Effect of Hormones on Transcription of the Gene for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) in Rat Kidney†

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ABSTRACT: The effect of hormones on the transcription rate of cytosolic phosphoenolpyruvate carboxykinase and level of mRNA for this enzyme in the rat kidney has been investigated. In renal nuclei isolated from rats given dibutyryladenosine cyclic 3′,5′-phosphate (Bt_2cAMP) or 8-bromoadenosine cyclic 3′,5′-phosphate (8-Br-cAMP), [^{32}P]UMP incorporation into hybridizable phosphoenolpyruvate carboxykinase mRNA increased severalfold within 1 h. Changes in the concentration of cytosolic phosphoenolpyruvate carboxykinase mRNA, measured by hybridization of [^{32}P]cDNA to poly(A)$^+$ mRNA, paralleled alterations in the transcription rate. Dexamethasone treatment of adrenalectomized rats increased the transcription rate and the level of phosphoenolpyruvate carboxykinase mRNA 3–4-fold after 4 h. Both parameters then declined to control values by 8 h. When dexamethasone (5 mg/kg) and Bt_2cAMP (25 mg/kg) were given together, the rate of phosphoenolpyruvate carboxykinase RNA synthesis and the level of cytosolic mRNA were not increased more than those with either drug alone. Transcription of the gene for renal phosphoenolpyruvate carboxykinase was not affected by diabetes or glucose refeeding but was increased 2-fold after 24 h of starvation and reduced by bicarbonate feeding after 2 h. We conclude that glucocorticoids and cAMP change the rate of transcription of the phosphoenolpyruvate carboxykinase gene in rat kidney, leading to changes of similar magnitude in mRNA level and, hence, enzyme activity. The results presented here and in previous work [Lamers, W., Hanson, R. W., & Meisner, H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5137] indicate that the transcription rate of the gene for phosphoenolpyruvate carboxykinase in liver and kidney responds to hormones in a tissue-specific manner.

Phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) is a gluconeogenic enzyme found in the cytosol and mitochondria of kidney and liver of most animals. The activity of the cytosolic enzyme changes rapidly in response to various hormones (Tilghman et al., 1976) in an organ-specific manner. In rat liver, the activity of this enzyme is reduced by insulin and induced by cAMP and cAMP-dependent hormones (Wicks et al., 1972; Tilghman et al., 1976), while the activity of the renal enzyme is unresponsive to cAMP and insulin but stimulated by glucocorticoids (Iynedjian et al., 1975). Changes in the level of hepatic phosphoenolpyruvate carboxykinase are regulated by alterations in the transcription of the gene for the enzyme (Lamers et al., 1982). Thus, Bt_2cAMP[1] stimulates hepatic phosphoenolpyruvate carboxykinase RNA synthesis 7–8-fold within 20 min, while glucose refeeding causes a 90% reduction over a 2-h period. These changes precede an increase or decrease in the concentration of enzyme mRNA and the rate of enzyme synthesis. Although hormonal control of the synthesis of phosphoenolpyruvate carboxykinase RNA in kidney has not been studied, steroid hormones have been shown to raise the level of phosphoenolpyruvate carboxykinase mRNA in this organ (Iynedjian & Hanson, 1977). We report here that not only dexamethasone but also cAMP increase the sequence abundance of renal phosphoenolpyruvate carboxykinase, an effect that can be accounted for by stimulation of the transcription rate of the gene.

Experimental Procedures

Male Sprague-Dawley rats weighing 200–300 g were obtained from Zivic-Miller Laboratories and fed rat chow ad libitum. The rats were adrenalectomized 4–5 days prior to experimental use and maintained on 0.9% saline. In order to minimize diurnal variation, all experiments were carried out on rats killed between 9:00 a.m. and 11:00 a.m.

Chemicals. [α-^{32}P]UTP (sp act. 410 Ci/mmol) was obtained from Amersham, [^{32}P]dCTP (400–600 Ci/mmol) was from New England Nuclear Corp., and [^{3}H]UTP was from ICN Inc. Nitrocellulose (BA-85) was from Schleicher & Schuell; DNase I (Worthington Biochemical, RNase free) was treated with 0.1% diethyl pyrocarbonate to remove contaminating RNase (Lis et al., 1981). Dexamethasone (Decadron) was obtained from Merck Sharp & Dohme.

Preparation of cDNA. A cloned 2.6-kb cDNA to phosphoenolpyruvate carboxykinase (pPCK10) inserted into the *Pst*I site of pBR322 (Yoo-Warren et al., 1983) was used in these studies.

Phosphoenolpyruvate Carboxykinase RNA Concentration. RNA was isolated by the guanidinium–hot phenol method (Maniatis et al., 1982), modified to include a 2 M LiCl wash to remove DNA. The yield of total RNA by this method averaged 1.6–1.8 mg/g of tissue. Poly(A)$^+$ RNA was prepared by oligo(dT)–cellulose affinity chromatography (Krystosek et al., 1975), bound to nitrocellulose filters, and hybridized to ^{32}P-labeled nick-translated pPCK10 (sp act. 0.5 $\times$ 10^8 cpm/μg) (Rigby et al., 1977; Thomas, 1980). Radioactivity bound to the filters was linear between 0.25 and 2 μg of added poly(A)$^+$ RNA, after correction for background.

RNA Transcription in Isolated Nuclei. Kidneys were disrupted at 4 °C in 0.3 M sucrose, 5 mM dithiothreitol, 5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5, and 0.1% Triton

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[1] Abbreviations: Bt_2cAMP, dibutyryladenosine cyclic 3′,5′-phosphate; 8-Br-cAMP, 8-bromoadenosine cyclic 3′,5′-phosphate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Table I: Effect of cAMP Analogues on Transcription of Phosphoenolpyruvate Carboxykinase Gene in Rat Kidney and Liver[a]

tissue	condition	total [^{32}P]RNA (cpm × 10^6)	pPCK10 (cpm)	pBR322 (cpm)	transcription rate (ppm)
kidney		3.8	920	350	280 ± 28
	saline	3.6	1190	510	340 ± 28
	8-Br-cAMP	3.0	1500	400	640 ± 110
	Bt_2cAMP	4.8	2500	520	835 ± 110
liver		13.0	1610	260	204–208
	Bt_2cAMP	16.2	17700	330	2090–2150

[a] Rats were fed glucose at 5 g/kg of body weight and 2 h later given 8-Br-cAMP (25 mg/kg) or saline intraperitoneally. Fifteen minutes later, the animals were killed and nuclei isolated from kidney and liver. Total [^{32}P]RNA was measured as radioactivity on a type GF/A glass filter after precipitation with 12% trichloroacetic acid and 20 mM sodium pyrophosphate. Transcription rates were determined as described under Experimental Procedures and are expressed as the mean ± SEM (four to five samples) in kidney or the range of duplicate samples in liver.

X-100, by several strokes in a Dounce homogenizer fitted with a type B pestle. After this was filtered through two layers of cheesecloth and centrifuged at 800g for 5 min, the resulting crude nuclear fraction was recentrifuged for 1 h at 25 000 rpm through 1.75 M sucrose and a layer of 2.3 M sucrose, 2 mM $MgCl_2$, and 10 mM Tris-HCl, pH 7.5, and the number of nuclei determined by phase microscopy. Recovery was about 0.5×10^8 nuclei/g wet wt of kidney. RNA synthesis was measured with either fresh nuclei or nuclei that had been frozen at −80 °C for up to 7 days. Both preparations gave similar results.

RNA synthesis by renal nuclei was measured at 24 °C as described for rat liver nuclei (Mory & Gefter, 1978; Lamers et al., 1982), with certain modifications. The effect of Mg^{2+} and Mn^{2+}, both of which are needed for RNA synthesis by RNA polymerase II in isolated sea urchin and rat liver nuclei (Roeder & Rutter, 1969, 1970) were studied in nuclei isolated from rat kidneys. We found that 10 mM Mg^{2+} alone gives nearly the same rate of incorporation of [^{3}H]UMP into total RNA as when Mn^{2+} was present. However, transcription due to RNA polymerase II measured by α-amanitin sensitivity is greater in the presence of Mn^{2+} and is optimal at 2 mM Mn^{2+} and 0.5 mM Mg^{2+}. At this Mn^{2+}/Mg^{2+} ratio, incorporation of [^{3}H]UMP to RNA is linear for less than 5 min and levels off after 20 min (data not shown). On the basis of these studies, we used a transcription medium containing 12.5% glycerol, 2 mM $MnCl_2$, 0.5 mM MgOAc, 0.03 mM EDTA, 50 mM Hepes, pH 7.5, 100 mM KCl, 4 mM dithiothreitol, 0.5 mM CTP, 0.5 mM GTP, 1.0 mM ATP, 0.04 mg of creatine phosphokinase/mL, 8.8 mM creatine phosphate, and $(2–3) \times 10^7$ nuclei in a 200-μL reaction volume. Transcription was started by adding [α-^{32}P]UTP (40–50 μCi) and terminated at 20 min with RNase-free DNase. After extraction with phenol/chloroform and a second DNase treatment, [^{32}P]RNA was hybridized to 2 μg of pPCK10. Hybridization efficiency was measured by including [^{3}H]cRNA [sp act. $(0.5–1) \times 10^6$ cpm/μg] (Lis et al., 1981) in each reaction. Transcription rates, expressed as ppm, were calculated from the following equation:

$$\text{ppm} = \frac{\text{cpm pPCK10} - \text{cpm pBR322}}{\text{cpm in total RNA}} \frac{100}{\text{efficiency}} \frac{2800 \text{ bp}}{2600 \text{ bp}}$$

This corrects for the size of the cDNA probe (2.6 kb) relative to full-length phosphoenolpyruvate carboxykinase mRNA and the efficiency of hybridization, which varied between 45 and 60%.

Results

The time course of the effect of Bt_2cAMP on the rate of transcription of PEPCK is shown in Figure 1. The transcription rate of the gene increases from 300 to 800 ppm in 15 min, and remains elevated up to 1 h after a single injection. The effect of cAMP analogues on the rate of transcription of

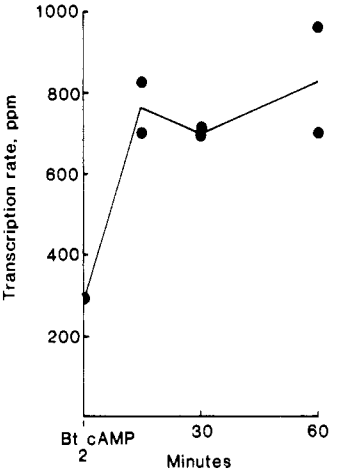

FIGURE 1: Effect of Bt_2cAMP on phosphoenolpyruvate carboxykinase transcription. Fed rats were given Bt_2cAMP (25 mg/kg) intraperitoneally, and kidneys were removed at the indicated times from groups of two to three animals. Nuclei were isolated and transcription rates measured in duplicate as described under Experimental Procedures.

phosphoenolpyruvate carboxykinase from kidney and liver is presented in Table I. In this experiment, fed rats were given oral glucose 2 hours prior to Bt_2cAMP injection. This treatment reduces the rate of hepatic phosphoenolpyruvate carboxykinase RNA transcription by 90% (Lamers et al., 1982). The basal rate of renal phosphoenolpyruvate carboxykinase RNA synthesis in isolated nuclei is 280 ppm, which is slightly higher than the 200 ppm found in liver nuclei isolated from glucose-fed rats. The transcription rate of saline-injected animals is 340 ppm in kidney, ruling out the possibility that handling of the rats, a procedure that raises catecholamine levels (Popper et al., 1977), is a factor. Both 8-Br-cAMP and Bt_2cAMP stimulate renal phosphoenolpyruvate carboxykinase transcription 2–2.5-fold within 15 min, which is considerably less than the 10-fold increase observed in liver.

The effect of a single dose of dexamethasone on the transcription rate and concentration of phosphoenolpyruvate carboxykinase RNA in kidneys of adrenalectomized rats is shown in Figure 2. In two separate experiments the rate of transcription did not increase until 1–2 h after glucocorticoid administration, reaching a peak of induction that is 3.5-fold above control values at 4 h. The transcription rate of the gene then declined to nearly normal after 8 h. The cytosolic phosphoenolpyruvate carboxykinase mRNA concentration, measured by hybridization of total poly(A)$^+$ RNA to nick-translated cDNA, follows a similar pattern of change. These data provide evidence that steroids increase the concentration of renal phosphoenolpyruvate carboxykinase mRNA by stimulating the rate of transcription of the gene for the enzyme.

Figure 3 (lower panel) shows that either dexamethasone or Bt_2cAMP alone stimulates transcription 2.5-fold and, when they are given together, they produce a 3-fold increase. We

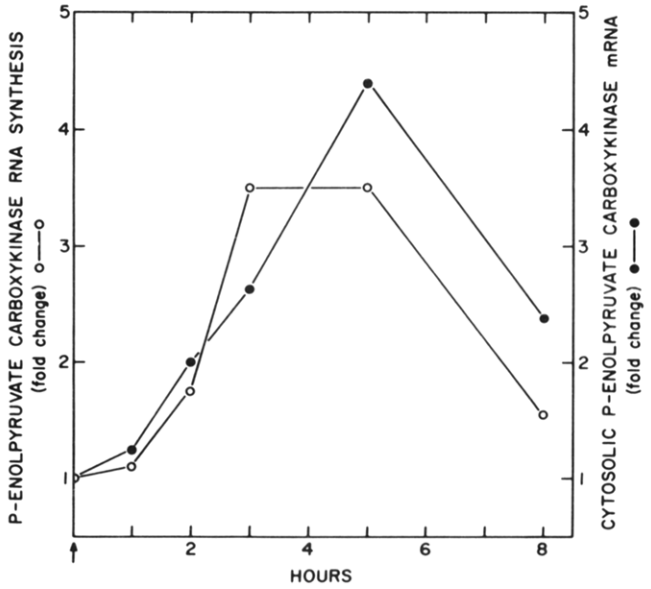

FIGURE 2: Effect of dexamethasone on phosphoenolpyruvate carboxykinase transcription and RNA level of kidneys of adrenalectomized rats. Dexamethasone (5 mg/kg) was injected intraperitoneally, and kidneys were removed from groups of three to four rats between the times indicated. Nuclei or poly(A)$^+$ RNA was prepared from contralateral kidneys as described under Experimental Procedures. Results are expressed as x-fold change from controls, which hybridize 70 cpm of nick-translated cDNA/μg of poly(A)$^+$ RNA.

Table II: Effect of Hormones and Diet on Phosphoenolpyruvate Carboxykinase Gene Transcription in Rat Kidney[a]

condition	total [^{32}P]RNA (cpm × 10^6)	synthesis rate (ppm)
fed	5.6	275–310
starved, 24 h	5.4	540–680
starved, 24 h; glucose, 2 h	3.4	660–880
starved, 24 h; bicarbonate, 2 h	4.6	410–520
streptozotocin, 5 days	5.6	360–400

[a] Rats were treated as described, and kidney nuclei were isolated. Where indicated, rats were refed glucose at 5 g/kg of body weight for 2 h and given $NaHCO_3$ (15 mmol/kg) by gavage or streptozotocin (60 mg/kg) by tail vein injection. The streptozotozin-treated rats had marked glucosuria within 5 days. Rates of transcription represent the range of duplicate samples. Efficiency of hybridization, determined in each sample with [^{3}H]cRNA, varied from 47 to 58%.

find (Figure 3, upper panel) that the level of phosphoenolpyruvate carboxykinase mRNA in the cytosol is raised 6- and 4-fold with Bt_2cAMP or dexamethasone alone and nearly 6-fold when both dexamethasone and Bt_2cAMP are added together. The increase in phosphoenolpyruvate carboxykinase mRNA by dexamethasone and Bt_2cAMP is further demonstrated in the northern blot of poly(A)$^+$ RNA (Figure 3, insert). In other experiments, the stimulation by these two hormones has varied between 3-fold and 7-fold, depending upon the basal level of phosphoenolpyruvate carboxykinase mRNA. Thus, no synergistic effect of dexamethasone and Bt_2cAMP was found in kidney, in contrast to the stimulation of the phosphoenolpyruvate carboxykinase RNA level caused by dexamethasone in primary cultures of hepatocytes (Salavert & Iynedjian, 1980).

The activity of renal phosphoenolpyruvate carboxykinase and its mRNA level are not altered significantly by insulin (Iynedjian et al., 1975; Cimbala et al., 1981). Table II shows that nuclei isolated from kidneys of rats made diabetic with streptozotocin (5 days after a single dose of 60 mg/kg) have rates of phosphoenolpyruvate carboxykinase transcription similar to those of untreated animals. Starvation, which el-

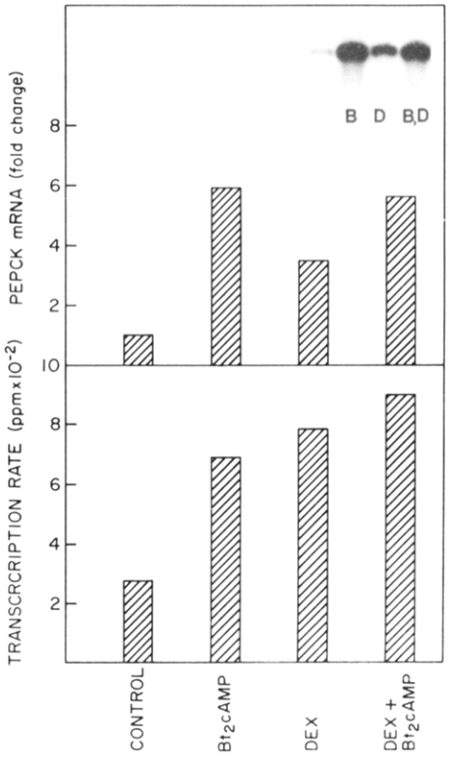

FIGURE 3: Effect of Bt_2cAMP and dexamethasone on phosphoenolpyruvate carboxykinase RNA synthesis and concentration in rat kidney. Rats were fed glucose at 5 g/kg of body weight and 2 h later given Bt_2cAMP (25 mg/kg) intraperitoneally. One hour later, the animals were killed and nuclei isolated from kidneys. Dexamethasone-treated rats were first adrenalectomized for 5 days and then given dexamethasone at 5 mg/kg for 4 h, with or without Bt_2cAMP for the last hour. Transcription rates and mRNA concentration were determined as described under Experimental Procedures. Results are the mean of two to five determinations. The control mRNA concentration was 40 cpm/μg, and the transcription rate was 285 ppm. The insert shows poly(A)$^+$ RNA (2 μg) that was electrophoresed in a 2.2 M formaldehyde–1.4% agarose gel, transferred to nitrocellulose, and hybridized to ^{32}P-labeled $pPCK_{10}$ cDNA.

evates the concentration of renal phosphoenolpyruvate carboxykinase RNA 2–3-fold in 24 h, also doubles the rate of mRNA synthesis. Glucose refeeding of starved rats, which causes a 90% drop in the rate of phosphoenolpyruvate carboxykinase transcription in liver, has no effect in kidney. Bicarbonate refeeding of starved rats reduces RNA synthesis within 2 h, consistent with the metabolic role of renal phosphoenolpyruvate carboxykinase in acidosis (Alleyne, 1970; Iynedjian et al., 1975). Although modest, the dietary and hormonal effects on transcription are reflected by similar changes in phosphoenolpyruvate carboxykinase mRNA concentration (Iynedjian & Hanson, 1977). These data support the claim that hormonally induced changes in phosphoenolpyruvate carboxykinase enzyme activity result from induction/deinduction of gene transcription.

Discussion

The stimulatory effect of cAMP analogues on renal phosphoenolpyruvate carboxykinase transcription was unexpected. Earlier studies, which measured phosphoenolpyruvate carboxykinase synthesis in vivo, showed that the kidney enzyme was relatively insensitive to cAMP and cAMP-dependent hormones, although a 20–30% stimulation by Bt_2cAMP was noted (Iynedjian et al., 1975). In the present study, we have found that Bt_2cAMP produces an immediate 2–3-fold stimulation of renal phosphoenolpyruvate carboxykinase RNA synthesis, which, although less than the 7–10-fold increase that

we have observed in liver (Lamers et al., 1982), shows similar kinetics. In both tissues, the stimulation of transcription occurs rapidly (a maximum activation is reached in less than 20 min) and lasts for at least 1 h. This stimulation is unrelated to a cAMP-mediated release of glucocorticoids, since adrenalectomized rats respond similarly (Figure 3). Theophylline causes no additional stimulation in either tissue (data not shown). Addition of [^{14}C]Bt_2cAMP to isolated rabbit cortical tubules raises the intracellular [^{14}C]cAMP level 4–5-fold (Boumendil-Podevin & Podevin, 1977), emphasizing that the smaller effect of Bt_2cAMP in kidney than in liver cannot be attributed to inability of the proximal tubules to convert dibutyryl- to monobutyryl-cAMP. Although increased globin gene transcription in Friend leukemia cells by butyrate has been observed (Leder & Leder, 1975), this compound is unlikely to be responsible for the activation of renal phosphoenolpyruvate carboxykinase RNA synthesis by Bt_2cAMP, since 8-Br-cAMP is also effective (Table I). The reason for the weaker stimulatory effect of Br_2cAMP on enzyme synthesis that was observed previously is obscure, although there is no reason that a stoichiometric relationship should be found between an increase of gene transcription and enzyme activity. For example, we consistently find that transcription of the gene and cytosolic mRNA level in hepatic tissue are increased 7–10-fold by Bt_2cAMP, yet a perusal of several earlier reports indicates only a 1.5–2.5-fold stimulation of phosphoenolpyruvate carboxykinase activity (Wicks et al., 1972; Gunn et al., 1975). If the renal enzyme follows a similar pattern, little activation would be expected when transcription is induced 2–3-fold. This emphasizes that other factors, including hnRNA attenuation and transport or mRNA turnover (Lowenhaupt & Lingrel, 1978; Wilson & Darnell, 1981; Brock & Shapiro, 1983), must also be considered in order to fully understand the cAMP-dependent hormone control of phosphoenolpyruvate carboxykinase gene expression.

Using in vitro RNA translation assays, previous work has shown that triamcinolone and dexamethasone raise the level of renal phosphoenolpyruvate carboxykinase mRNA 4–6-fold over a period of 6–10 h (Iynedjian & Hanson, 1977; Iynedjian & Jacot, 1980; Cimbala et al., 1982). We demonstrate here that, after an initial lag period of 1–2 h, dexamethasone induces a 3–4-fold increase in phosphoenolpyruvate carboxykinase RNA synthesis in rat kidney. The concentration of cellular mRNA for phosphoenolpyruvate carboxykinase is also increased to a similar extent and follows the same time course. The requirement of a period of several hours before a maximum stimulation of phosphoenolpyruvate carboxykinase transcription is reached differentiates the effect of glucocorticoids from the immediate activation of this gene by Bt_2cAMP- and cAMP-dependent hormones. Our data are consistent with the concept (Swaneck et al., 1979) that the primary mechanism of steroid hormone action in on RNA synthesis, although accompanying changes in RNA turnover cannot be discounted.

Other dissimilar responses in the transcription of this gene to hormones have been found between liver and kidney. In contrast to the marked stimulation of phosphoenolpyruvate carboxykinase transcription in the kidney, glucocorticoids cause only a minimal increase of transcription of the gene in liver (Lamers et al., 1982). This difference may be partly due to the deinduction of hepatic phosphoenolpyruvate carboxykinase transcription by insulin, which is released in response to glucocorticoids (Gunn et al., 1975). The most distinct difference between kidney and liver phosphoenolpyruvate carboxykinases is the response to glucose, an effect that is presumably mediated via insulin secretion. Glucose feeding of starved rats causes a 90% decrease in liver phosphoenolpyruvate carboxykinase RNA synthesis within 2 h (Lamers et al., 1982), whereas no effect was observed in kidney. This lack of responsiveness of phosphoenolpyruvate carboxykinase transcription in kidney after glucose refeeding or diabetes (Table II) may be a property of the proximal tubule cells, where the enzyme is localized (Schmidt & Guder, 1976). The proximal tubule reabsorbs and degrades insulin rapidly and completely in a nonsaturable manner (Chamberlian & Stimmler, 1967), so that under physiological conditions the concentration of insulin may be inadequate to deinduce the phosphoenolpyruvate carboxykinase gene. The degradation of insulin in liver is less extensive (Terris & Steiner, 1976) and is saturable (Sodoyez et al., 1980), which may explain the effectiveness of glucose feeding on the enzyme in liver.

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Interaction of the 43K Protein with Components of *Torpedo* Postsynaptic Membranes†

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ABSTRACT: Interactions of the major M_r 43 000 peripheral membrane protein (43K protein) with components of *Torpedo* postsynaptic membranes have been examined. Treatment of membranes with copper *o*-phenanthroline promotes the polymerization of 43K protein to dimers and higher oligomers. These high molecular weight forms of 43K protein can be converted to monomers by reduction with dithiothreitol and do not contain any of the other major proteins found in these membranes, including the subunits of the acetylcholine receptor, as shown by immunoblotting with monoclonal antibodies. To study directly its interactions with the membrane, the 43K protein was radioiodinated and purified by immunoaffinity chromatography. Purified 43K protein binds tightly to pure liposomes of various compositions in a manner that is not inhibited by KCl concentrations up to 0.75 M. The binding can be reversed by adjusting the pH of the reaction to 11, the same treatment that removes 43K protein from postsynaptic membranes. Unlabeled 43K protein solubilized from *Torpedo* membranes with cholate can be reconstituted with exogenously added lipids in the absence of the receptor. The results suggest that 43K protein molecules are amphipathic and that they may interact with each other and with the lipid bilayer. These interactions cannot explain the coextensive distribution of 43K proteins with acetylcholine receptors in situ. However, they could account for the association of the 43K protein with the postsynaptic membrane and may contribute to the maintenance of the structure of the cytoplasmic specialization of which this protein is a major component.

A peripheral membrane protein of M_r 43 000 (43K protein)[1] is a major component of purified postsynaptic membranes from *Torpedo* electric organ (Sobel et al., 1978; Neubig et al., 1979; Elliot et al., 1980). Immunocytochemical studies using monoclonal antibodies have shown that in situ the 43K protein and the acetylcholine receptor are coextensively distributed in the postsynaptic membrane (Sealock et al., 1984) and that a component related immunologically to the *Torpedo* 43K protein is found at the mammalian neuromuscular junction (Froehner et al., 1981; Porter & Froehner, 1983; Froehner, 1984). The 43K protein is located on the cytoplasmic side of the membrane (Wennogle & Changeux, 1980; St. John et al., 1982; Porter & Froehner, 1983; Nghiem et al., 1983; Sealock et al., 1984), is present in the membranes in concentrations similar to that of the receptor (Burden et al., 1983), and is biochemically and immunologically distinct from actin and creatine kinase, both of which are found in some *Torpedo* nicotinic membrane preparations (Barrantes et al., 1983; Gysin et al., 1983; Porter & Froehner, 1983).

Although the function of this synaptic protein has not been directly demonstrated, some evidence suggests that it may be involved in anchoring receptors in the postsynaptic membrane so as to maintain a high concentration of them directly beneath the sites of transmitter release. Removal of the 43K protein from *Torpedo* membranes can be accomplished with alkaline

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[1] Abbreviations: 43K protein, the basic, membrane-bound M_r 43 000 protein of *Torpedo* postsynaptic membranes; AChR, nicotinic acetylcholine receptor; LIS, lithium diiodosalicylate; SDS, sodium dodecyl sulfate; PC, dioleoylphosphatidylcholine; PA, dioleoylphosphatidic acid; DTT, dithiothreitol; BSA, bovine serum albumin; CuP, copper *o*-phenanthroline; NEM, *N*-ethylmaleimide; EDTA, (ethylenedinitrilo)-tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,-*N*′,*N*′-tetraacetic acid; mab, monoclonal antibody; MOPS, 3-(*N*-morpholino)propanesulfonic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; TCA, trichloroacetic acid.

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