Proc. Nat. Acad. Sci. USA
Vol. 73, No. 2, pp. 568–572, February 1976
Cell Biology

Regulation by steroid hormones of phosphorylation of specific protein common to several target organs

(estradiol/testosterone/cyclic adenosine 3′:5′-monophosphate/endogenous protein phosphorylation)

ALICE Y.-C. LIU AND PAUL GREENGARD

Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510

Communicated by Alfred Gilman, December 4, 1975

ABSTRACT **The effect of *in vivo* administration of steroid hormones on the endogenous phosphorylation of individual proteins in cell sap from several target tissues has been studied using the technique of discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The hormones studied (and their respective target organs) were: 17β-estradiol [1,3,5(10)-estratriene-3, 17β-diol] (uterus); testosterone (17β-hydroxy-4-androsten-3-one) (ventral prostate and seminal vesicle); cortisol (11β,17α, 21-trihydroxy-4-pregnene-3,20-dione) (liver); aldosterone (the 18,11-hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al) (toad bladder). In each of the five target organs studied, pretreatment with the appropriate hormone reduced the amount of ^{32}P incorporated from [γ-^{32}P]ATP into an apparently common protein band present in the cytosol fraction. The endogenous phosphorylation and dephosphorylation of this protein was also regulated by cAMP. This protein, designated SCARP (steroid and cyclic adenosine 3′:5′ monophosphate regulated phosphoprotein), was estimated to have an apparent molecular weight of 54,000 in the gel electrophoresis system used. The effect of the steroid hormones in decreasing the phosphorylation of SCARP was specific for their respective target tissues. The effect of 17β-estradiol and of testosterone on SCARP could be observed as early as two hours after a single dose of the steroid. A protein synthesis inhibitor, cycloheximide, abolished the effect of the steroid hormones, but not that of cAMP, on the endogenous phosphorylation of SCARP. The results suggest that steroid hormones regulate either the amount of SCARP or its ability to become phosphorylated. This regulation of a single species of protein by several types of steroid hormones in different target organs raises the possibility that this common biochemical action may be a component of the mechanism by which these steroids achieve some of their biological effects.**

Antidiuretic hormone (vasopressin), cyclic adenosine 3′:5′-monophosphate (cAMP) and its derivatives, and aldosterone (the 18,11-hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al) each cause an increase in the sodium permeability of toad bladder epithelium (1–3) and also cause a decrease in the level of phosphorylation of a specific protein in this tissue (4–7). A variety of evidence (4–7) suggests that the actions of antidiuretic hormone, cAMP, and aldosterone on sodium permeability may be related to their actions on protein phosphorylation. The possible involvement of protein phosphorylation in the physiological action of aldosterone led us to examine other steroid hormones for effects on phosphorylation of specific proteins in their respective target tissues.

METHODS

Preparation of Animals. Charles River rats weighing 150–250 g were used. Females were bilaterally ovariectomized via the dorsal route, and males were bilaterally castrated via the scrotal route under pentobarbital anesthesia (40 mg per kg, i.p.). Steroids were administered as solutions in 1 ml of sesame oil. Control animals received sesame oil only. In experiments in which the effect of a single dose of estrogen or androgen was studied, females were ovariectomized 1 week and males were castrated 3 days before sacrifice; 17β-estradiol [1,3,5(10)-estratriene-3,17βdiol] (20 μg per rat) was administered i.p. to females and testosterone acetate [17β-hydroxy-4-androsten-3-one] (2 mg per rat) was administered i.p. to males 2 hr before sacrifice [the higher dose of testosterone acetate than of 17β-estradiol is based on the difference in potency of these two steroids (8)]. In experiments in which the effect of chronic steroid treatment was studied, animals were given 20 μg of 17β-estradiol or 2 mg of testosterone acetate at 0, 25, and 50 hr after surgery, and were sacrificed 2 hr after the third injection. Cycloheximide, when used, was injected i.p. (300 μg, dissolved in 0.5 ml of 0.9% NaCl solution) 30 min prior to each administration of steroid. Adrenalectomized male rats, obtained from Charles River, were sacrificed 5 days after surgery; half of these rats were given hydrocortisone 21-sodium succinate (cortisol succinate; 11β,17α,21-trihydroxy-4-pregnene-3,20-dione 21-succinate) (10 mg per rat) in 1 ml of 0.9% NaCl at 24, 22, 19, 15, and 2 hr prior to sacrifice.

Animals were killed by cervical dislocation. The uterus, ventral prostate, and seminal vesicle were quickly removed, cleaned of secretions, dissected free of adhering fat and connective tissue, and placed in a solution of 0.32 M sucrose–10 mM Tris·HCl, pH 7.5 (sucrose–Tris medium), at 0°. Livers were perfused with 20 ml of sucrose–Tris medium *in situ*, then quickly removed and placed in sucrose–Tris medium.

Preparation of Cytosol Fractions. All procedures were carried out at 4°. Tissues from five to six animals were pooled, weighed, and chopped thoroughly with a McIlwain tissue chopper (200-μm setting). The resulting pieces were suspended in 5–10 volumes of sucrose–Tris medium and homogenized at 2000 rpm with a glass–Teflon homogenizer. The homogenate was centrifuged at 11,500 × *g* for 10 min, and the precipitate was washed once and recentrifuged. The combined supernatants were filtered through cheese cloth and centrifuged at 100,000 × *g* for 60 min. The 100,000 × *g* supernatant was used as the cell sap. Protein was determined by the method of Lowry *et al.* (9) with bovine serum albumin as standard, prior to carrying out the phosphorylation assay.

Standard Assay. The standard reaction mixture used for studying endogenous protein phosphorylation contained 50 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes) (pH 6.2), 5 mM $ZnCl_2$, 2–4 μM [γ-^{32}P]ATP [specific activity 10–20 Ci/mmol, prepared by the method of Post and Sen (10)], and 200 μg of cytosol protein, with or without 10 μM cAMP, in a

Abbreviations: cAMP, cyclic adenosine 3′:5′-monophosphate; SCARP, steroid and cyclic adenosine 3′:5′-monophosphate regulated phosphoprotein; $NaDodSO_4$, sodium dodecyl sulfate; M_r, molecular weight.

final volume of 100 μl. The phosphorylation reaction was initiated by the addition of [γ-^{32}P]ATP, and was carried out at 30° for 1 min in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 20 μl of a sodium dodecyl sulfate ($NaDodSO_4$)-containing stop solution and heating at 100° as described previously (6).

In preliminary experiments it was found that the dephosphorylation, but not the phosphorylation, of the protein of interest was abolished in the presence of 5 mM Zn^{++}, in subcellular fractions from both steroid-treated and untreated animals, in agreement with earlier studies on normal tissues (11, 12). Therefore, the standard assay system for studying the effects of steroid hormones and of cAMP on endogenous protein phosphorylation included 5 mM $ZnCl_2$, since any effects observed in the presence of this metal ion are not attributable to actions on protein phosphatase. Where specified, assays were carried out in the presence of 10 mM $MgCl_2$ rather than 5 mM $ZnCl_2$. The absolute level of phosphorylation was greater in the presence of Mg^{++} than in the presence of Zn^{++}. However, in the presence of Mg^{++}, both protein kinase and protein phosphatase activity were present, complicating the interpretation of results.

Gel Electrophoresis and Autoradiography. Following termination of the phosphorylation reaction, the entire sample was subject to polyacrylamide gel electrophoresis and autoradiography in order to determine the amount of phosphate incorporated into individual protein bands. The standard $NaDodSO_4$ polyacrylamide gel electrophoresis system used was the discontinuous, high pH system of Maizel (13) with modifications (14). Gels, prepared from a stock solution of 30% acrylamide–0.8% *N,N'*-bismethylene acrylamide, were polymerized chemically in the presence of 0.05% (vol/vol) *N,N,N',N'*-tetraethylmethylenediamine and 0.01% ammonium persulfate. The separation gel contained 0.375 M Tris (pH 8.9), 0.1% $NaDodSO_4$, and 10% acrylamide in 0.125 M sucrose, and the slabs were 1.6 mm thick and 16 cm in length. [In a few experiments, the results were confirmed using a gradient gel system in which the separation gel contained 0.375 M Tris (pH 8.9), 0.1% $NaDodSO_4$, and a linear gradient prepared from 5% acrylamide–0.125 M sucrose and 15% acrylamide—0.5 M sucrose, and the slabs were 1.1 mm thick and 20 cm in length.] The stacking gel (4–5 cm length) contained 0.06 M Tris (pH 6.7), 0.1% $NaDodSO_4$, and 3% acrylamide, and was polymerized in the presence of 0.05% *N,N,N',N'*-tetraethylmethylenediamine–0.1% ammonium persulfate. The electrode buffer contained 0.05 M Tris (pH 8.6), 0.192 M glycine, and 0.1% $NaDodSO_4$. Electrophoresis was carried out at a total current of 50 mA until the tracking dye pyronin Y reached the bottom of the gel.

After electrophoresis, the slab gels were stained for protein with 0.025% Coomassie blue, destained, and dried, and autoradiography was carried out as described previously (15). Autoradiographs were scanned with a Canalco G-II microdensitometer and the peak heights of the optical density tracings were used as a quantitative measure of the incorporation of ^{32}P into individual protein bands. All results are representative of at least three separate experiments.

RESULTS

Effect of Acute Pretreatment with Steroid Hormone on Phosphorylation of Cytosol Proteins. When the cytosol fraction of rat uterus was incubated in the presence of [γ-^{32}P]ATP for 1 min, a major radioactive band seen on autoradiographs was a protein with an apparent molecular weight of 54,000, designated SCARP for reasons discussed below. In a typical experiment, after 1 min of phosphorylation under standard conditions, the amount of ^{32}P incorporated into SCARP in the cytosol fractions of uterus (and of ventral prostate and seminal vesicle) was approximately 1000–2000 cpm per gel slot. The effect of ovariectomy and of acute estrogen pretreatment on the incorporation of ^{32}P from [γ-^{32}P]ATP into SCARP in the cytosol fraction of rat uterus is shown as a function of incubation time in Fig. 1. Under standard assay conditions, [^{32}P]phosphate in SCARP reached a maximum level within 30 sec of incubation and remained at that level for incubation periods up to 2 min. Ovariectomy caused an increase in the maximal level of phosphorylated SCARP, and this effect was partially reversed by treatment, 2 hr prior to sacrifice, with a single 20 μg dose of 17β-estradiol. The effects of ovariectomy and 17β-estradiol treatment were observed whether the assays were carried out in the absence or in the presence of 10 μM cAMP. In addition, cAMP caused a substantial reduction in the extent of phosphate incorporation into SCARP.

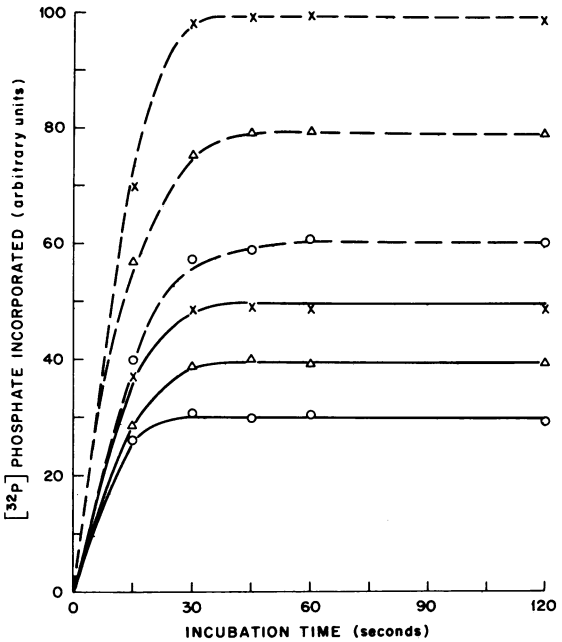

FIG. 1. Time course of endogenous phosphorylation of SCARP present in the cytosol fraction from uterus of normal rats, ovariectomized rats, and ovariectomized rats treated 2 hr prior to sacrifice with a single dose of 17β-estradiol. The cytosol preparations were incubated at 30° with [γ-^{32}P]ATP for the indicated times in the absence or presence of 10 μM cAMP in the standard reaction mixture. Cytosol fraction prepared from uterus of normal rats (○), ovariectomized rats (×) or ovariectomized rats treated with 17β-estradiol (△) was incubated in the absence (broken lines) or presence (solid lines) of cAMP. The scale of arbitrary units used was the same under all conditions.

Effects of castration, of acute pretreatment with testosterone acetate, and of incubation with cAMP, qualitatively similar to those shown in Fig. 1, were observed on SCARP phosphorylation in cytosol fractions from ventral prostate and seminal vesicle of male rats. However, the magnitude of the effect of testosterone (also given 2 hr prior to sacrifice) was smaller than that of 17β-estradiol.

The effect of cAMP, but not that of steroid administration, on SCARP phosphorylation was dependent on the nature of the cation present in the reaction mixture. Thus, cAMP caused an inhibition of SCARP phosphorylation in

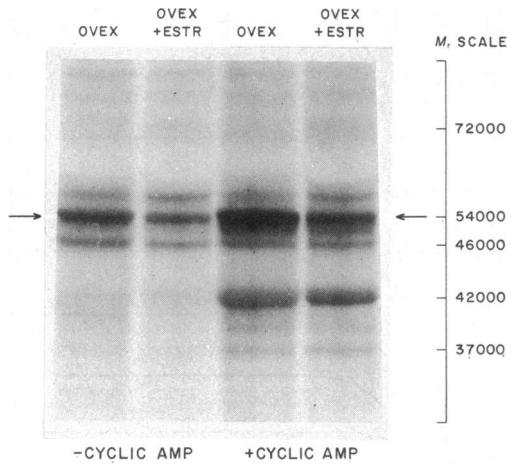

FIG. 2. Autoradiograph illustrating endogenous phosphorylation of cytosol proteins from uterus of ovariectomized rats (OVEX) and ovariectomized rats treated 2 hr prior to sacrifice with a single dose of 17β-estradiol (OVEX + ESTR). The cytosol preparations were incubated at 30° for 1 min with [γ-^{32}P]ATP in the absence or presence of 10 μM cAMP, in the standard reaction mixture except that 10 mM $MgCl_2$ was used instead of 5 mM $ZnCl_2$. The arrows identify the position on the gel of phosphorylated SCARP. M_r is molecular weight.

the presence of Zn^{++} (Fig. 1), whereas it caused a stimulation of SCARP phosphorylation in the presence of Mg^{++} (Fig. 2), under otherwise standard assay conditions, in agreement with previous observations on various mammalian tissues (12). In contrast, pretreatment with 17β-estradiol caused a decrease in SCARP phosphorylation when assays were carried out in the presence of either Zn^{++} (Fig. 1) or Mg^{++} (Fig. 2), in the absence or presence of 10 μM cAMP.

Effect of Chronic Pretreatment with Steroid Hormone on Phosphorylation of Cytosol Proteins. The effect of chronic administration of 17β-estradiol, testosterone acetate, or hydrocortisone 21-sodium succinate on the incorporation of ^{32}P from [γ-^{32}P]ATP into cytosol proteins from four target tissues is shown in Fig. 3. In every instance, steroid pretreatment caused a decrease in SCARP phosphorylation in the absence or presence of cAMP. Moreover, cAMP inhibit-

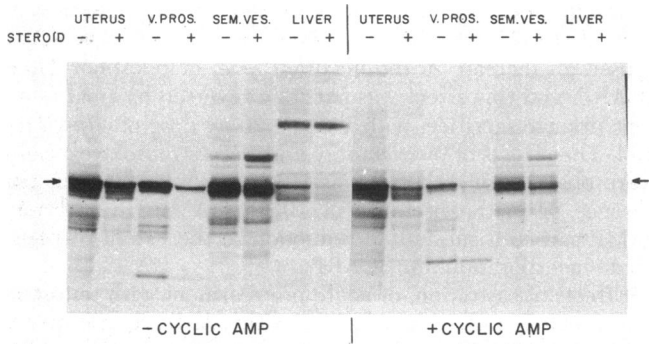

FIG. 3. Autoradiograph illustrating effect of chronic steroid pretreatment on the endogenous phosphorylation of cytosol proteins from four different rat tissues. The effect of 17β-estradiol on uterus of ovariectomized rats, of testosterone on ventral prostate (V. Pros.) and seminal vesicle (Sem. Ves.) of castrated rats, and of hydrocortisone on liver of adrenalectomized male rats was studied as described under *Methods*. The cytosol preparations were incubated at 30° for 1 min with [γ-^{32}P]ATP in the absence or presence of 10 μM cAMP in the standard reaction mixture. The arrows identify the position on the gel of phosphorylated SCARP.

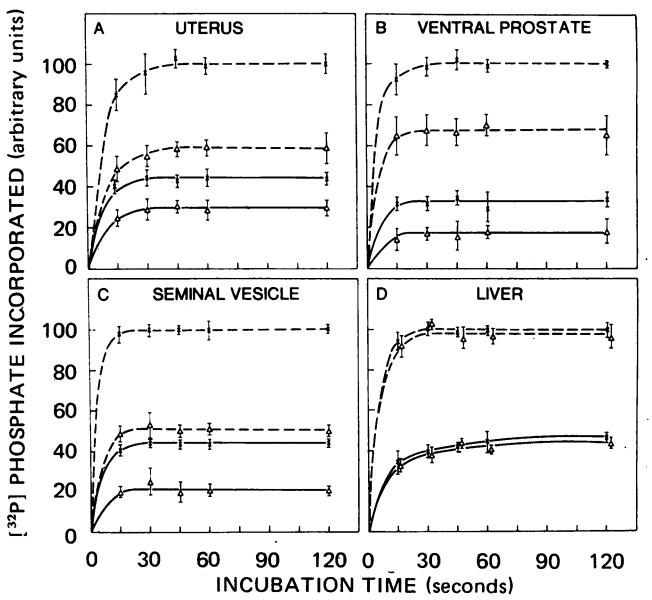

FIG. 4. Effect of chronic steroid pretreatment on the endogenous phosphorylation of SCARP present in the cytosol fraction of various rat tissues. The effect of 17β-estradiol on uterus (A) of ovariectomized rats, and of testosterone acetate on ventral prostate (B), seminal vesicle (C), and liver (D) of castrated rats was studied as described under *Methods*. Cytosol fractions prepared from tissues of untreated (×) or steroid-treated (△) rats were incubated at 30° for the indicated times in the absence (broken lines) or presence (solid lines) of 10 μM cAMP in the standard reaction mixture. The scale of arbitrary units used was the same under all conditions for a given tissue. The data represent mean ± SEM for three separate determinations.

ed SCARP phosphorylation in cytosol fractions prepared either from steroid-treated or nontreated animals. The effects of chronic treatment with steroid hormone were qualitatively similar to, but greater in magnitude than, those obtained upon acute steroid administration: the effect of ovariectomy, of castration, and of adrenalectomy on SCARP phosphorylation could be completely reversed by the chronic administration of 17β-estradiol, testosterone, and hydrocortisone, respectively (data not shown), in contrast to the partial reversal in the acute steroid experiments.

The effect of chronic pretreatment with 17β-estradiol or with testosterone on SCARP phosphorylation in cytosol fractions from uterus, ventral prostate, and seminal vesicle was studied as a function of incubation time. In these time-course experiments, liver was used as a control tissue to study the specificity of the effect of 17β-estradiol and testosterone. Under the standard assay conditions, maximal phosphorylation of SCARP in the cytosol fraction of all four tissues was achieved within 30 sec after initiation of the phosphorylation reaction (Fig. 4). In each case, steroid pretreatment caused a significant reduction in the level of SCARP phosphorylation in the target tissues at all incubation times studied, both in the absence and presence of cAMP. In contrast to these effects of 17β-estradiol and testosterone (Fig. 3; Fig. 4A, B, and C), and the action of hydrocortisone on SCARP phosphorylation in liver cytosol (Fig. 3; A. Liu, J. Sporn, and P. Greengard, manuscript in preparation), neither testosterone (Fig. 4D) nor 17β-estradiol pretreatment affected SCARP phosphorylation in the cytosol fraction from liver, nor did hydrocortisone pretreatment affect SCARP phosphorylation in the cytosol fraction of seminal vesicle. In addition, none of those three steroids affected the

Table 1. Blockade by cycloheximide of steroid-induced decrease in amount of phosphorylated SCARP in various rat tissues

	cAMP			
	−		+	
	Steroid		Steroid	
	−	+	−	+
	[^{32}P]phosphate incorporated, %			
Uterus				
−Cycloheximide	100	58	50	29
+Cycloheximide	100	97	53	49
Prostate				
−Cycloheximide	100	64	24	17
+Cycloheximide	112	99	29	28
Seminal vesicle				
−Cycloheximide	100	55	41	22
+Cycloheximide	86	78	31	32

The effect of 17β-estradiol on uterus of ovariectomized rats and of testosterone acetate on ventral prostate and seminal vesicle of castrated rats was studied as described under *Methods*. Cytosol fractions were incubated at 30° for 1 min with [γ-^{32}P]ATP in the absence or presence of 10 μM cAMP in the standard reaction mixture. The scale of arbitrary units used was the same under all conditions for a given tissue.

phosphorylation of a similar species of protein present in the cytosol fraction of heart that is also regulated by cAMP.

In some experiments, the effects of chronic steroid administration and of cAMP were studied in the presence of $MgCl_2$, rather than $ZnCl_2$, using cytosol fractions from uterus, ventral prostate, seminal vesicle, and liver. For all four tissues, the results obtained in the presence of this cation were qualitatively similar to those observed in the acute experiments with uterus (Fig. 2), i.e., administration of the appropriate steroid hormone decreased, whereas cAMP increased, SCARP phosphorylation.

SCARP was the only protein band whose phosphorylation was found to be altered by the appropriate steroid in each of the four mammalian target tissues studied. In addition to this common effect on SCARP, certain other bands present in only one target tissue, or common to two or three of the four target tissues, were also affected by the appropriate steroid. Steroid pretreatment caused increased phosphorylation of some of these bands and decreased phosphorylation of others. However, the total amount of ^{32}P incorporated into cytosol protein, as measured by trichloroacetic acid precipitation, was not significantly affected by steroid pretreatment.

In some experiments, phosphorylated cytosol samples from uterus, ventral prostate, seminal vesicle, and liver of steroid-treated and untreated animals were incubated with Pronase (0.2 mg per ml, 15 min at room temperature) before the standard procedures of electrophoresis and autoradiography were performed. When this was done, neither protein-staining bands nor radioactive bands were observed in the gels, whereas ribonuclease treatment (0.2 mg per ml, 15 min at room temperature) had no effect. These results indicate that the radioactive bands studied, including SCARP, represent phosphoproteins on the gels.

The effects of 17β-estradiol and testosterone in lowering SCARP phosphorylation cannot be ascribed to a steroid-induced alteration in ATPase activity or ATP level in the cytosol (manuscript in preparation).

Ability of Cycloheximide to Abolish the Effect of Steroid Hormones on the Incorporation of ^{32}P into SCARP. Steroid hormones appear to exert many of their effects through control of the synthesis of RNA and protein in their target organs (16). In the present study, cycloheximide, a protein synthesis inhibitor, was found to abolish the inhibitory effect of 17β-estradiol and of testosterone on the incorporation of ^{32}P into SCARP in the cytosol fractions of uterus, ventral prostate and seminal vesicle, both in the absence and presence of 10 μM cAMP (Table 1). In contrast, cycloheximide did not alter the inhibitory effect of cAMP on SCARP phosphorylation. In accordance with the idea that protein synthesis is required for the effect of the steroids on SCARP phosphorylation, addition of 17β-estradiol, testosterone, or hydrocortisone to the standard reaction mixture did not affect SCARP phosphorylation.

DISCUSSION

The protein whose phosphorylation was markedly affected by cAMP and by steroid pretreatment, in all target tissues studied, had an apparent molecular weight (M_r) of 54,000 in the discontinuous pH gel electrophoresis system employed in the present experiments. In some experiments, for comparative purposes, the $NaDodSO_4$-polyacrylamide gel system described by Fairbanks *et al.* (17) was also employed, and gave an apparent M_r of 49,000 for this protein (data not shown). A protein with the same electrophoretic mobility in the system of Fairbanks *et al.*, whose endogenous phosphorylation and dephosphorylation were affected by cAMP, was found in both the soluble and microsomal fractions from each of 15 vertebrate tissues examined (12). In both the previous (12) and present studies, in all of the tissues examined, this phosphoprotein contained a substantial proportion of the radioactive phosphate observed on $NaDodSO_4$-polyacrylamide gels. cAMP inhibited the endogenous phosphorylation of this protein when assays were carried out in the presence of Zn^{++}, and cAMP stimulated the endogenous phosphorylation of this protein when assays were carried out in the presence of Mg^{++}. Moreover, in both the previous investigation (12) and in the present study (data not shown), the dephosphorylation of this protein by an endogenous protein phosphatase was markedly stimulated by cAMP in the cytosol and microsomal fractions of all tissues studied. Although the higher resolution gel electrophoresis system used in most experiments of the present study gave an apparent molecular weight for this protein different than the one given by the Fairbanks system used previously, it is clear that the same species of protein was examined in both studies. The fact that the apparent M_r of this protein depends upon the gel electrophoresis system used makes it no longer appropriate to use a terminology (12) for this protein based upon its apparent M_r. Until more is established about the molecular properties and functional significance of this protein, we propose to refer to it by the acronym SCARP, representing "steroid and cyclic adenosine 3′:5′-monophosphate regulated phosphoprotein."

Antidiuretic hormone, monobutyryl cAMP, and aldosterone, applied to intact cell preparations of toad bladder epithelial tissue, and cAMP, applied to subcellular fractions of this tissue, each caused a decrease in the level of phosphorylation of a protein with the characteristics of SCARP (4–7). Interestingly, in this amphibian tissue aldosterone caused only a slight (10%) but consistent decrease in SCARP phosphorylation and a several-fold increase in rate of SCARP de-

phosphorylation, in contrast to the marked decrease in SCARP phosphorylation observed in mammalian tissues in the present study in response to steroid hormones. The effects of aldosterone pretreatment and of cAMP on SCARP phosphorylation and dephosphorylation in toad bladder were confirmed in the present study (unpublished observations), using the discontinuous pH $NaDodSO_4$-polyacrylamide gel electrophoresis system. Thus, it is clear that steroids can decrease the level of phosphorylated SCARP *via* either of two phenomenologically distinct mechanisms, i.e., a decrease in phosphorylation or an increase in dephosphorylation. It was not possible in the present study to evaluate accurately the effect of steroid pretreatment on SCARP dephosphorylation in mammalian target tissues because of the large difference in level of phosphorylated SCARP in steroid-treated versus control tissues.

The mechanism(s) by which steroid hormones affect SCARP metabolism is not yet known. One fact which must be taken into account is that ovariectomy, castration, and steroid pretreatment affect the *final* level of phosphorylation of SCARP, even under conditions in which protein phosphatase activity is abolished (Figs. 1 and 4). These results suggest that steroid hormones regulate either the amount of SCARP or its ability to become phosphorylated. Certain considerations discussed elsewhere (12) suggest that SCARP may be the regulatory subunit of a cAMP-dependent protein kinase and that it may undergo autophosphorylation catalyzed by the catalytic subunit of the enzyme. [Such an autophosphorylation reaction has been shown for purified protein kinase from heart (18) and brain (19).] In view of this possibility, it would seem important to determine whether administration of steroid hormones might lead to an alteration in the regulatory subunit and/or catalytic subunit of the kinase, or whether it might have some other effect on the interaction between the two subunits. It is important to emphasize that the steroid hormones also affect the phosphorylation of protein bands other than SCARP in various target tissues. It would be of interest to elucidate the mechanism by which the steroids exert their effects on those other phosphoproteins, and to study the possible physiological significance of these effects.

cAMP (and presumably those hormones that work through cAMP) is able to regulate the phosphorylation and dephosphorylation of SCARP (ref. 12, present results). The effect of cAMP on SCARP phosphorylation in the presence of Mg^{++} can be either stimulatory or inhibitory, depending on a variety of experimental conditions (12, 20). It now appears that steroid hormones are also capable of regulating the SCARP system. These combined results suggest the possibility of a widespread and significant role for the SCARP kinase/SCARP/SCARP phosphatase system in biological regulation. It would be premature to speculate in detail about whether the SCARP system is a direct link in the primary chain of molecular events by which steroids elicit their biological effects, or whether this system is involved, instead, in some secondary role in the target tissue, such as regulation of metabolic rate or feedback control of steroid hormone action. However, the possibility should at least be mentioned that the well-documented synergism (21, 22) between the biological actions of the steroid hormones and the biological actions of those hormones whose effects are mediated through cAMP may be related to their common action on the SCARP system.

This work was supported by U.S. Public Health Service Grants NS-08440 and MH-17387.

1. Bentley, P. J. (1965) *Biol. Rev.* **41**, 275–316.
2. Orloff, J. & Handler, J. (1967) *Am. J. Med.* **42**, 757–768.
3. Crabbé, J. (1961) *J. Clin. Invest.* **40**, 2103–2110.
4. DeLorenzo, R. J., Walton, K. G., Curran, P. F. & Greengard, P. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 880–884.
5. Walton, K. G., DeLorenzo, R. J., Curran, P. F. & Greengard, P. (1975) *J. Gen. Physiol.* **65**, 153–177.
6. Liu, A. Y.-C. & Greengard, P. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3869–3873.
7. DeLorenzo, R. J. & Greengard, P. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1831–1835.
8. Williams-Ashman, H. G. (1965) *J. Cell. Comp. Physiol.* **66**, no. 2 (Part II), 111–124.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
10. Post, R. L. & Sen, A. K. (1967) in *Methods in Enzymology*, eds. Estabrook, R. W. & Pullman, M. E. (Academic Press, New York), Vol. X, pp. 773–776.
11. Maeno, H. & Greengard, P. (1972) *J. Biol. Chem.* **247**, 3269–3277.
12. Malkinson, A. M., Krueger, B. K., Rudolph, S. A., Casnellie, J. E., Haley, B. E. & Greengard, P. (1975) *Metabolism* **24**, 331–341.
13. Maizel, J. V. (1971) in *Methods in Virology*, eds. Maramorosh, D. & Koprowski, A. (Academic Press, New York), Vol. V, pp. 179–244.
14. Hubbard, A. L. & Cohn, Z. A. (1975) *J. Cell Biol.* **64**, 438–460.
15. Ueda, T., Maeno, H. & Greengard, P. (1973) *J. Biol. Chem.* **248**, 8295–8305.
16. O'Malley, B. W., Woo, S. L. C., Harris, S. E., Rosen, J. M. & Means, A. R. (1975) *J. Cell. Physiol.* **85**, 343–356.
17. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617.
18. Rosen, O. M., Erlichman, J. & Rubin, C. S. (1974) in *Advances in Cyclic Nucleotide Research*, eds. Greengard, P. & Robison, G. A. (Raven Press, New York), Vol. V, pp. 253–264.
19. Maeno, H., Reyes, P. L., Ueda, T., Rudolph, S. A. & Greengard, P. (1974) *Arch. Biochem. Biophys.* **164**, 551–559.
20. Ueda, T., Rudolph, S. A. & Greengard, P. (1975) *Arch. Biochem. Biophys.* **170**, 492–503.
21. Thompson, E. B. & Lippman, M. E. (1975) *Metabolism* **23**, 159–202.
22. Wicks, W. D. (1974) in *Advances in Cyclic Nucleotide Research*, eds. Greengard, P. & Robison, G. A. (Raven Press, New York), Vol. IV, pp. 335–438.