0.76±0.12 (6)	1.09	0.33±0.05 (6)	1.03

1.6-fold over that found in the original homogenate and had a specific activity of nearly twice that of the microsomal fragments.

The fact that cytoplasmic fractions have a high intrinsic protein kinase indicates the necessity of ensuring that particulate subcellular fractions are washed free of cytoplasmic contaminants before their intrinsic protein kinase activity is measured.

Distribution of histone and phosvitin kinase activities

It may be seen from Table II that no significant histone kinase activity was found in preparations of synaptic vesicles ($P > 0.1$) and only slight, if any, activity

TABLE II

DISTRIBUTION OF HISTONE AND PHOSVITIN KINASE ACTIVITIES IN SUBCELLULAR FRACTIONS OF RAT BRAIN

Enzyme activities were determined as described in the text and are shown as means ±standard deviations with the number of observations in parenthesis.

Fraction	Protein kinase activity (nmol phosphate transferred/mg protein per min)			
	Phosvitin kinase		Histone kinase	
	Activity	Enrichment	Activity	Enrichment
Total homogenate	0.085±0.02 (6)	–	1.10 ±0.05 (6)	–
Synaptic vesicles	0.09 ±0.025 (4)	1.06	0.025±0.04 (4)	0.023
Synaptic membranes	0.002±0.003 (6)	0.23	0.064±0.05 (6)	0.058
Microsomes	0.093±0.013 (6)	1.1	0.96 ±0.15 (5)	0.87
Synaptic soluble	0.83 ±0.22 (6)	9.8	1.94 ±0.2 (5)	1.76
Primary soluble	0.54 ±0.1 (6)	6.3	2.3 ±0.3 (6)	2.1

was found in preparation of synaptic membrane fragments (only 0.058 of that found in the total homogenate). Most activity was found in the primary or synaptosomal soluble fractions of the original homogenate. 2–3 % contamination of the synaptosomal plasma membrane fractions with soluble material could account for the histone kinase activities. While this is higher than the contamination detected using lactate dehydrogenase as a marker [12], other markers (choline acetyltransferase and glutamate decarboxylase) have suggested that the soluble contamination may be of this size.

These results differ markedly from those obtained by Maeno et al. [10]. Although they report essentially similar activities in the original homogenate and show an enrichment of 1.75-fold in the primary soluble fraction they claim that their synaptosome membrane fragments have an activity of about 0.5 nmol phosphate transferred/mg protein per min; nearly 10-fold higher than we have observed. This difference is almost certainly due to the fact that our preparations of synaptic membrane fragments were much more highly purified than those used by Maeno et al. and were free from cytoplasmic contaminants.

Phosvitin kinase activity, like histone kinase, was predominantly soluble showing 6.3- and 9.8-fold enrichments in the primary and synaptosomal soluble fractions respectively. No significant activity was found in the synaptosome membrane fragments ($P > 0.1$) but, by contrast with histone kinase activity, the level of phosvitin kinase activity in synaptic vesicles was almost the same as in the original homogenate.

In view of the fact that the levels of histone and phosvitin kinase activity detected in the synaptosomal plasma membrane fractions can easily be explained by low levels of soluble contamination, it is highly unlikely that these enzymes are responsible for the intrinsic protein kinase activity of the membrane fragments.

The fact that preparations of synaptic vesicle contain phosvitin kinase, but not histone kinase, activity is of some interest since the intrinsic protein kinase activity of these preparations is, like phosvitin kinase activity, not affected by cyclic AMP or cyclic GMP. The possibility that the intrinsic protein kinase activity of this fraction is due to the presence of an enzyme which can also catalyse the phosphorylation of phosvitin is worth further investigation.

Effect of Triton X-100 on protein kinase activities

Treatment of membrane fragments with low concentrations of Triton X-100 causes an increase in apparent histone kinase activity [10] presumably by releasing occluded enzyme. Triton X-100 markedly increases the apparent histone kinase activity of synaptosome membrane and microsomal fragments (Tables III and IV). Intrinsic protein kinase activity is not, however, stimulated and, in the case of synaptosomal membranes fragments, is somewhat inhibited by the detergent. Phosvitin kinase activity of the microsomal fraction is stimulated by Triton X-100 but, even after detergent treatment, the activity of the enzyme in synaptosomal membrane fragments is too low to permit accurate determination. No histone kinase activity could be detected in preparation of synaptic vesicles even after homogenisation in 0.2 % Triton X-100.

After centrifugation of the detergent-treated synaptosome membrane fragments 90 % of the histone kinase activity is found in the soluble fraction and no

TABLE IIIa

DISTRIBUTION OF PROTEIN KINASE ACTIVITIES ON TREATMENT OF SYNAPTIC MEMBRANE FRAGMENTS WITH TRITON X-100

Fraction	Intrinsic protein kinase (nmol *P* transferred/mg protein per 0.5 min)		Histone kinase (nmol *P* transferred/mg of protein per min)	Phosvitin kinase (nmol of *P* transferred/mg of protein per min)
	−Cyclic AMP	Cyclic AMP stimulated		
Untreated synaptic membranes	0.92±0.1	0.4 ±0.1	0.019±0.05	0.01±0.01
Triton X-100 treated				
Synaptic membranes	0.6 ±0.04	0.34±0.02	0.14 ±0.05	0.03±0.02
Soluble fraction	0.02±0.01	0	0.3 ±0.05	0.03±0.01
Pellet	1.0 ±0.1	0.6 ±0.05	0.002±0.003	0

TABLE IIIb

PERCENTAGE DISTRIBUTION OF PROTEIN KINASE ACTIVITIES AFTER TREATMENT OF SYNAPTOSOME MEMBRANE FRAGMENTS WITH TRITON X-100

Samples of synaptosome membrane fragments were homogenised at a concentration of about 1 mg of protein/ml in 0.2 % Triton X-100, 10 mM Tris · HCl (pH 7.4) and then centrifuged for 1 h at 100 000 × *g*. Protein kinase activities of the material before and after homogenisation in detergent and of the supernatant and pellet after centrifugation were determined as described in the text. Results are shown as means ±S.D. and are taken from 4 separate observations. Cyclic AMP stimulated intrinsic protein kinase activity is defined as the difference between activities measured in the presence and absence of cyclic AMP (10 μM).

Fraction	Protein (%)	Intrinsic protein kinase (% of activity in Triton-treated homogenate)		Histone kinase (%)
		−Cyclic AMP	Cyclic AMP stimulated	
Soluble	40±5	1.3± 0.06	0	90 ±12
Insoluble	60±4	97 ±10	106±11	0.85± 1.0

significant ($P > 0.2$) activity in the pellet. Conversely, although some 40 % of protein is solubilised by the detergent 97 % of the basal intrinsic protein kinase activity and 106 % of the cyclic AMP stimulated activity is found in the pellet. No intrinsic protein kinase activity is found in the soluble fraction.

It has already been reported that treatment of cerebral membrane fragments with detergents which solubilise large proportions of the protein leave the intrinsic protein kinase activity in the insoluble fraction [17]. These results demonstrate quite clearly that enzyme which catalyses the intrinsic protein kinase activity of synaptosome membrane, and probably microsome fragments is not the same as the soluble enzyme which catalyses the phosphorylation of histones and phosvitin.

TABLE IVa

DISTRIBUTION OF PROTEIN KINASE ACTIVITY ON TREATMENT OF MICROSOMAL FRAGMENTS WITH TRITON X-100

Fraction	Intrinsic protein kinase (nmol *P* transferred/mg of protein per 0.5 min)		Histone kinase (nmol *P* transferred/mg protein per min	Phosvitin kinase nmol *P* transferred/mg protein per min
	– Cyclic AMP	Cyclic AMP stimulated		
Untreated microsomes	0.8 ± 0.1	0.24 ± 0.5	1.1 ± 0.08	0.072 ± 0.03
Triton X-100 treated				
Microsomes	0.9 ± 0.12	0.3 ± 0.04	2.3 ± 0.2	0.27 ± 0.04
Soluble fraction	0.04 ± 0.02	0.025 ± 0.01	7.0 ± 0.5	0.65 ± 0.04
Pellet	1.0 ± 0.13	0.45 ± 0.03	0.12 ± 0.05	0.05 ± 0.02

TABLE IVb

PERCENTAGE DISTRIBUTION OF PROTEIN KINASE ACTIVITY AFTER TREATMENT OF MICROSOMAL FRAGMENTS WITH TRITON X-100

Samples of cerebral microsomal fragments were homogenised at a concentration of about 8 mg of protein/ml in 0.2 % Triton X-100, 10 mM Tris · HCl pH 7.4 and then centrifuged for 1 h at 100 000 × *g*. Protein kinase activities of the material, before and after homogenisation in detergent, and of the supernatant and pellet after centrifugation were determined as described in the text. Results are shown as means ± S.D. and are taken from 4 separate observations. Cyclic AMP stimulated intrinsic protein kinase activity is defined as the difference between activities measured in the presence and absence of cyclic AMP (10 μM).

Fraction	Protein (%)	Intrinsic protein kinase (% of activity in Triton-treated homogenate)		Histone kinase (%)	Phosvitin kinase (%)
		– Cyclic AMP	Cyclic AMP stimulated		
Soluble	27 ± 3	1.3 ± 0.4	2.3 ± 1	90 ± 12	80 ± 15
Insoluble	70 ± 4	79 ± 14	105 ± 16	3.5 ± 1.4	12 ± 8

DISCUSSION

We have shown in this paper that preparations of synaptosome plasma membrane fragments contain only very low levels of histone kinase and no phosvitin kinase activity but are rich in cyclic AMP-stimulated intrinsic protein kinase activity which catalyses the phosphorylation of endogenous proteins. Treatment with Triton X-100 solubilises the slight histone kinase activity and results in a preparation rich in cyclic AMP-stimulated intrinsic protein kinase activity but completely lacking phosvitin kinase and histone kinase activities. Similar, though somewhat less clear cut results were also obtained with cerebral microsomal fragments. These results contrast with those of Maeno et al. [10] who reported that preparations of cerebral membrane fragments contain quite large levels of histone kinase activity even after treat-

ment with detergent. The difference is almost certainly due to the fact that we have used much more highly purified preparation of synaptosome membrane fragments.

Our results demonstrate that the intrinsic protein kinase activity of cerebral membrane fragments is due to the presence of a protein kinase which can act on endogenous membrane proteins but not on histones or phosvitin. The intrinsic protein kinase activity of cerebral membrane fragments is thus not due to the presence of the widely studied, soluble, protein kinase which catalyses the phosphorylation of histones (as well as a number of other proteins) and which is stimulated by cyclic AMP. In further support of this conclusion it has been shown that a specific protein inhibitor of histone kinase activity does not affect the intrinsic protein kinase activity of synaptosome membrane fragments [3]. The intrinsic protein kinase, moreover, differs from histone kinase with respect to its sensitivity to Ca^{2+} and Mn^{2+} [17, 21] as well as to FMN [3]. Similarly the intrinsic protein kinases of cerebral membrane fragments differ from phosvitin kinase activity since the former is stimulated by cyclic AMP and is insensitive to Na^{+}, while the latter is unaffected by cyclic AMP [1] and stimulated by Na^{+} [6]. It has, moreover, been shown that a purified preparation of cerebral phosvitin kinase activity cannot catalyse the phosphorylation of cerebral membrane fragments [22].

The time-course of the phosphorylation of synaptosomal membrane fragments described in this paper (Fig. 1) is completely different to that reported by Ueda et al. [3] who examined the phosphorylation of individual synaptosome membrane proteins, separating them by polyacrylamide gel electrophoresis after phosphorylating the intact membrane fragments. They reported that the phosphorylation of only two of the proteins was stimulated by cyclic AMP, and that the phosphorylation reached a maximum at 5 s and then declined. On the basis of these results they concluded that the reaction was rapid and took only a few seconds to saturate all the acceptor sites on the protein. Since they quoted their results in arbitrary units it is impossible to tell how much phosphate was incorporated. The ATP concentration which they used, however, was only 5 μm in a volume of 0.2 ml. The Mg^{2+} ATPase activity of an average preparation of synaptosome membrane fragments is about 250 nmol ATP hydrolysed/mg protein per min, so after 5 s incubation there would no longer be any ATP present and protein phosphorylation would obviously cease. In fact since the membrane fragments contain protein phosphatase activity [17] there will be a decrease in the apparent level of phosphorylation. Under these conditions it is quite obvious that the time taken for protein phosphorylation to reach a peak is a function of the ATP concentration initially present. If still lower concentrations of ATP had been used the time would be even less. In Fig. 1 the time course of phosphorylation of total synaptosome membrane proteins is shown using a concentration of ATP (0.5 mM) which will not be completely hydrolysed for some time. Under these conditions it may be seen that the synaptosome membrane proteins are not saturated with phosphate until after about 2 min. In the case of ox brain synaptosome membrane fragments the reaction is much slower, and it takes about 30 min to saturate all the membrane proteins [17].

The presence of a bound protein kinase which catalyses the phosphorylation of endogenous membrane proteins has now been reported in preparations of membrane fragments from heart [20, 23, 24], ovary [25], kidney [20], liver [20] and erythrocytes [25]. It is tempting to suggest that in all these cases the reaction is due to the

presence of a protein kinase which acts specifically on membrane proteins. Preparations of microsomal fragments from heart which contain cyclic AMP-stimulated intrinsic protein kinase activity are not inhibited by a specific protein inhibitor of histone kinase [23] and do not, in fact contain histone kinase activity [24]. A protein kinase has also been isolated from retinal rod outer segments which catalyses the phosphorylation of a retinal rod outer segment protein (rhodopsin) but which does not catalyse the phosphorylation of casein, histone, or phosvitin, the only other proteins so far tested [27].

The function of the phosphorylation of membrane proteins is uncertain. The most attractive possibility is that changes in the state of phosphorylation of particular membrane proteins alters the passive permeability of the membrane to certain ions by opening or closing pores. In support of this hypothesis it has been shown that the time-course of the increase in phosphorylation of a particular protein in the erythrocyte membrane, induced by cyclic AMP, is similar to the time-course of the increase in Na^+ permeability [29]. In addition, an increased phosphorylation of protein in the retinal rod outer segment membranes lowers the permeability to Ca^{2+} [30, 31] (a process which may be important in dark adaptation [32]) and it has been suggested that changes in the state of phosphorylation of certain membrane proteins may have a general role in the control of Ca^{2+} permeability and/or binding [31].

It has similarly been suggested that changes in the state of phosphorylation of synaptosome membrane proteins, by altering the passive permeability to certain ions, may alter the ease of passage of the nervous impulse [20, 33]. An increase in cyclic AMP concentration occurs in brain slices on electrical stimulation [34], or on treatment with certain neurohormonal agents [35], and this increase is associated with an increased phosphorylation of membrane proteins [20, 36, 37], presumably due to the stimulation of the bound protein kinase. It thus seems likely that changes in the state of phosphorylation of cerebral membrane proteins will occur in vivo and may alter the permeability to certain ions. We have in fact shown that increased phosphorylation of synaptosome membrane proteins causes a decrease in permeability to Ca^{2+} [38]. These changes in ion permeability could have profound affect on the ease of passage of a nervous impulse through the affected synapse. We have shown in this paper (in contrast to the results of Ueda et al. [37]) that the phosphorylation of membrane proteins catalysed by the intrinsic protein kinase is a rather slow reaction taking minutes rather than seconds.

Changes in the state of phosphorylation of synaptic membrane proteins will thus occur relatively slowly but, since the intrinsic protein phosphatase is also slow acting [17], will be long lasting.

ACKNOWLEDGEMENT

M. Weller gratefully acknowledges receipt of a fellowship from the U.K. Science Research Council.

REFERENCES

1 Weller, M. and Rodnight, R. (1970) Nature 225, 187–188
2 Johnson, E. M., Maeno, H. and Greengard, P. (1971) J. Biol. Chem. 246, 7731–7739
3 Ueda, T., Maeno, H. and Greengard, P. (1973) J. Biol. Chem. 248, 8295–8305
4 Ehrlich, Y. H. and Routtenberg, A. (1974) FEBS Lett. 45, 237–243
5 Miyamoto, E., Kuo, I. F. and Greengard, P. (1969) Science 165, 63–65
6 Rodnight, R. and Lavin, B. E. (1900) Biochem. J. 93, 84–91
7 Langan, T. A. in Advances in Cyclic Nucleotide Research (Greengard, P. and Robison, G. A., eds.), Vol. 3, pp. 99–153, Raven Press, New York
8 Walinder, O. (1973) Biochim. Biophys. Acta 293, 140–149
9 Baggio, B., Pinna, L. A., Moret, V. and Siliprandi, N. (1970) Biochim. Biophys. Acta 212, 515–517
10 Maeno, H., Johnson, E. M. and Greengard, P. (1971) J. Biol. Chem. 246, 134–142
11 Weller, M. and Rodnight, R. (1973) Biochem. J. 132, 483–492
12 Morgan, I. G., Wolfe, L. S., Mandel, P. and Gombos, G. (1971) Biochim. Biophys. Acta 241, 737–751
13 Morgan, I. G., Zanetta, J. P., Breckenridge, W. C., Vincendon, G. and Gombos, G. (1973) Brain Res. 62, 405–411
14 Glynn, I. M. and Chappell, J. B. (1964) Biochem. J. 90, 147–149
15 Rodnight, R., Hems, D. A. and Lavin, B. A. (1966) Biochem. J. 101, 502–515
16 Waehneldt, T. V. and Mandel, P. (1972) Brain Res. 40, 419–436
17 Weller, M. and Rodnight, R. (1971) Biochem. J. 124, 393–406
18 Maeno, H. and Greengard, P. (1972) J. Biol. Chem. 247, 3269–3277
19 Weller, M. (1974) Biochim. Biophys. Acta 343, 565–583
20 Rodnight, R. and Weller, M. (1972) in Effects of Drugs of Cellular Control Mechanisms (Rabin, B., R. and Freedman, R. B., eds.), pp. 175–197, MacMillan Press Ltd
21 Weller, M. and Rodnight, R. (1974) Biochem. J. 142, 603–609
22 Weller, M. (1969) M. Phil. Thesis, London University
23 Wray, H. L., Gray, R. R. and Olson, R. A. (1973) J. Biol. Chem. 248, 1496–1498
24 Krause, E. G., Will, H., Pelouch, V. and Wollenberger, A. (1973) Acta Biol. Med. Germ. 31, K37–43
25 Bavelo, J. and Rieber, M. (1973) FEBS. Lett. 37, 37–41
26 Guthrow, C. E., Allen, J. E. and Rasmussen, H. (1972) J. Biol. Chem. 247, 8145–8153
27 Weller, M., Virmaux, N. and Mandel, P. (1975) Proc. Natl. Acad. Sci. U.S. 72, 381–385
28 De Lorenzo, R. J., Walton, K. G., Curron, P. F. and Greengard, P. (1973) Proc. Natl. Acad. Sci. U.S. 70, 880–884
29 Rudolph, S. A. and Greengard, P. (1974) J. Biol. Chem. 249, 2684–2687
30 Weller, M. and Virmaux, N. (1975) Exp. Eye Res. 20, 195
31 Weller, M., Virmaux, N. and Mandel, P. (1975) Nature 256, 68–70
32 Weller, M., Gordidis, C., Virmaux, N. and Mandel, P. (1975) Exp. Eye Res., in the press
33 Greengard, P., Mcafee, D. A. and Kebabion, J. (1972) in Advances in Cyclic Nucleotide Research, (Greengard, P., Paoletti, R. and Robison, G. A., eds.), Vol. 1, pp. 337–355, Raven Press, New York
34 Kakiuchi, S., Rall, T. W. and McIlwain, H. (1969) Neurochemistry 16, 458–489
35 Drummond, G. I. (1900) Progress in Neurobiology, Vol. 2, pp. 121–176
36 Reddington, M., Rodnight, R. and Williams, M. (1973) Biochem. J. 132, 475–482
37 Williams, M. and Rodnight, R. (1974) Brain Res. 77, 502–506
38 Weller, M. and Morgan, I. (1975) Abstract No. 107 Fifth International Meeting of the ISN, Barcelona, Spain