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A COMPARISON OF THE INTRINSIC PROTEIN KINASE ACTIVITIES OF MEMBRANE PREPARATIONS FROM VARIOUS TISSUES

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Summary

The ability of membrane preparations from different tissues to catalyse the phosphorylation of their endogenous protein (intrinsic protein kinase activity) was determined. It was found that membrane fragments prepared from a large variety of tissues contain this activity although the actual level varies quite widely. Preparations from vas deferens and brain have nearly ten times more activity than preparations from heart, kidney, or erythrocytes. Plasma membranes from skeletal muscle have no detectable activity.

The intrinsic protein kinase activity of membrane fragments from most tissues is stimulated by cyclic AMP although the phosphorylation of proteins in preparations of kidney microsomes or heart plasma membranes is not affected. Cyclic GMP (10 μM) has no effect on the intrinsic protein kinase activity of any membrane preparation examined.

A specific inhibitor of soluble, cyclic AMP-stimulated, protein kinase has no effect on the intrinsic protein kinase activity of any of the membrane preparations examined. This suggests that the intrinsic protein kinase activity of membrane preparations may be due to the presence of a specific protein kinase.

It is suggested that an examination of the distribution of membrane-bound intrinsic protein kinase activity among different tissues may be helpful in determining the function of the reaction.

Introduction

It is well established that synaptic membrane fragments contain a tightly bound protein kinase which catalyses the phosphorylation of endogenous pro-

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teins [1—3]. There have also been a number of reports which show that membrane preparations from several tissues such as heart [4—6], fat cells [7] and kidney [8—10] similarly have intrinsic protein kinase activity [11].

All mammalian cells contain a soluble, cyclic AMP-stimulated, protein kinase (type a_1 protein kinase) which catalyses the phosphorylation of histones, protamines, phosphorylase kinase, glycogen synthetase and a number of other proteins [11]. It appears, however, that this enzyme is not responsible for the intrinsic protein kinase activity of membrane fragments from brain [12] and heart [6] since a specific protein inhibitor of type a_1 protein kinase activity does not inhibit the intrinsic protein kinase activity of these preparations. In addition the properties of the intrinsic protein kinase activity of synaptic plasma membranes differ in a number of ways from those of the type a_1 protein kinase [11,22] and well washed preparations of membrane fragments from heart [4], ovaries [14] and brain [13] have unimpaired intrinsic protein kinase although they lack histone kinase activity.

The function of the phosphorylation of membrane proteins is uncertain. In the case of heart muscle [15—17] and slow-, but not fast-, contracting skeletal muscle [18,19] the phosphorylation of a certain membrane protein increases active Ca^{2+} transport by stimulating Ca^{2+}-ATPase. In addition phosphorylation of proteins in the synaptic membrane [20], in retinal rod outer segment disc membranes [21] and in erythrocyte plasma membranes [22,23] lowers Ca^{2+} permeability.

In the case of the toad bladder epithelial cells cyclic AMP and vasopressin (acting by increasing the concentration of cyclic AMP) apparently stimulate the dephosphorylation of membrane proteins [25] and on addition of vasopressin to the cells there is a correlation between the time courses of dephosphorylation and increase in Na^+ permeability [26]. This was originally interpreted as suggesting that the state of phosphorylation of certain membrane proteins regulates Na^+ premeability [26]. Later work, however, showed that much higher concentrations of vasopressin are needed to stimulate the dephosphorylation of membrane proteins than are needed to increase Na^+ permeability [27]. It thus appears that phosphorylation of membrane proteins in toad bladder epithelial cells does not control Na^+ permeability. It has similarly been shown that cyclic AMP increases the Na^+ permeability of the avian erythrocyte membrane by a direct effect not mediated by protein phosphorylation [23].

In view of these various results it seemed desirable to carry out a program designed to investigate the effects of phosphorylation of membrane proteins on the permeability and transport properties of the membranes. An important part of this program is obviously to establish the distribution of membrane-bound intrinsic protein kinase activity in different tissues. For this reason we prepared different types of membrane fragments from a number of different tissues and examined their intrinsic protein kinase activities.

Methods

Preparation of membrane fragments. Membrane fragments from a number of different tissues were prepared by the standard techniques listed below, all procedures being carried out at 0—4°C. The final pellets from all the prepara-

tions were always suspended in 10 mM Tris-HCl (pH 7.4) at a concentration of approx. 0.5 mg protein/ml and centrifuged at 120 000 × *g* for 30 min. This procedure was repeated and the pellets finally suspended in 0.32 M sucrose/10 mM Tris-HCl (pH 7.4) at a concentration of approx. 10 mg protein/ml and stored in small lots at −20°C. (Separate experiments showed that freezing did not affect the intrinsic protein kinase activity of membrane preparations from brain, liver or kidney, and it was assumed that the activity in other preparations would similarly not be affected.) The washing procedure helped to remove cytoplasmic contamination.

Synaptic plasma membranes were prepared by the technique of Morgan et al. [28] from rat cerebral cortex.

Brain microsomes were prepared and purified from rat cerebral cortex by the technique of Rodnight [29].

Liver and kidney microsomes were prepared by the method of Stanbury et al. [30]. Rat liver or kidney was homogenised in 0.38 M sucrose/0.1 M Tris-HCl (pH 7.3) (3.3 ml/g tissue). The homogenates were centrifuged at 12 000 × *g* for 15 min and the supernatants centrifuged at 105 000 × *g* for 60 min. The final, microsomal pellets were washed with 10 mM Tris-HCl as described above.

Heart microsomes were prepared by a slight modification of the method of Fanburg and Gergely [31]. Rats were killed and 6 g of heart muscle ground in liquid nitrogen with a pestle and mortar. The powder was then homogenised in 40 ml 0.25 M sucrose/10 mM Tris-HCl (pH 7.6) and the homogenate centrifuged at 12 000 × *g* for 20 min. The pellet was suspended in 0.25 M sucrose/10 mM Tris-HCl (pH 7.6) and again centrifuged at 12 000 × *g* for 20 min. The supernatant from this centrifugation was combined with the first supernatant and centrifuged at 80 000 × *g* for 40 min. The final, microsomal, pellet was washed with 10 mM Tris-HCl as described above.

Liver plasma membranes were prepared by the method of Neville [32]. Rat livers were homogenised in 1 mM $NaHCO_3$, pH 7.5, (5 ml/g tissue) and the homogenate diluted to a concentration of 24 ml/g tissue, then centrifuged at 1500 × *g* for 10 min. The pellet was resuspended in bicarbonate buffer and centrifuged at 1220 × *g* for 10 min. The fluffy top layer of the pellet was again suspended in bicarbonate buffer and centrifuged once more at the same speed. The process was repeated several times until the pellet (now almost white) consisted of only one layer. This pellet was then suspended in 44% (w/w) sucrose. The suspension was layered under 37% (w/w) sucrose and centrifuged at 100 000 × *g* for 25 min. The band between the 44 and 37% sucrose layers, which contained the plasma membranes, was collected and 10 mM Tris-HCl (pH 7.4) added to reduce the sucrose concentration to about 0.3 M. The mixture was then centrifuged at 100 000 × *g* for 30 min. The final pellet was washed with 10 mM Tris-HCl as described above.

Kidney plasma membranes were prepared by the method of Fitzpatrick et al. [33]. Rat kidneys were homogenised in 3 volumes of 0.25 M sucrose/1 mM Tris-EDTA and the homogenate centrifuged at 1475 × *g* for 10 min. The pellet was suspended in 2 M sucrose (1 ml/g original tissue) and centrifuged at 13 300 × *g* for 10 min. The supernatant, which contained plasma membranes and mitochondria, was diluted with 7 volumes of water and centrifuged at 35 000 × *g* for 15 min. The upper layer of the pellet was collected in 0.25 M sucrose/1 mM

Tris-EDTA and centrifuged at 35 000 × *g* for 15 min. The process was repeated several times until the pellet gave only one layer. The final pellet of plasma membranes was washed with 10 mM Tris-HCl as described above.

Heart plasma membranes were prepared by the method of Kidwai et al. [34]. Rat hearts were homogenised in 0.25 M sucrose (5 ml/g) using a Polytron Type PT 10-35 homogeniser (Kinematica GmbH, Luzern, Switzerland) for 15 s at half maximal speed. The homogenate was then centrifuged at 100 000 × *g* for 30 min. The pellet was suspended in a small volume of 0.25 M sucrose and loaded onto a discontinuous gradient of 31% (w/w) sucrose and 25% (w/w) sucrose. The sample was then centrifuged at 112 000 × *g* for 90 min and the band at the junction of the two layer sucrose layers diluted with 10 mM Tris-HCl (pH 7.4) to give a sucrose concentration of 0.25 M. This sample was then centrifuged at 100 000 × *g* for 30 min and the pellet of plasma membranes washed with 10 mM Tris-HCl as described above.

Spleen, lung, uterus and vas deferens microsomes were prepared by a method which was essentially that of Bisby and Fillenz [35]. The rat tissues were homogenised in 10 volumes of 0.3 M sucrose/1 mM EDTA/0.1 mM potassium phosphate buffer, pH 7.4 (uteri and vas deferens were first ground in liquid nitrogen). The homogenates were centrifuged at 600 × *g* for 10 min and the supernatants taken and centrifuged at 100 000 × *g* for 120 min. The microsomal pellets were then washed with 10 mM Tris-HCl as described above.

Skeletal muscle microsomes were prepared by the method of Bondani and Karler [36]. Rat quadriceps muscle was dissected free of fat and connective tissue and ground in liquid nitrogen. The powder was then homogenised in 0.25 M sucrose/0.05 M Tris-HCl, pH 7.8 (10 ml/g powder). The homogenate was centrifuged at 10 000 × *g* for 15 min, the supernatant taken and centrifuged at 100 000 × *g* for 45 min. The microsomal pellet was then washed with 10 mM Tris-HCl as described above.

Gut microsomes were prepared by the method of Hurwitz et al. [37]. Rat ileum was cleaned in 0.25 M sucrose, homogenised in 30 volumes of 0.25 M sucrose, and centrifuged at 1500 × *g* for 10 min. The supernatant was taken and centrifuged at 27 000 × *g* for 10 min and the new supernatant centrifuged at 105 000 × *g* for 60 min. The final, microsomal, pellet was then washed with 10 mM Tris-HCl as described above.

Skeletal muscle plasma membranes were prepared by the method of Peter [38]. Rat quadriceps muscle (20 g) was dissected free of fat and connective tissue and ground in liquid nitrogen. The powder was then suspended in 50 mM $CaCl_2$ (8 ml/g powder) and homogenised in a Polytron type PT 10-35 homogeniser (Kinematica GmbH, Luzern, Switzerland). The suspension was decanted free from any unbroken connective tissue and centrifuged at 200 × *g* for 3 min. The pellet was then suspended in 45 mM KCl/30 mM $KHCO_3$/2.5 mM histidine/2.5 mM Tris-HCl (pH 7.8) and centrifuged at 120 × *g* for 3 min. The new pellet was suspended in 50 ml of the same buffer, filtered through glass wool and left for 20 h at 4°C. After centrifugation at 200 × *g* for 3 min the pellet was suspended in 250 ml of 0.01 mM Na_2EDTA (pH 7.0) and centrifuged at 1475 × *g* for 10 min. This washing procedure, which extracted all the actomyosin, was repeated six times. The final pellet was washed with 10 mM Tris-HCl as described above.

Erythrocyte plasma membranes were prepared by a modification of the method of Dodge et al. [41] and Romero [42]. Human blood (40 ml) was taken in heparinised tubes from various healthy colleagues and centrifuged at 1000 × *g* for 20 min. The supernatant and 'Buffy coat' were removed and the contents of the tube made up to 40 ml with buffer I (155 mM NaH_2PO_4 adjusted to pH 7.4 with 103 mM Na_2HPO_4) and centrifuged at 1000 × *g* for 20 min. The pellet was washed twice more with the same buffer and then suspended in 200 ml of buffer II (buffer I diluted 1 : 20, with $MgCl_2$ added to give a final concentration of 1 mM) and centrifuged at 31 000 × *g* for 30 min. The new pellet was washed twice in buffer II, then suspended in 40 ml of buffer I diluted 1 : 20 (N.B. with no $MgCl_2$) and centrifuged at 120 000 × *g* for 30 min. The final pellet of plasma membranes was washed with 10 mM Tris-HCl as described above.

Determination of intrinsic protein kinase activity. This was determined as previously described [43]. Samples of membrane fragments (100—200 μg protein) were incubated in a volume of 0.5 ml with 1 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.4) 0.5 mM [γ-^{32}P]ATP (specific radioactivity approx. $1 \cdot 10^7$ cpm/μmol) in the presence or absence of 10 μM cyclic AMP or cyclic GMP for stated times. Reactions were stopped by the addition of 2 ml ice-cold 15% trichloroacetic acid, the denatured protein separated by centrifugation and washed twice by resuspension in and centrifugation from 10% trichloroacetic acid/1 M orthophosphoric acid. The pellets were then suspended in 2 ml 10% trichloroacetic acid, heated in a boiling water bath for 10 min and centrifuged again (this procedure removes any protein-bound acyl phosphate, phosphohistidine or phospholysine). The pellets were suspended in 0.5 ml 0.1 M NaOH, warmed at 37°C for 10 min and 2 ml 10% trichloroacetic acid added. The samples were then centrifuged and the pellets finally washed by resuspension in, and centrifugation from, 1-ml lots of ethanol/diethyl ether (1 : 1, v/v). The pellets were then dissolved in 0.1 M NaOH by heating at 100°C for 10 min and the protein-bound radioactivity counted by measuring the Cerenkov radiation [42].

Estimation of protein kinase inhibitor activity. Aliquots of a crude preparation of type a_1 protein kinase from beef heart [62] were incubated at 37°C in the presence or absence of protein kinase inhibitor (Sigma, St. Louis, MO, U.S.A.) in 0.5 ml 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.4), 10 μM cyclic AMP, 0.1 mM [γ-^{32}P]ATP (specific radioactivity approx. $1 \cdot 10^7$ cpm/μM) and 2 mg of histone (Type a_2 Sigma). Reactions were stopped after 5 min by the addition of 2 ml ice-cold 15% trichloroacetic acid and the protein precipitate washed and counted as described for the determination of intrinsic protein kinase activity. A 'Unit of inhibitor' is defined as that quantity which inhibits the transfer of 1 pmol of phosphate to protein in 1 min under the above conditions of incubation.

Determination of RNA. Samples of microsomes were dissolved in 0.1 M NaOH and the amount of RNA present determined spectrophotometrically.

Lactate dehydrogenase. Was determined by the method of Johnson [56].

Cytochrome oxidase. Was determined by the method of Duncan and Mackler [57].

Rotenone-insensitive NADPH cytochrome reductase. Was determined by the method of Lu et al. [58] in the presence of 10 μg rotenone.

ATPase activities. Were determined as described by Rodnight and Lavin [59]. $(Na^+ + K^+)$-ATPase activity is defined as the difference between the activity observed in the presence of Na^+ and K^+ and the activity observed in the absence of these ions.

Results

Characteristics of the membrane preparations

In order to estimate the purity of the various membrane preparations the activity of several marker enzymes was determined in the original homogenates and in the final preparations. Lactate dehydrogenase was used as a marker for cytoplasm, cytochrome oxidase for mitochondria, rotenone-insensitive NADPH cytochrome reductase for endoplasmic reticulum and $(Na^+ + K^+)$-ATPase for plasma membranes.

The activities found in the various preparations are shown in Table I and from these values it is possible to estimate the approximate purity of the preparations (Table II). The percentage contamination with cytoplasm, endoplasmic reticulum and plasma membranes was estimated by expressing the activity of the appropriate marker enzyme found in the preparation under investigation as a percentage of the activity found in the appropriate purified fraction. The values given are of course overestimates, particularly in the case of contamination with endoplasmic reticulum, since the calculation assumes (incorrectly) that the preparations of microsomes are pure endoplasmic reticulum and that the preparations of plasma membranes are also completely pure. It is rather more difficult to estimate the degree of contamination with mitochondria. In the case of liver, kidney, heart and brain it was possible to compare the cytochrome oxidase activity of the various membrane preparations with those found in purified mitochondria [60,61,34,28]. In the case of the other tissues it was assumed that mitochondria would account for 20% of the protein in the total homogenate. The cytochrome oxidase activity of purified mitochondria would thus be five times that of the total homogenate. This is, of course, a gross approximation but it does allow a very approximate estimate of contamination.

It may be seen that in no case were any of the membrane preparations contaminated with cytoplasm or mitochondria to a greater extent than 5 or 14%, respectively. As pointed out above these figures represent an absolute upper limit and are probably considerable over estimates. The purified preparations of plasma membranes were only slightly contaminated with endoplasmic reticulum but, as would be expected, the microsomal preparations were, where this was examined, found to be fairly heavily contaminated with plasma membranes. Determination of the concentration of RNA in the various membrane preparations indicated that none of them were contaminated with ribosomes to a greater extent than 5%.

The occurrence of intrinsic protein kinase activity in membrane preparations from different tissues

The phosphorylation of proteins in a membrane preparations is controlled by the protein phosphatase activity of the preparation and the initial state of phosphorylation of the membrane proteins as well as by the intrinsic protein

TABLE I

THE DISTRIBUTION OF CERTAIN MARKER ENZYMES IN MEMBRANE PREPARATIONS FROM VARIOUS TISSUES

	Lactate dehydrogenase		Cytochrome oxidase		NADPH cytochrome reductase		Mg^{2+}-ATPase		$(Na^+ + K^+)$-ATPase	
	Activity *	RSA ***	Activity *	RSA ***	Activity *	RSA ***	Activity **	RSA ***	Activity **	RSA ***
Brain										
Homogenate	340	—	200	—	7	—	25	—	10	—
Microsome	42	0.12	15	0.08	18	2.6	16	—	40	4
Liver										
Homogenate	579	—	179	—	13.8	—	25	—	<2	—
Microsome	90	0.23	10.3	0.086	165	18	22	—	3	>2
Plasma membranes	64	0.11	27.6	0.15	5	0.36	28	—	10	>5
Kidney										
Homogenate	241	—	248	—	2.5	—	22	—	2	—
Plasma membranes	16	0.066	20.6	0.08	<0.5	<0.24	30	—	60	30
Microsomes	32	0.12	41	0.15	21	7.8	50	—	19	9.4
Heart										
Homogenate	466	—	138	—	2.6	—	32	—	3.5	—
Microsomes	48	0.1	95	0.7	6	2.3	70	—	10	4
Plasma membranes	<10	<0.02	34	0.25	1	<0.4	77	—	16	5
Spleen										
Homogenate	257	—	41	—	7.6	—	35	—	1	—
Microsomes	50	0.2	<5	0.006	28	3.8	—	—	—	—
Lung										
Homogenate	193	—	11	—	10	—	—	—	—	—
Microsomes	34	0.18	5.1	0.5	40	4.5	—	—	—	—
Skeletal										
Homogenate	1350	—	14	—	10	—	50	—	0.5	—
Microsomes	60	0.045	10	0.7	15	1.5	—	—	—	—
Plasma membranes	20	0.015	—	—	—	—	12	—	25	50
Gut										
Homogenate	771	—	21	—	8	—	30	—	1	—
Microsomes	128	0.17	<5	<0.24	10.3	1.3	—	—	—	—
Vas deferens										
Homogenate	128	—	14	—	<0.5	—	—	—	—	—
Microsomes	16	0.12	<5	0.36	10.3	>20.4	—	—	—	—
Uterus										
Homogenate	233	—	44	—	6.9	—	—	—	—	—
Microsomes	34	0.145	<5	<0.11	15	2.17	—	—	—	—

TABLE II

ESTIMATES OF THE PURITIES OF MEMBRANE PREPARATIONS FROM VARIOUS TISSUES

Enrichment was calculated as the activity of the appropriate marker enzyme in the preparation under investigation/activity in the original homogenate.

	Contamination (%)				Enrichment	
	Endoplasmic reticulum	Mitochondria	Cytoplasm	Plasma membrane	$(Na^+ + K^+)$-ATPase	NADPH cytochrome reductase
Kidney plasma membrane	2.3	3.6	1.9	—	30	—
Kidney microsomes	—	7.1	3.9	31	—	9
Liver plasma membrane	<3	7	2	—	>5	—
Liver microsomes	—	2.6	2.8	30	—	12
Gut microsomes	—	<5	5	—	—	1.3
Vas deferens microsomes	—	<7	3.8	—	—	>20
Uterus microsomes	—	<2.5	4.3	—	—	2.2
Skeletal muscle microsomes	—	14	1.3	—	—	1.5
Skeletal muscle plasma membrane	—	—	0.5	—	50	—
Lung microsomes	—	13	5	—	—	4
Spleen microsomes	—	<2	5	—	—	3.7
Heart microsomes	—	9.3	<0.6	15	—	2.3
Heart plasma membrane	<16	3.1	3	—	5	—
Brain microsomes	—	0.4	3.7	45	—	2.6
Brain synaptic * plasma membranes	<10	<10	<1	—	10—15	—

* Figures taken from ref. 28.

kinase activity of the preparation. The initial rate of phosphorylation of the membrane proteins can, however, be taken as an estimate of the kinase activity [45,46]. The time courses of the phosphorylation of the various membrane preparations were initially determined [47] and it was found that the amount of phosphate transferred to protein during 30 s incubation with [γ-^{32}P]ATP could be taken as an estimate of the initial rate of protein phosphorylation. A comparison of the activities found in the various membrane preparations (Table III) showed that most activity is found in synaptic plasma membrane fragments and in microsomal preparations from brain and vas deferens. Microsomal fragments from uterus, lung and spleen also contain quite high activities. Membrane preparations from other tissues contain relatively low activities and we were unable to detect any activity in preparations of plasma membrane fragments from skeletal muscle. The latter result is in agreement with the observations of Sulahke and Drummond [49] who found no detectable intrinsic protein kinase activity in preparations of sarcolemma from rabbit skeletal muscle. It has similarly been reported that plasma membrane fragments prepared from rat diaphragm have only a very low level (not more than 10 pmol of phosphate transferred/mg of protein per min) of intrinsic protein kinase activity and this is not affected by cyclic AMP [49]. Andrew et al. [50] however, reported that sarco-

TABLE III

THE DISTRIBUTION OF INTRINSIC PROTEIN KINASE ACTIVITIES IN MEMBRANE FRAGMENTS PREPARED FROM VARIOUS TISSUES

Intrinsic protein kinase activities were determined as described in the text, samples being phosphorylated for 0.5 min. The results are shown as the amount of ^{32}P transferred to protein (pmol phosphate/mg protein per 0.5 min) and are given as means ± S.D. and are taken from ten observations with five different preparations. Unless otherwise stated all preparations were obtained from rat tissues. A Student's *t*-test showed that cyclic AMP significant ($P < 0.01$) stimulated the phosphorylation of all membrane preparations except heart plasma membranes and kidney microsomes.

Tissue	Basal activity (no cyclic nucleotides)	+ Cyclic AMP (10 μM)	+ Cyclic GMP (10 μM)
Brain synaptic plasma membranes	210 ± 12	340 ± 20	205 ± 16
Vas deferens microsomes	157 ± 15	191 ± 8	154 ± 15
Brain microsomes	145 ± 15	220 ± 20	148 ± 8
Uterus microsomes	83 ± 8	103 ± 12	81 ± 7
Lung microsomes	80 ± 14	96 ± 7	78 ± 16
Spleen microsomes	76 ± 8	108 ± 7	75 ± 6
Liver plasma membranes	47 ± 5	62 ± 16	47 ± 7
Skeletal muscle microsomes	40 ± 6	62 ± 4	40 ± 8
Gut microsomes	29 ± 5	53 ± 6	39 ± 4
Heart plasma membranes	35 ± 4	26 ± 5	35 ± 2
Kidney microsomes	28 ± 3	24 ± 1	30 ± 5
Liver microsomes	28 ± 8	45 ± 13	29 ± 10
Human erythrocyte plasma membranes	25 ± 5	45 ± 6	23 ± 4
Heart microsomes	23 ± 4	42 ± 3	22 ± 6
Kidney plasma membranes	20 ± 3	25 ± 2	20 ± 6
Skeletal muscle plasma membranes	— *	— *	— *

* No significant activity (less than 2 pmol).

lemma from rat skeletal muscle contains high intrinsic protein kinase activity (350 pmol of phosphate transferred/mg of protein per min).

We considered the possibility that the method which we used for preparing plasma membrane fragments from skeletal muscle, though one of the mildest available, could have inactivated the intrinsic protein kinase enzyme. The intrinsic protein kinase activity of cerebral membranes is certainly rather sensitive to Ca^{2+} [51]. A sample of rat brain microsomes put through the procedure used to prepare skeletal muscle plasma membrane fragments, however, yielded a preparation with a nearly normal level of intrinsic protein kinase activity although sensitivity to cyclic AMP was lost. The reasons for the difference between the results of Andrew et al. [50] and those of our, and other, studies is thus not clear.

Other workers have reported the occurrence of intrinsic protein kinase activity in microsomal preparation from heart [4,6]; kidney [8,9]; vas deferens, uterus and small intestine [52]; and in plasma membranes from erythrocytes [53,54] and kidney [10]. Owing to differences in the techniques used for the determination of kinase activities, however, it is impossible to compare the results obtained in these studies with the data in Table I.

Effect of cyclic nucleotides on intrinsic protein kinase activity

It may be seen from Table III that cyclic AMP in general stimulates the

intrinsic protein kinase activity of membrane preparations, although its effect is often slight. It did not, however, significantly affect the phosphorylation of heart plasma membranes or kidney microsomes.

In our hands cyclic GMP has no effect on the phosphorylation of any of the membrane preparations examined. This is in contrast to the results of Casnellie and Greengard [52] who reported that cylcic GMP stimulates the phosphorylation of proteins in microsomal preparations from ductus deferens, uterus and small intestine. Like us, however, De Jonge [55] found that the intrinsic protein kinase activity of microsomal preparations from small intestine is not affected by cyclic GMP.

Lack of effect of Triton X-100 on intrinsic protein kinase activity

It seemed possible that the act of preparing the membrane fragments could have resulted in preparations in which the substrate was no longer accesible to the kinase. In order to unmask possible latent activity preparations were homogenised in 0.3% Triton X-100 before determining kinase activity (final concentration of detergent in the kinase assay 0.06%). In no case did this treatment produce a change of activity of more than 10% measured in the presence or absence of cyclic AMP.

Lack of effect of an inhibitor of soluble, cyclic AMP-stimulated protein kinase on the intrinsic protein kinase activity of various membrane preparations

It may be seen from Table IV that a specific inhibitor of the soluble, cyclic AMP-stimulated protein kinase which catalyses the phosphorylation of histones and a number of other proteins (type a_1 protein kinase) [11] has no effect on the intrinsic protein kinase activity of any of the membrane preparations investigated. Other workers have previously reported that the inhibitory does not affect the intrinsic protein kinase activity of synaptic plasma membranes [12] or heart microsomes [6].

TABLE IV

THE EFFECT OF AN INHIBITOR OF SOLUBLE CYCLIC AMP-STIMULATED PROTEIN KINASE ON THE INTRINSIC PROTEIN KINASE ACTIVITY OF MEMBRANE PREPARATIONS FROM VARIOUS TISSUES

Crude protein kinase inhibitor was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and its activity measured as described in Methods. A "unit of inhibitor" is that quantity which inhibits the transfer of 1 pmol of phosphate to protein (histone) in 1 min. Phosphorylation was carried out for 0.5 min in the presence of 1 mM $MgCl_2$/0.5 mM [γ^{32}P]ATP and 50 mM Tris-HCl (pH 7.4) as described in the text. The results are also shown as the amount of ^{32}P transferred to protein in 0.5 min (pmol/mg protein) and are given as the means ± S.D. of six observations with three separate preparations.

Tissue	— Inhibitor	+ Inhibitor (30—50 units)
Brain microsomes	230 ± 15	228 ± 11
Uterus microsomes	67 ± 9	70 ± 8
Spleen microsomes	112 ± 10	106 ± 7
Liver plasma membrane	105 ± 3	111 ± 9
Skeletal muscle microsomes	47 ± 5	51 ± 4
Gut microsomes	64 ± 8	62 ± 7
Kidney microsomes	58 ± 9	59 ± 7
Liver microsomes	26 ± 6	24 ± 3
Heart microsomes	41 ± 5	39 ± 6

These results indicate that the intrinsic protein kinase activity of membrane fragments is probably not caused by the presence of bound type a_1 protein kinase.

Discussion

The results described above indicate that membrane fragments prepared from a variety of tissues contain a bound protein kinase which catalyses the phosphorylation of endogenous proteins in a reaction which is generally stimulated by cyclic AMP. This indicates that the phosphorylation of membrane proteins is a reaction which serves some generally important role in membrane function. The fact that plasma membrane from skeletal muscle lack significant amounts of the enzyme may provide a clue to the nature of the function as may an examination of the relative activities of the enzyme in membrane preparations from different tissues.

One of the most likely possibilities would appear to be that phosphorylation of certain membrane proteins regulates membrane permeability and/or transport processes.

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References

1 Weller, M. and Rodnight, R. (1970) Nature 225, 187—188
2 Weller, M. and Rodnight, R. (1973) Biochem. J. 132, 483—492
3 Earlich, Y.H. and Routtenberg, A. (1974) FEBS Lett. 45, 237—243
4 Krause, E.G., Will, H., Pelough, V. and Wollenberger, A. (1973) Acta Biol. Med. Ger, 31K, 37—43
5 Rodnight, R. and Weller, M. (1972) in Effects of Drugs on Cellular Control Mechanisms (Rabin, B.R. and Freedman, R.B., eds.), pp. 175—192, MacMillan Press, London
6 Wray, H.C., Gray, R.R. and Olson, R.A. (1973) J. Biol. Chem. 248, 1496—1498
7 Chang, K-J., Marcus, N.A. and Cuatrecasas, P. (1975) J. Biol. Chem. 249, 1199—1206
8 Dousa, T.P., Walter, R., Schwartz, I.C., Sands, H. and Hechter, O. (1971) Adv. Cyclic Nucleotide Res. 1, 121—135
9 About-Issa, H., Mendicino, J., Leibach, F. and Pillion, I. (1975) FEBS Lett. 50, 121—124
10 Kinne, R., Shlatz, L.J., Kinne-Saffran, E. and Schwartz, I.L. (1975) J. Membr. Biol. 24, 145—159
11 Weller, M. (1977) Protein Phosphorylation, Pion Press, London, in the press
12 Ueda, T., Maeno, H. and Greengard, P. (1973) J. Biol. Chem. 248, 8295—8505
13 Weller, M. and Morgan, I. (1976) Biochim. Biophys. Acta 433, 223—228
14 Bavelo, J. and Rieber, M. (1973) FEBS Lett. 37, 37—41
15 Will, H., Shirpke, D. and Wollenberger, A. (1973) Acta Biol. Med. Ger. 31K, 45—52
16 Kirchberger, M.A. and Chu, G. (1976) Biochim. Biophys. Acta 419, 559—562
17 Hoi, C-W., Drummond, M. and Drummond, G.I. (1976) Arch. Biochem. Biophys. 173, 415—427
18 Sulahke, P. and Drummond, G.I. (1974) Arch. Biochem. Biophys. 161, 448—455
19 Kirchberger, M.A. and Tada, M. (1976) J. Biol. Chem. 251, 725—729
20 Weller, M. and Morgan, I.G. (1977) Biochim. Biophys. Acta 465, 527—534
21 Weller, M., Virmaux, N. and Mandel, P. (1975) Nature 256, 68—70
22 Weller, M. (1978) Mol. Cell. Biochem., in the press
23 Weller, M. and Laing, W. (1978) Mol. Cell. Biochem., in the press
24 Weller, M. and Rodnight, R. (1971) Biochem. J. 124, 393—406
25 De Lorenzo, R.J. and Greengard, P. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1831—1835
26 De Lorenzo, R.J., Walton, U.G., Curran, P.E. and Greengard, P. (1973) Proc. Natl. Acad. Sci. U.S. 70, 880—884

27 Ferguson, D.R. and Twite, B.R. (1974) J. Endocrin. 61, 501—507
28 Morgan, I.G., Wolfe, L.S., Mandel, P. and Gombos, G. (1971) Biochim. Biophys. Acta 241, 737—751
29 Rodnight, R. (1970) Biochem. J. 120, 1—13
30 Stanbury, J.B., Morris, M.L., Corrigan, H.J. and Lassiter, W.E. (1960) Endocrinology 67, 353—362
31 Fanburg, B. and Gergely, J. (1965) J. Biol. Chem. 240, 2721—2728
32 Neville, D.M. (1960) J. Biophys. Biochem. Cytol. 8, 413—422
33 Fitzpatrick, D.F., Davenport, G.R., Forte, L. and Landon, E.J. (1969) J. Biol. Chem. 244, 3561—3569
34 Kidwai, A.M., Radcliffe, M.A., Daniel, E.E. (1971) Biochim. Biophys. Acta 233, 538—549
35 Bisby, M.A. and Fillenz, M. (1971) J. Physiol. 215, 163—179
36 Bondani, A. and Karler, R. (1969) J. Cell. Physiol. 75, 199—212
37 Hurwitz, L., Fitzpatrick, D.F., Debbas, G. and Landon, E.J. (1973) Science 179, 384—386
38 Peter, J.B. (1970) Biochem. Biophys. Res. Commun. 40, 1362—1367
39 Smith, A.D. and Winkler, H. (1969) Biochem. J. 103, 480—482
40 Winkler, H., Hörtnagl, H. and Smith, A.D. (1970) Biochem. J. 118, 303—310
41 Dodge, J.T., Mitchell, D. and Hanaham, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
42 Romero, P.J.B. (1974) Biochim. Biophys. Acta 339, 116—125
43 Weller, M. and Morgan, I.G. (1976) Biochim. Biophys. Acta 436, 675—685
44 Haviland, R.T. and Bieber, L.L. (1970) Anal. Biochem. 33, 323—334
45 Weller, M. (1974) Biochim. Biophys. Acta 343, 565—583
46 Weller, M. (1977) J. Theor. Biol. 64, 391—399
47 Carstens, M. (1977) M.Sc. Thesis, Stellenbosch University, South Africa
48 Sulahke, P.V. and Drummond, G.T. (1974) Arch. Biochem. Biophys. 161, 448—455
49 Pinkett, M.O. and Perlman, R.C. (1974) Fed. Proc. Fed. Am. Soc. Exp. Biol. 33, 401
50 Andrew, C.G., Roses, A.D., Almon, R.R. and Appel, S.H. (1973) Science 182, 927—928
51 Weller, M. and Rodnight, R. (1974) Biochem. J. 142, 605—609
52 Casnellie, J.E. and Greengard, P. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1891—1895
53 Guthrow, C.E., Allen, J.E. and Rasmussen, M. (1972) J. Biol. Chem. 247, 8145—8153
54 Rubin, C.J., Rosenfeld, R.D. and Rosen, O.M. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3735—3738
55 De Jonge, H.R. (1976) Nature 262, 590—592
56 Johnson, M.K. (1960) Biochem. J. 77, 610—618
57 Duncan, H.M. and Mackler, B. (1966) J. Biol. Chem. 241, 1694—1697
58 Lu, A.Y.H., Junk, K.W. and Coon, M.J. (1960) J. Biol. Chem. 244, 3714—3721
59 Rodnight, R. and Lavin, B.E. (1966) Biochem. J. 101, 495—505
60 Aranson, M.N. and Touster, O. (1974) Methods Enzymol. 31, 90—102
61 Wattiaux-De Coninck, S., Rutgeerts, M-J. and Wattiaux, R. (1965) Biochim. Biophys. Acta 105, 446—459
62 Miyamoto, E., Kuo, J.F. and Greengard, P. (1969) J. Biol. Chem. 244, 6395—6402