are presented as mean ± SE. Kinetic paraments (K_t and V_{max}) and SE for these values were calculated as described by Wilkinson[10]. Significant differences were assessed by Student's two-tailed t-test. p-Values less than 0.05 were considered to be significant.

Results and discussion. Thiamine has been shown to be transported by two mechanisms in isolated rat hepatocytes; one is a saturable mechanism with a K_t of 34.1 μM and V_{max} of 20.8 pmol/10^5 cells per 30 s, the second is a nonsaturable mechanism[5]. The table shows the inhibitory effect of several lipophilic cations on thiamine uptake by the saturable mechanism. DDA, triphenylmethylphosphonium, tetraphenylphosphonium and tetraphenylarsonium at the concentration 10 μM inhibited 10 μM thiamine uptake by 91, 98, 98 and 98%, respectively. Barts et al.[8] reported that these lipophilic cations inhibited thiamine uptake in yeast cells. In their experiments, these compounds at the concentration 100 μM inhibited 0.8 μM thiamine uptake by 70-87%. These findings indicate that lipophilic cations are much stronger inhibitors of the thiamine transport system in isolated rat hepatocytes than in yeast cells. Previously DDA was found to be a potent inhibitor with a K_i of 0.64 μM, whose affinity is much higher than that of other quaternary amonium compounds, and to share a common binding site for thiamine in isolated rat hepatocytes[7]. Therefore, we performed Lineweaver-Burk analyses of thiamine uptake in the presence or absence of these lipophilic cations. As shown in the figure, these compounds were also competitive inhibitors.

The values of K_t and V_{max} for thiamine in the absence or presence of 0.2 μM tetraphenylphosphonium were 48.4 ± 3.53 and 202 ± 18.2 μM ($p < 0.001$), 17.4 ± 0.593 and 17.1 ± 1.12 pmol/10^5 cells per 30 s (n.s.), respectively. In the case of tetraphenylarsonium inhibition, the values of K_t and V_{max} in the absence or presence of 0.2 μM tetraphenylarsonium were 48.4 ± 3.53 and 265 ± 38.8 μM ($p < 0.001$), 17.4 ± 0.593 and 19.0 ± 2.18 pmol/10^5 cells per 30 s (n.s.), respectively. K_i values of tetraphenylphosphonium and tetraphenylarsonium were calculated to be 0.06 μM and 0.05 μM, respectively. The apparent affinities of these compounds for the thiamine binding site are about ten times that of DDA. Since these compounds at a concentration of 10 μM almost completely inhibited thiamine uptake, these lipophilic cations were probably also purely competitive inhibitors. These results indicated that lipophilic cations share a common binding site for thiamine in isolated rat hepatocytes.

Although several studies[2-7, 11-17] have been carried out on the thiamine transport system in mammalian cells, no evidence has been provided that lipophilic cations other than DDA share a common binding site for thiamine. The finding in isolated rat hepatocytes is the first such evidence. However, in yeast cells, lipophilic cations have been used to measure the membrane potentials, and the relation between lipophilic cations and thiamine binding sites has been demonstrated[8]. In mammalian cells, lipophilic cations such as tetraphenylphosphonium and triphenylmethylphosphonium have also been used to measure the membrane potentials in thyroid cells[18] and macrophages[19]. Although the thiamine transport system in these cells is still unclear, we should be careful when measuring the membrane potentials with lipophilic cations in mammalian cells as described for yeast cells. Furthermore, our findings indicate that some compounds which have a monovalent cationic group other than the quaternary nitrogen share a common binding site for thiamine in isolated rat hepatocytes.

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The in vitro characterization of the inhibition of mouse brain protein kinase-C by retinoids and their receptors

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Summary. The mechanism of the in vitro inhibition of Ca^{2+}-, phosphatidylserine-dependent protein kinase C (PK-C)[2] by the purified *holo* (ligand-saturated) forms of cellular retinol-binding protein (cRBP) and cellular retinoic acid-binding protein (cRABP) was studied. We report here that i) the PK-C-inhibitory action of *holo*-cRBP and *holo*-cRABP is due to their respective ligands, all-*trans*-retinol and all-*trans*-retinoic acid; ii) the reduced phosphorylation of the *holo*-retinoid-binding proteins and brain cytosolic proteins is not the result of a retinoid-induced soluble phosphatase or protease activity; iii) retinoids reduce PK-C affinity for calcium and phosphatidylserine in vitro; and iv) the structure-function activity of the retinoids and the specific interaction of these compounds with their binding proteins are important in blocking the activity of PK-C. These observations suggest that the inhibitory effect of retinoids on plasma membrane-associated PK-C activity pays a significant role in defining the early epigenetic aspects of PK-C-dependent tumor promotion and may be a physiological mechanism by which retinoids induce terminal differentiation in cell types that do not express soluble retinoid-binding proteins.
Key words. Retinoids; protein kinase-C; receptors.

Chemical carcinogenesis is a multistep process involving at least two phases, *initiation* and *promotion*. Initiation in the mouse skin model is achieved by the application of a subcarcinogenic dose of a chemical (such as dimethylbenz[a]anthracene); tumors are elicited by repeated application of an incomplete carcinogen, the promoter, to initiated skin[3].

The phorbol esters have been extensively studied in this model system and the tumor promoting potential of these compounds, particularly 12-O-tetradecanoylphorbol-13-acetate (TPA), correlates with their ability to bind to plasma membrane receptors characterized as calcium-, phosphatidylserine-dependent PK-C, and with PK-C-dependent phosphorylation of numerous cellular proteins[4-6]. Additionally, TPA induces numerous tumor promotion markers in mouse skin such as L-ornithine decarboxylase (ODC, E.C. 4.1.1.17) and S-adenosyl-L-methionine decarboxylase (E.C. 4.1.1.50)[7,8]. In contrast, the vitamin A compounds (retinoids), all-*trans*-retinol and especially all-*trans*-retinoic acid, are highly effective as anti-tumor-promoters and inhibit TPA-promoted tumorigenesis almost completely in the mouse skin system[7]. Moreover, there is evidence that the biological activity of retinoids is mediated by their receptors or binding proteins (RBPs), cRBP and cRABP. These receptors provide a mechanism for the specific interaction of retinoids with plasma membrane, endoplasmic reticulum, and the cell nucleus[9,10]. Although in some retinoid-responsive cell lines, such as HL-60 and LLTC, RBPs are not detectable by conventional ligand binding methods (presumably due to phosphorylation and/or tight nuclear binding of RBPs)[11], generally, cytodifferentiation in retinoid-dependent cell lines correlates with the expression of RBPs and the relative binding affinity of RBPs for retinoids[12-14].

In an experiment designed to characterize the interaction between the retinoids (all-*trans*-retinol and all-*trans*-retinoic acid), their binding proteins (cRBP and cRABP), and the phosphorylating capacity of the phorbol ester receptor (PK-C), we observed that the *apo* forms of cRBP and cRABP could act as substrates for PK-C in vitro while the *holo* forms blocked the phosphorylation of themselves as well as the 19, 22, 67, 78, 130, and 150 kDa protein substrates in the mouse brain cytosol fraction[11,15]. We examine here whether the retinoid inhibition of PK-C activity was due to the presence of retinol or retinoic acid, or whether inhibition of PK-C was dependent on the presence of the *holo* form of the retinoid-binding proteins per se. We also examined the possible induction of other systems that can directly or indirectly affect PK-C activity.

Materials and methods. cRBP and cRABP were purified to homogeneity from calf liver and calf uterus, respectively, as previously described[15-17]. Bovine serum albumin (BSA, fraction V, fatty acid free) and ovalbumin (Type 5, Sigma Chemical Co.) were used as non-specific retinoid-binding protein controls and were added at concentrations of 100 and 65 µg/ml, respectively, in the final incubation mixtures.

Soluble PK-C was prepared from TPA-exposed mouse brain (100,000 × g) supernatant fraction prepared from female CD-1 mice (Charles River Breeder's), and PK-C-dependent phosphorylation of histone III-S (Sigma Chemical Co.) was determined as previously described[4,15,18]; the assay reaction was initiated by the addition of the [γ-^{32}P] ATP (0.06-0.25 Ci/mmol, Amersham Searle Co.).

Histone III-S phosphorylation was also determined colorimetrically by the modified method of Hess and Derr[19]. The PK-C dependent phosphorylation of the histone III-S substrate was carried out in the presence of either unlabeled retinylphosphate or unlabeled ATP. Phosphate was assayed as the PO_4^{3-} removed from histone III-S by alkaline phosphatase treatment. Briefly, the PK-C reaction mixture in this experiment was transferred to 25 mm DE81 filter paper disks (Whatman Inc.) contained in glass scintillation vials, and air dried. The vials were heated to 65 °C for 30 min, then cooled to 25 °C. Tris-HCl buffer (10 mM, pH 7.6, 5 °C) was used to wash the filter disks (5X, 3 ml) followed by acetone and ether washing as previously described[4,18]. After the final wash the filters were air dried and 1.0 ml of 10 mM Tris buffer, pH 8.7, containing 1 mM $MgCl_2$, and 0.1 mM $ZnCl_2$ was added to each vial. Alkaline phosphatase (15 units in 0.1 ml, type VII; Sigma Chemical Co.) was added and the mixture was incubated on a shaker bath of 37 °C for 20 h. An aliquot of the reaction mixture was removed and assayed for the liberation of PO_4^{3-}.

TPA-induced mouse epidermal ODC was assayed in a 30,000 × g supernatant fraction as previously described in detail by Cope et al.[20], and Verma et al.[21] with slight modification. DL-[1-^{14}C]-Ornithine (50 mCi/mmol, New England Nuclear) was used as the assay substrate at a concentration of 100 mM in a final volume of 2.0 ml. Reactions were allowed to proceed for 1 h at 37 °C and were stopped by the addition of 1 ml of 2 M citrate solution; the incubation was continued for an additional 1 h at 25 °C. The evolved [^{14}C]-CO_2 was trapped in ethanolamine and radioactivity was determined by liquid scintillation spectrometry.

Protein concentrations were determined by the Coomassie Blue-binding assay using lysozyme as a protein standard[22].

Results and discussion. The retinoic acid and *holo*-cRABP inactivation curve of PK-C is shown in figure 1. The addition of free all-*trans*-retinoic acid to the PK-C reaction mixture inhibited phosphorylation of the histone III-S fraction, a standard assay substrate, in a concentration-dependent manner. When similar concentrations of this retinoid were added as a *holo*-complex with cRABP, the PK-C-inhibitory response was reduced, with a q-fold increase in the IC_{50} for retinoic acid + cRABP (1.52 µM; table 1a and b). In addition, retinol also inhibited (0.52 µM) PK-C-dependent phosphorylation of the histone substrate while the *holo*-cRBP complex was 3-fold less active in this regard. These observations demonstrate that the retinoid ligands are the effective components which abrogate PK-C activity and can function in our in vitro system without RBPs. Nevertheless, *holo*-RBPs are effective at concentrations that can be considered physiologically significant[10,15].

The efficacy of other retinoids in the presence and absence of proteins that are unrelated to the RBPs was also tested (table 1a and b). The addition of the all-*trans*-furyl analog of retinoic acid, a retinoid incapable of reversing keratinization in tracheal epithelial cells[23] and inhibiting TPA-promoted tumorigenesis[21], did not result in a significant loss of PK-C activity nor did the concomitant addition of bovine serum albumin (BSA) alter the kinase activity. The addition of retinylphosphate (table 1a), the retinoid active in lipid-linked oligosaccharide synthesis, reduced the incorporation of [32P] into the histone III-S substrate while the potent retinoidal derivative of benzoic acid, TTNPB (4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1E-propenyl] benzoic acid) was the most effective retinoid compound tested. It should be noted here that the relative effectiveness of these compounds to inhibit PK-C correlates with their ability to induce terminal differentiation in murine F-9 teratocarcinoma cells in culture[24]. The inclusion of BSA (table 1b) in the PK-C reaction mixture reduced (30-fold) the efficacy of free all-*trans*-retinoic acid as did the addition of ovalbumin which was maximally effective (78-fold) in this regard. Taken together, these data suggest that the anti-PK-C effect of retinoids is associated with the biological function of these compounds and that the reversible and specific binding relationship of retinoids with RBPs may be biologically significant in mediating their interaction with the PK-C-inhibiting site(s) in vivo.

Moreover, since a particular biological function of retinoids is the induction of alkaline phosphatase[25], we determined whether or not the induction of this enzyme, as found in the 100,000 × g mouse brain supernatant fraction, was a factor in reducing PK-C-dependent incorporation of [^{32}P] into the histone III-S substrate. This was achieved by assaying the supernatant fraction for PK-C activity as outlined but without the initial addition of retinoids or their *holo*-binding proteins. After 5 min, the usual assay time, the retinoid compounds or their *holo*-binding pro-

teins were added and the incubation was continued for an additional 5 or 10 min. This postincubation of the reaction mixtures in the presence of the retinoids or their *holo*-binding proteins produced no significant change from the values in table 1 of retinol, retinoic acid, or either *holo*-binding protein with regard to incorporation of the [^{32}P] into the histone III-S substrate, suggesting that the PK-C-inhibitory action of the retinoids was not mediated by the in vitro activation of a soluble phosphatase. Additionally, incubation of the mouse brain supernatant fraction with leupeptin (a thiol protease inhibitor) prior to addition to the assay mixture did not appreciably alter the inhibitory capacity of either retinoic acid or retinol (table 1b). Thus, the inhibition of PK-C was not due to retinoid stimulation of PK-C-inactivating proteases. This result is consistent with other experiments that in fact, show that PK-C is transiently *activated* by leupeptin-sensitive proteases that promote the loss of diacylglycerol, phosphatidylserine, and Ca^{2+} binding sites and result in the release of an unregulated active PK-C fragment[26]. Moreover, our observations are consistent with those of Anderson et al.[27]

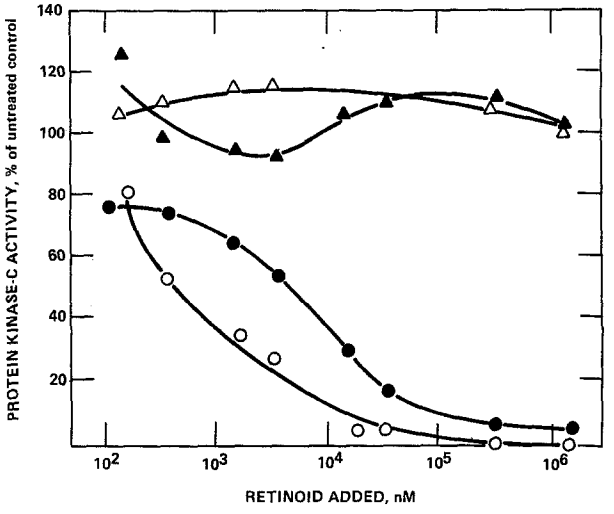

Figure 1. In vivo inhibition of protein kinase-C derived from TPA-treated mouse brain. PK-C was assayed as described. The reduction in PK-C activity in the mouse brain supernatant was plotted as a function of the amount of retinoid added. All-*trans*-retinoic acid (○); *holo*-cRABP, (●); all-*trans*-furyl retinoic acid (△); all-*trans*-furyl retinoic acid + BSA (▲). Each point represents the mean of five determinations with a coefficient of variation not exceeding 18%. (See table 1 for a list of all tested variables.)

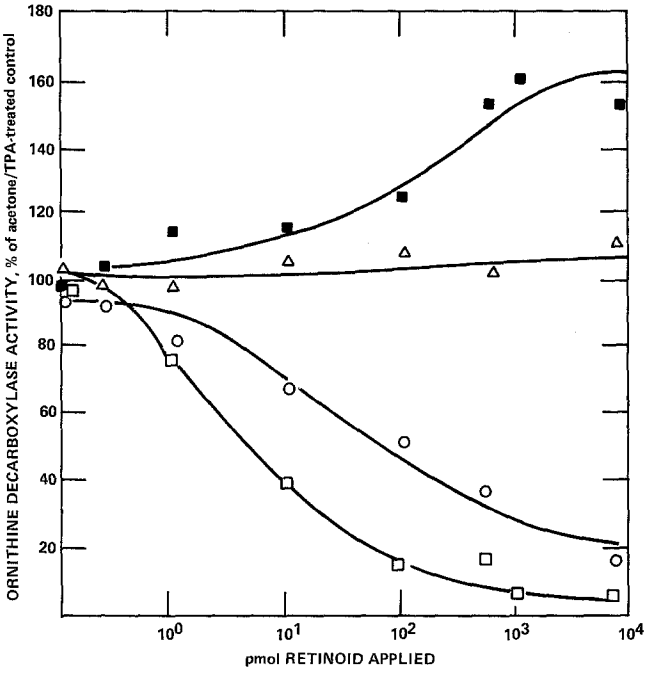

Figure 2. In vivo inhibition of TPA-induced ornithine decarboxylase activity in mouse epidermis by retinoids. TPA-induced mouse epidermal ornithine decarboxylase (E.C. 4.1.1.17) was assayed in a 30,000 × g supernatant fraction as described. The change in ODC activity of the retinoid/TPA-treated mice to the acetone/TPA-treated mice was plotted as a function of the amount of retinoid applied to the animals. All-*trans*-retinoic acid (○); all-*trans*-furyl analog of retinoic acid (△); TTNPB, retinoidal derivative of benzoic acid (□); and retinylphosphate, (■). Each point represents the mean of five determinations with a coefficient of variation not exceeding 13%.

and suggest that retinoids are particularly effective against PK-C in the presence of TPA or diacylglycerol.

Because of the lipophilicity of PK-C and because the retinoids are hydrophobic, we determined whether or not these compounds could displace the enzymatic co-factor, phosphatidylserine, from PK-C and thereby reduce its affinity for Ca^{2+} and its enzyme activity. Table 1 shows that, in the presence of optimum phosphatidylserine and Ca^{2+}, the IC_{50} for the retinoids (column a) is increased in the presence of their respective binding proteins (column b) and that the all-*trans*-furyl analog is inactive in blocking PK-C activity. When the phosphatidylserine (column c) or Ca^{2+} (column d) level increased to a point at which these

Table 1. Summary of the IC_{50} of retinoids and retinoid-protein complexes for soluble mouse brain PK-C activity in vitro*

Additional assay component	(a)	(b)	(c)	(d)
All-*trans*-retinoic acid	0.27 ± 0.05 μM	2.51 ± 0.45 μM (+ cRABP)	1.62 ± 0.30 μM	0.52 ± 0.08 μM
	–	8.25 ± 1.25 μM (+ BSA)	–	–
	–	21.05 ± 3.01 μM (+ ovalbumin)	–	–
	–	0.38 ± 0.07 μM (+ leupeptin)	–	–
All-*trans*-retinol	0.52 ± 0.09 μM	1.52 ± 0.19 μM (+ cRBP)	5.20 ± 0.78 μM	1.56 ± 0.23 μM
	–	0.68 ± 0.10 μM (+ leupeptin)	–	–
All-*trans*-furyl analog of retinoic acid	≫ 1 mM	≫ 1 mM (+ BSA)	–	–
Arotinoid TTNPB	0.015 ± 0.003 μM	–	–	–
Retinylphosphate	2.00 ± 0.25 μM	–	–	–

*The IC_{50} (concentration at which 50% inhibition of PK-C was achieved) was determined by the titrative reduction of [^{32}P]-incorporation into histone III-S substrate in the presence of the additional assay component(s). Data are expressed as the μM concentration of the added component determined in five experiments ± SD. Mouse brain cytosol was used as the source of PK-C as described. Experiments which included leupeptin (Sigma Chemical Co.) were accomplished by the addition of 50 μg/ml of this protease inhibitor to the 100,000 × g mouse brain supernatant fraction 15 min (at 25 °C) prior to inclusion of the supernatant fraction (the source of PK-C) into the assay mixture. Column (a) indicates the PK-C assay completed in the presence of free retinoids; column (b) indicates that the assay included the specified protein. Columns (a) and (b) reflect the concentration of phosphatidylserine (PS; 10 μg/tube) and Ca^{2+} (0.7 mM) determined to be conductive for maximum PK-C activity in the absence of additional assay components while those in (c) and (d) are the maximum PK-C-permissible concentrations (< 15% drop in PK-C activity; column c- PS, 100 μg and Ca^{2+}, 0.7 mM; column d- PS, 10 μg and Ca^{2+}, 3.5 mM).

components become inhibitory to PK-C, the amount of free retinol or retinoic acid required to achieve the same level of PK-C inhibition is increased 3 to 10-fold and 2 to 6-fold, respectively. However, additional phosphatidylserine or Ca^{2+} had no effect on the concentration of retinoid required to maximally inhibit PK-C. This indicates that a part of the inhibitory phenomenon of retinoids can be attributed to an effect on PK-C affinity for phosphatidylserine and Ca^{2+}.

We also questioned whether or not the inhibition of PK-C by the retinoids tested here correlated with their ability to inhibit ODC, a TPA-dependent, and presumably a PK-C-induced tumor promotion marker[28]. Figure 2 shows that all-*trans*-retinoic acid and the arotinoid TTNPB both inhibited TPA-induced epidermal ODC activity with ID_{50} values (dose at which a 50% reduction in ODC activity was achieved) of 68.0 pmol and 7.8 pmol, respectively; furyl retinoic acid was not effective in blocking ODC induction. Verma et al.[21] have observed that retinol has an ID_{50} of 250 pmol, and it is therefore significantly less effective than other retinoids tested here. Thus, inhibition of ODC in this experiment correlates with the relative efficacy of these compounds to inhibit PK-C. However, retinylphosphate synergistically increased TPA-induced ODC activity (150% at 880 pmol) when applied in conjunction with TPA. The application of retinylphosphate alone, under these conditions, had no effect on ODC activity.

To determine a basis for this response, we conducted PK-C assays in which retinylphosphate was substituted for ATP in the reaction mixture and examined the PO_4^{3-} incorporation into the histone III-S substrate. Phosphorylation of the histone substrate was reduced by only one-third when contrasted with the amount incorporated when ATP was the PO_4^{3-} donor (table 2a). Additionally, the phosphorylation of the histone III-S fraction substrate was sensitive to the exclusion of Ca^{2+} and phosphatidylserine regardless of the PO_4^{3-} donor used. Table 2b also indicates that both ATP and retinylphosphate were hydrolyzed during the reaction. It is quite possible that the hydrolysis of retinylphosphate led to the information of a novel intermediate PO_4^{3-} donor, although this was not determined. In any case, these observations suggest to us that retinylphosphate competitively reduces [^{32}P] incorporation in the histone III-S substrate in vitro as in table 1, while in vivo, it enhances a rate-limiting PO_4^{3-} pool that is used for PK-C-dependent phosphorylation of proteins associated with tumor promotion, thus maximizing the induction of ODC (fig. 2). This concept is consistent with the fact that retinylphosphate is less effective than would be expected for an active retinoid metabolite and is probably not an in vivo metabolite, and that other glycosol intermediates are reduced in chemically-induced hepatocellular carcinoma, perhaps as a result of their increased degradation[29].

Given the observations that retinylphosphate could act as a PO_4^{3-} donor (directly or not), that retinoids block PK-C activity in a concentration-dependent manner, and that retinoids do not compete for the TPA- or other phorbol ester-binding sites on PK-C[30,31], retinoids most likely have a direct effect on PK-C by either 1) interacting with the enzymatic site of PK-C, 2) interacting with a site(s) independent of any ligand (Ca^{2+}, phosphatidylserine, or TPA) binding, 3) associating with plasma membrane lipids (in vitro) or micelles (in vitro) and thus altering PK-C conformational status important to the kinase activity or 4) interacting with some protein or cofactor required for PK-C activity.

If we integrate the observations presented here with those of the current theory of the two-stage tumor promotion model (in which retinoids are unable to block the primary stage of TPA-induced promotion or the *memory phase* but inhibit the secondary or *promoter reexposure phase* of TPA-induced tumorigenesis), we conclude 1) that the primary stage of promotion involves TPA-induced cell changes, of which PK-C is not necessarily a required mediator, and 2) that the induction of PK-C by TPA is required in secondary stage tumor promotion. Miyake et al.[32] have shown that mezerein, an impotent primary-stage but strong secondary-stage tumor promoter, activates PK-C. Thus, PK-C appears to be indispensible in secondary-stage tumor promotion. This fact further suggests that retinoids are acting during this period and is consistent with the observation that a high cellular PK-C concentration per se and its induction are not sufficient for tumorigenesis[20]. Finally, the cellular ubiquity of PK-C, its suppression by retinoids, and the apparent role of phosphorylation in controlling protein-DNA interactions[33,34] provides the basis for an attractive hypothesis: retinoid-dependent cytodifferentiation is mediated epigenetically and genetically by retinoid/PK-C interactions. Thus, genes under the influence of retinoids may be controlled by the relative phosphorylation (positive or negative) of their associated proteins, which in turn control DNA secondary structure and gene expression.

Table 2. The in vitro assay of PK-C-dependent incorporation of PO_4^{3-} into the histone III-S substrate and hydrolysis of retinylphosphate*

PO_4^{3-} Donor Added or deleted assay component	Retinyl-phosphate nmol PO_4^{3-}/min/mg supernatant protein	5'-ATP
(a) Total alkaline phosphatase-labile histone PO_4^{3-}	3.05 ± 0.35	4.55 ± 0.38
+ polymyxin B	0.55 ± 0.09	0.55 ± 0.07
− supernatant fraction	≤ 0.05	0.15 ± 0.03
− Ca^{2+},-phosphatidylserine	0.65 ± 0.08	0.75 ± 0.13
− histone	0.25 ± 0.04	0.15 ± 0.03
+ polymyxin B, − Ca, − phosphatidylserine	≤ 0.05	≤ 0.05
(b) Total PO_4^{3-} hydrolyzed (w/supernatant − w/o supernatant)	4.05 ± 0.37	6.25 ± 1.03

* Soluble mouse brain PK-C-dependent phosphorylation of histone III-S (a) was assayed as described in a 1.0 ml final volume with the addition of either unlabeled retinylphosphate (25 μM) or unlabeled ATP (25 μM) and the added or deleted component(s) indicated above. Polymyxin B (10 μM) was added as a specific inhibitor of PK-C[35]. Phosphate generated as a result of the hydrolysis of either ATP or retinylphosphate during the assay (b) was determined by assaying duplicate incubations as in (a) for free PO_4^{3-} at the end of the PK-C enzyme assay, typically 5 min. The amount of hydrolysis was determined by the difference in free PO_4^{3-} liberated in the presence and absence of mouse brain 100,000 × g supernatant. Data are expressed as nmol PO_4^{3-} incorporated on the histone III-S substrate (a), or released (b) as free PO_4^{3-}/min/mg supernatant protein ± SD; the limits of PO_4^{3-} detectability in this assay are 0.05 nmol/min/mg protein. Values are the means of five determinations.

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2 Abbreviations used: PK-C, Ca^{2+}-, phosphatidylserine-dependent protein kinase-C; cRBP, cellular retinol-binding protein; cRABP, cellular retinoic acid-binding protein; TPA, 12-O-tetradecanolphorbol-13-acetate; ODC, ornithine decarboxylase (E.C.4.1.1.17); RBPs, retinoid-binding proteins; TTNPB, 4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1E-propenyl]-benzoic acid; IC_{50}, concentration at which 50% inhibition of PK-C control activity was achieved; ID_{50}, dose at which a 50% reduction in ODC activity was achieved; PS, phosphatidylserine.

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Effects of 18-hydroxydeoxycorticosterone on central nervous system excitability

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Summary. The effects of 18-hydroxydeoxycorticosterone (18-OH-DOC) on central nervous system excitability were studied in adrenalectomized rats. Sixty-four evoked potentials (EP) recorded from the pontine reticular formation were averaged before and after the injection of vehicle and hormone. 750 µg of 18-OH-DOC dissolved in 0.5 ml of a 4:1 saline Cremophor-EL solution were injected i.v. A decrease of 55.7 ± 6.1% in the amplitude of the EPs was observed with the hormone 16.3 min ± 2.7 (SE) after injection. Amplitude values returned to baseline levels 38 min ± 6.8 (SE) after injection. The secretion of 18-OH-DOC is greatly increased by ACTH and might modulate central nervous system function.
Key words. 18-OH-DOC; brain; excitability.

Nervous systems are sensitive targets for steroid hormones[1,2]. Thus, besides their well-known effects on neuroendocrine feedback mechanisms, steroid hormones modulate CNS excitability and affect mood and sexual behavior[3]. Within the adrenal steroids the glucocorticoid type hormones have received the most attention in the past.

Recent work has demonstrated that mineralocorticoid type hormones are taken up and metabolized by nervous tissue[4]. Moreover, DOC, as well as its ring-A reduced metabolites DHDOC and THDOC significantly reduced brain excitability in the rat[5]. DOC, however, is not the only mineralocorticoid hormone in the rat. The rat adrenal is unique in secreting, as its second most prominent steroid after corticosterone, 18-OH-DOC[6]. 18-OH-DOC is a sodium-retaining compound produced mainly in the zona fasciculata of the adrenal cortex under the control of ACTH[6]. Radiochemical studies, as well as measurement of the endogenous hormone, revealed that 18-OH-DOC is widely distributed in the brain[7,8].

We therefore thought it appropriate to examine the possible effects of 18-OH-DOC on brain excitability.

Methods. Adrenalectomized male Sprague-Dawley rats weighing 250–300 g were used. Adrenalectomies were carried out in our laboratory 2 days prior to the experiments and the rats were maintained on Purina Chow and physiologic saline ad libitum. The rats were anesthetized with urethane (1.1 g/kg) given i.v. under ether anesthesia. A trachea cannula was positioned and the femoral artery and vein were cannulated. The femoral arterial pressure and the percentage CO_2 in the expired air were monitored throughout the experiments with a Statham P23 pressure transducer and a Godart Statham capnograph, connected to the bridge mode of a Grass DC pre-amplifier respectively. Expired CO_2 was maintained at 3.8–4%. The animals were paralyzed with gallamine triethiodide (Flaxedil, Poulenc) 10 mg/kg and maintained under artificial respiration. The rectal temperature was servoregulated to 37 °C with a Yellow Springs Instrument feedback system attached to a DC heating source. In preparation for recording, a bone flap was removed between the lamboidal suture and bregma and the dura was retracted. The sciatic nerve contralateral to the recording site was prepared for stimulation and maintained in a pool of warm paraffin oil.

Field activity was recorded from bipolar electrodes stereotaxically positioned in regions corresponding to the pontine reticular formation. Evoked potentials were amplified through a Tektronix 122 preamplifier (band width of 0.2 Hz to 10 kHz). After further amplification, the potentials were displayed on a Tektronix 565 oscilloscope. They were averaged with an Enhancetron digital memory oscilloscope (Nuclear Data Inc.) and displayed on a Tektronix 5000 series oscilloscope and photographed from it. Anodal electrolytic DC lesions, 20 µa for 15 s, were made for histological verification of recording sites. 18-OH-DOC was dissolved in a 4:1 saline:cremophor-EL solution.

(Cremophor EL, a polyoxyethylated castor oil, was purchased from Sigma). Only one injection (750 µg/0.5 ml) was given in each experiment to avoid dose summation. The injection was