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THE EFFECT OF INTERMEDIATE FILAMENT INHIBITORS ON
STEROIDOGENESIS AND CYTOSKELETON IN Y-1
MOUSE ADRENAL TUMOR CELLS

THESIS

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By

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When Y-1 mouse adrenal tumor cells were treated with sodium orthovanadate, an intermediate filament (IF) inhibitor in BHK21-F cells, there was no change in the amount of 20 α -dihydroprogesterone produced. A neurofilament inhibitor, β,β' -iminodipropionitrile (IDPN), enhanced the ability of Y-1 cells to produce steroid in response to ACTH by acting on the plasma membrane. Electron microscopy of Y-1 cells extracted with Triton X-100 revealed that both vanadate and IDPN caused the aggregation of cytoskeletal and granular structures in the perinuclear area. The steroidogenic effects of IDPN suggest that the perinuclear aggregation of cytoskeletal structures may result from the detachment of IF from the plasma membrane, while the reason for the cytoskeletal changes by vanadate is unknown.

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CHAPTER I

INTRODUCTION

It is now well established that a variety of different vertebrate cells contain intermediate filaments as well as microfilaments and microtubules (Uehara et al., 1971; Goldman and Knipe, 1973; Small and Sobieszek, 1977; Starger and Goldman, 1977). These cytoskeletal structures are mainly involved in the positioning of organelles, cellular movement, and the transport of intracellular materials (Goldman, 1971; Malaisse et al., 1972; Lehto et al., 1978).

Cells in the zona fasciculata region of adrenal cortex produce increased amounts of steroid in response to adrenocorticotropin (ACTH), $N^6, O^{2'}$ -dibutyryl adenosine 3',5'-monophosphate (dibutyryl-cAMP), and cholera toxin (Kowal, 1970; Wishnow et al., 1975). In response to stimulation, microfilaments contributed to the transport of cytoplasmic cholesterol to the mitochondrial side-chain cleavage enzyme complex, increasing steroidogenesis (Garren et al., 1971; Mrotek and Hall, 1975; 1977; Mrotek et al., 1982). Controversy exists over the role of microtubules in steroid

production (Temple and Wolff, 1973; Mrotek and Hall, 1978; Clark and Shay, 1981); thus, it is unclear whether microtubules are required for the steroidogenic response of these cells to ACTH and dibutyryl-cAMP.

Even though intermediate filaments, together with microtubules (Holmes and Choppin, 1968), are known to be involved in the positioning of organelles in non-adrenal cells (Lehto et al., 1978), their cellular functions are relatively unclear. In the past, this lack of clarity was partially due to the absence of the inhibitors which could be used as intermediate filament probes. Recently it was found in baby hamster kidney cells (BHK21-F) that sodium orthovanadate caused perinuclear aggregation of intermediate filaments by separating them from microtubules (Wang and Choppin, 1981). Therefore vanadate may be an intermediate filament inhibitor. Similarly, β,β' -iminodipropionitrile (IDPN) caused the accumulation of neurofilaments in the proximal part of axons and the degeneration of neurofilaments in dendrites (Goldberg, 1977; Deshmukh and Taylor, 1978; Griffin et al., 1978; Shimono et al., 1978; Clark et al., 1980). Considering that non-neuronal intermediate filaments morphologically resemble vertebrate neurofilaments

(Lazarides, 1981) and that a major molecular subunit (68,000 dalton) of neurofilaments also exists in intermediate filaments of non-neuronal cells, including skeletal muscle myofibrils (Wang et al., 1980), IDPN may represent another intermediate filament inhibitor.

Since the Y-1 mouse adrenal tumor cell represents a good model system for studying the intracellular changes occurring during steroid production (Kowal, 1970; Masui and Garren, 1971; Temple and Wolff, 1973; Mrotek and Hall, 1975; Cuprak et al., 1977; Mrotek and Hall, 1977; Mrotek et al., 1982), it was selected as the cell to study the effects of vanadate and IDPN and ultimately to determine whether intermediate filaments are involved in steroidogenesis. Using a radioimmunoassay (Maroulis and Abraham, 1975) for the 20 α -hydroxypregn-4-en-3-one (20 α -dihydroprogesterone, 20-DHP) produced by the Y-1 cell (Kowal, 1970), the effects of vanadate and IDPN on non-stimulated and ACTH-stimulated steroidogenesis were determined. In order to examine the cytoskeletal changes, a non-ionic detergent, Triton X-100, was employed to remove the plasma membrane, cytoplasmic ground substances, and almost all the subcellular organelles (Small and Celis, 1978; Schliwa and Blerkom,

1981). Inhibitors of microfilaments (Wessels et al., 1971) or microtubules (Borisy and Taylor, 1967) were also used to study the interrelationship between intermediate filaments and microfilaments or microtubules.

CHAPTER II

MATERIALS AND METHODS

Cell Culture

Y-1 mouse adrenal tumor cells (American Type Culture Collection, Rockville, MD, USA) were grown in Eagle's Minimal Essential Medium (MEM) supplemented with 10 mM N-2-hydroxyethylpiperazine N-1,2-ethanesulfonic acid (HEPES), 25 mM NaHCO₃, 12.5% (v/v) horse serum (Lot #010680), 2.5% (v/v) fetal calf serum (Lot #007443), 0.025% (w/v) L-glutamine, and 0.0125% (w/v) gentamycin (pH 7.2-7.4). Cells were incubated at 37°C at 100% relative humidity and a 5% (v/v) CO₂, 95% (v/v) air atmosphere. Medium was changed every two to three days. Cells were subcultured with 0.05% (w/v) trypsin and 10 mM ethyleneglycol-bis-(β -aminoethyl ether) N,N'-tetraacetic acid (EGTA) every seven to fifteen days (split ratio, 1:2).

Experimental Solutions

To standardize solvents to a uniform volume, treatment stock solutions were prepared in concentrations which would

allow injection of 20 μ l into the experimental medium at the time of treatment. Experimental medium consisted of Eagle's MEM containing 10 mM HEPES and 25 mM NaHCO_3 without serum. Cytochalasin B was prepared in dimethyl sulfoxide/absolute ethanol (1:1, v/v), ACTH was dissolved in 1% (w/v) bovine serum albumin/saline, while cholera toxin, dibutyryl cAMP, and colchicine were directly dissolved in the experimental medium. A stock solution of sodium orthovanadate was made in 0.1 M NaOH as described by Wang and Choppin (1981). The IDPN was dissolved in saline to make a stock solution. Unless otherwise stated final concentrations in experimental medium for the various treatments were: Cytochalasin B, 10^{-5} M; ACTH, 0.5 IU/ml; cholera toxin, 10^{-4} M; dibutyryl cAMP, 10^{-3} M; colchicine, 10^{-5} M; sodium orthovanadate, 10^{-6} - 10^{-4} M; and IDPN, 0.0001-1% (v/v).

Experimental Treatment Procedure

During experimental incubations at 37°C, Eagle's MEM was used without serum supplements. At half-hourly intervals, incubated medium was removed and fresh medium added. The first two intervals served as a preincubation to remove serum residues, the third provided a measure of basal steroid production, while the fourth and subsequent intervals

were conducted using media containing various experimental solutions. When cells were preincubated with vanadate during the fourth period, the duration of the incubation was 16 hours (Wang and Choppin, 1981). Each experiment was performed in duplicate three times.

Cell Viability

Following experimental incubations, cells were washed with Hank's buffer and 1 ml of 0.04% (w/v) trypan blue solution was added to each plate. After 5 minutes, cells were briefly washed with Hank's buffer and stained cells were counted as dead (Phillips, 1973).

Radioimmunoassay

Radioimmunoassay for 20-DHP was done according to the procedure of Maroulis and Abraham (1975) as modified by Mrotek and Hall (1977). Tritium-labeled 20-DHP was obtained from New England Nuclear Corp. (Boston, MA, USA), and 20-DHP antiserum was a gift from Dr. Guy Abrahams (Harbor General Hospital, Torrance, CA, USA).

Statistical Tests

T-test ($\alpha=0.05$) was used for the values in Figure 4 and 5. Analysis of variance with Duncan's multiple range test

($\alpha=0.05$) was used for the values in Table I, II, Figure 2, and 3 (Steel and Torrie, 1960).

Cell Extraction and Electron Microscopy

After experimental incubations, cells grown on carbon- and Formvar-coated grids (Ladd Research Industries, Burlington, VT, USA) were briefly washed with a buffer (PHEM) which contained 60 mM 1,4-piperazine diethylsulfonic acid (PIPES), 25 mM HEPES, 10 mM EGTA and 2 mM MgCl_2 (pH 6.9), and then lysed at 37°C with PHEM buffer supplemented with 1% (v/v) Triton X-100 for five to ten minutes (Schliwa and Blerkom, 1981). Extracted cells were washed three times with PHEM buffer, fixed with 1% (v/v) glutaraldehyde in PHEM buffer for 20 minutes, and washed again with PHEM buffer. After staining for two to three minutes with 0.5% (w/v) osmium tetroxide dissolved in PHEM buffer, cells were washed three times with deionized water, and dehydrated in an ethanol series (15, 30, 50, 70, 95, 100, 100, 100%, v/v). Cells were critical point-dried using an ethanol/liquid carbon dioxide transition fluid, then examined with a RCA EMU-3 electron microscope.

Chemicals

Adrenocorticotropin, N⁶,O^{2'}-dibutyryladenine 3',5'-monophosphate, cholera toxin, sodium orthovanadate, and cytochalasin B were purchased from Sigma (St. Louis, MO, USA), and colchicine from Fisher Scientific Co. (Fair Lawn, NJ, USA). Eastman Kodak (Rochester, NY, USA) provided the β,β' -iminodipropionitrile. All serums were from Irvine Scientific Co. (Santa Ana, CA, USA), and Eagle's minimal essential medium was from KC Biological Inc. (Lenexa, KS, USA).

CHAPTER III

RESULTS

Following the procedures used by Wang and Choppin (1981) for BHK21-F cells, Y-1 adrenal cells were preincubated with vanadate for 16 hours prior to measuring the effects of vanadate on steroidogenesis by control and ACTH-treated cells. There were no significant differences ($P > 0.05$) in the levels of basal steroid production when non-treated and vanadate-treated cells were compared (Table I).

TABLE I
BASAL STEROID PRODUCTION BY Y-1 ADRENAL
CELLS INCUBATED WITH VANADATE

Vanadate Treatments (μM) ^a	20-DHP (ng/ml) Produced ^b	
	16hr Preincubation	1hr incubation
0	7.9 $\pm$ 0.6	2.5 $\pm$ 0.4
1	7.5 $\pm$ 0.4	2.4 $\pm$ 0.3
10	7.5 $\pm$ 0.2	2.6 $\pm$ 0.1
40	7.4 $\pm$ 0.3	2.5 $\pm$ 0.2
100	7.8 $\pm$ 0.3	2.3 $\pm$ 0.2

^aY-1 cells were preincubated for 16 hours with the indicated treatments and the preincubation medium was assayed for 20-DHP. Cells from the preincubation were then washed and new medium containing the indicated treatments was incubated with these cells for 1 hour before measuring steroid production.

^bValues are expressed as the mean $\pm$ SEM. Individual values for each incubation period were not significantly different ($P > 0.05$).

In addition, vanadate did not significantly affect ($P > 0.05$) the stimulation of steroidogenesis by ACTH (Table II).

TABLE II
EFFECT OF VANADATE ON ACTH-STIMULATED STEROID
PRODUCTION BY Y-1 ADRENAL CELLS

Treatments ^a			20-DHP (ng/ml) Produced ^b	
16hr Preincubation	1hr Incubation			
Vanadate (μ M)	Vanadate (μ M)	ACTH (IU/ml)	16 hrs	1 hr
0	0	0	7.3 $\pm$ 1.2	2.3 $\pm$ 0.3
0	0	0.5	7.0 $\pm$ 0.5	8.7 $\pm$ 0.6
1	1	0.5	6.9 $\pm$ 0.5	8.5 $\pm$ 0.7
10	10	0.5	6.7 $\pm$ 0.7	9.1 $\pm$ 0.5
40	40	0.5	7.1 $\pm$ 0.4	8.4 $\pm$ 0.6
100	100	0.5	6.8 $\pm$ 0.7	8.7 $\pm$ 0.3

^aY-1 cells were preincubated for 16 hours with the indicated treatments and the preincubation medium was assayed for 20-DHP. Cells from the preincubation were then washed and new medium containing the indicated treatments was incubated with these cells for 1 hour before measuring steroid production.

^bValues are expressed as the mean $\pm$ SEM. Individual values for each incubation period were not significantly different ($P > 0.05$).

To determine whether vanadate affected the arrangement of the cytoskeletal structures, Triton X-100 extracted control and vanadate-treated Y-1 cells were examined by transmission electron microscopy. The perinuclear cytoplasmic area of a control cell is seen in Figure 1A. Bundles of microfilaments (stress fibers) used for maintaining close attachment of the cell to the growth substrate and evenly distributed granular structures were observed in the

cytoplasmic area.

In contrast, a Y-1 cell incubated for 16 hours in the presence of vanadate is shown in Figure 1B. In this vanadate-treated cell, detergent-resistant granules, together with cytoskeletal structures and attached polyribosomes were aggregated in the perinuclear area.

Fig. 1. Detergent extracted cytoskeletons from non-treated (A) and vanadate-treated (B) Y-1 adrenal cells. Note the stress fibers and even distribution of granules in A. The granules in B were aggregated in the perinuclear area. (G) Granules, (N) nucleus, (P) polyribosome, (SF) stress fibers. x4,250.

To determine whether other intermediate filament perturbing agents affected steroidogenesis or the arrangement

of cytoskeletal structures in Y-1 cells, experiments were conducted using IDPN. When the Y-1 cells were incubated with increasing concentrations of IDPN, basal steroid production was unchanged ($P > 0.05$); however, ACTH-stimulated steroid production was increasingly enhanced by IDPN at concentrations between 0.0001 and 0.01% (v/v) (Figure 2). Although 0.01% (v/v) IDPN significantly increased ($P < 0.05$) ACTH-stimulated steroid production, 1% (v/v) IDPN

Fig. 2. Effect of IDPN on basal and ACTH-stimulated steroid production by Y-1 adrenal cells. ^aValues are the mean $\pm$ SEM. *Significantly different ($P < 0.05$).

significantly reduced steroidogenesis ($P < 0.05$). Determination of cell viability for cells treated with ACTH plus IDPN (0.1 and 1%, v/v) was inconclusive because of cell thickness due to rounding; however, no dead cells were seen in any of the flat-growing Y-1 control cells treated with IDPN (0.1 and 1%, v/v).

The cooperative effects between IDPN and ACTH were reversible when IDPN was removed from the medium and incubation was continued (data not shown).

In order to localize the effect of IDPN on the physiological sequence of events leading to steroidogenesis, two different agents which by-passed steps occurring in the plasma membrane were used to stimulate steroid production. When plasma membrane adenyl cyclase was by-passed using dibutyryl cAMP (Grahame et al., 1967; O'Hare and Neville, 1973), IDPN (0.0001-0.1%) had no effect on cAMP-induced steroidogenesis ($P > 0.05$) (Figure 3). Cholera toxin which by-passes ACTH receptors and directly stimulates adenyl cyclase (Wishnow et al., 1975) was used to further localize the effect of IDPN; in this experiment IDPN (0.0001-0.1%, v/v) still had no effect on the stimulation of steroidogenesis ($P > 0.05$). On the other hand, 1% IDPN significantly

($P < 0.05$) reduced both cAMP-stimulated and cholera toxin-stimulated steroidogenesis (Figure 3).

Fig. 3. Effect of increasing concentrations of IDPN on cAMP-stimulated or cholera toxin-stimulated steroid production by Y-1 adrenal cells. ^avalues are the mean $\pm$ SEM. *Significantly different ($P < 0.05$).

From these previous experiments it appeared that IDPN enhanced the interaction of ACTH with its adrenal cell receptors; an experiment was, therefore, designed to determine whether this enhancement occurred because IDPN prevented the time-dependent desensitization of Y-1 cells to ACTH. When

cells were incubated with ACTH or ACTH plus IDPN for six consecutive half-hour periods, IDPN did not prevent the time-dependent desensitization of Y-1 cells to ACTH (Figure 4).

Fig. 4. Time-dependent changes in the effect of 0.01% IDPN on ACTH-stimulated Y-1 cells. ^aValues are the mean $\pm$ SEM. *Significantly different ($P < 0.05$).

Since IDPN acted at the cell surface, the possibility was tested that IDPN enhanced ACTH stimulation by affecting membrane-attached intermediate filaments which were interacting with membrane-attached microtubules or

microfilaments. Cells were incubated with ACTH plus IDPN in the presence of colchicine or cytochalasin B. IDPN significantly enhanced ($P < 0.05$) the effect of ACTH, but did not significantly alter ($P > 0.05$) the effect of ACTH when either colchicine or cytochalasin B was present in the medium (Figure 5).

Fig. 5. Effect of colchicine or cytochalasin B on ACTH-stimulated and ACTH plus IDPN-stimulated steroid production. ^aValues are the mean $\pm$ SEM. *Significantly different ($P < 0.05$). (A) ACTH, (I) 0.01% IDPN, (C) colchicine, (B) cytochalasin B.

A micrograph of the cytoskeleton of Triton-extracted IDPN-treated cells is shown in Figure 6. Cytoskeletal structures were aggregated in the perinuclear area and detergent-resistant granules tended to accumulate around nucleus.

Fig. 6. Triton extraction of Y-1 adrenal cell treated with 0.01% IDPN. Note the aggregation of filamentous structures (F) and granules (G) around the nucleus (N). x2,300.

When the Y-1 cell was stimulated with ACTH, the cytoskeleton had a characteristic stellate morphology which resulted from the retraction of the peripheral margins of the cell (Figure 7A). Few stress fibers were visible and detergent-resistant granular structures were evenly distributed over the cytoplasmic area of cells treated with ACTH before

extraction (Figure 7B). In contrast, when cells were extracted after treatment with ACTH plus IDPN, the stellate shape remained (Figure 7D) and a dense aggregation of cytoskeletal and granular structures was seen in perinuclear area (Figure 7C and 7D).

Fig. 7. Detergent extracted cytoskeletons from Y-1 adrenal cells treated with ACTH or ACTH plus IDPN. (A) ACTH-treated whole cell. x2,300. (B) Nuclear area (N) of an ACTH-treated cell. x4,250. (C) Whole cell treated with ACTH plus IDPN. x2,300. (D) Nuclear area of ACTH plus IDPN-treated cell. x4,250. Note the aggregation of granular structures (G) in the perinuclear area (C and D).

CHAPTER IV

DISCUSSION

Up to now, the absence of intermediate filament probes similar to cytochalasin for microfilaments (Wessels et al., 1971) and colchicine for microtubules (Borisy and Taylor, 1967) has impeded investigation of the intracellular functions of intermediate filaments. The present studies give evidence that sodium orthovanadate and IDPN may perturb intermediate filaments in Y-1 cells.

Both vanadate and IDPN caused the perinuclear aggregation of cytoskeletal structures and an accompanying perinuclear aggregation of detergent-resistant granules. Since the perinuclear density of the cytoskeletal and granular structures interfered with specific, electron microscopic identification of perinuclear cytoskeletal elements, it is uncertain that intermediate filaments were solely responsible for the perinuclear aggregation of these two structures. However, indirect evidence suggests that intermediate filaments could be involved in their aggregation. Intermediate filaments are characteristically located in

the perinuclear area in non-adrenal cells (Franke, 1971; Franke et al., 1978; Aubin et al., 1980; Lazarides, 1980; Kim and Okada, 1981). Secondly, vanadate causes perinuclear aggregation of intermediate filaments in BHK21-F cells (Wang and Choppin, 1981). Since intermediate filaments are often associated with cellular organelles (Lehto et al., 1978; Small and Celis, 1978; Lee et al., 1979; Virtanen et al., 1979; David-Ferreira and David-Ferreira, 1980), the migration of the Y-1 cell granular organelles to the perinuclear area after vanadate or IDPN treatment is consistent with the hypothesis that intermediate filaments withdraw toward the perinuclear area in response to these two treatments.

When Y-1 cells were treated with 0.5 IU/ml ACTH, a concentration which maximally stimulated steroidogenesis, together with 0.01% IDPN, steroidogenesis was significantly enhanced ($P < 0.05$). However, IDPN had no effect ($P > 0.05$) on cAMP-stimulated steroid production, suggesting that IDPN did not enhance the effect of ACTH by acting after intracellular cAMP production. Because IDPN did not affect ($P > 0.05$) cholera toxin-stimulated steroid production, it is assumed that IDPN perturbed plasma membrane events occurring

prior to the interaction of the ACTH-receptor complex with the adenyl cyclase moiety.

Although 0.1 and 1% IDPN inhibited cholera toxin-, cAMP-, and ACTH-stimulated steroid production, this effect may have resulted from cytotoxicity. On the other hand, these concentrations of IDPN, by itself, did not cause reduced basal steroid production or cell death. The explanation for these contradictory observations was not apparent.

The reason for the effects of IDPN on the adrenal cell external surface was unclear. Several explanations were considered, including the possibilities that IDPN altered the time-dependent desensitization of Y-1 cells to ACTH, or that IDPN affected ACTH receptor protein-associated intermediate filaments which were interacting with membrane integral protein-associated microfilaments or microtubules.

It is well established that prolonged exposure of adrenal cells to ACTH causes a progressive decrease in steroidogenesis (Schulster et al., 1970; Chen and Auersperg, 1976; Mrotek et al., in press). This decrease may be due to receptor internalization (Brown and Goldstein, 1976; Carpenter and Cohen, 1976; Amsterdam et al., 1979; 1980; Nolin,

1980), desensitization of adenylyl cyclase (Lamprecht et al., 1973; Marsh et al., 1973; Selstam et al., 1976; Lamprecht et al., 1977) or agonist-induced depletion of receptor sites, the so-called down regulation (Harwood et al., 1978). Even though IDPN seemed to enhance the interaction of ACTH with its receptors, the present study indicated that this enhancement did not result from inhibiting the time-dependent desensitization of adrenal cells to ACTH (Figure 4).

Microtubules and microfilaments are attached to the integral glycoprotein receptors of plasma membranes and contribute to the distribution and mobility of these receptors within the membrane (Nicolson, 1976a; 1976b; Bray, 1978; Flanagan and Koch, 1978; Koch and Smith, 1978), particularly during the clustering of membrane receptor proteins in response to bound peptide hormones (Jarett and Smith, 1974; Orci et al., 1975). If similar cytoskeleton-membrane protein interactions occur in Y-1 cells, the possibility exists that IDPN affected the interaction of plasma membrane molecules with intermediate filaments, microtubules, and microfilaments. Experiments were designed to test whether the IDPN-sensitive processes within the Y-1 cell were affected by the microtubule perturbing

agent (colchicine) or the microfilament perturbing agent (cytochalasin B). Colchicine or cytochalasin B prevented the effects of IDPN from being expressed. Cytochalasins inhibit the intracellular cAMP-dependent steroidogenic steps of Y-1 cells that require microfilaments (Mrotek and Hall, 1975; 1977). The similarity in effects of cytochalasin B on both ACTH- and ACTH plus IDPN- stimulated steroidogenesis is consistent with the view that IDPN acts on one of the initial events occurring during the response to ACTH. This similarity suggests that microfilaments mediate the effects of IDPN. The interpretation of the results obtained using colchicine and IDPN is more complicated. Using time-lapse videomicroscope observations, this laboratory demonstrated that ACTH-treated Y-1 cells actively extended filopodia from various points on the cell periphery (Voorhees and Mrotek, 1981, unpublished data). In addition, it is known that tubulin is assembled into microtubules during ACTH stimulation of Y-1 cells (Clark and Shay, 1981). Since filopodia contain parallel arrays of microtubules (Mrotek et al., 1982), the tubulin assembly and time-lapse studies argue that microtubules within the filopodia may grow as extensions from nucleating sites in the cell periphery

during ACTH stimulation. The fact that colchicine prevented the cooperativity between ACTH and IDPN is consistent with a suggestion that the effects of IDPN may depend on the assembly of microtubules in response to ACTH. In other words, microtubules serve as guides for the directed perinuclear migration of intermediate filaments. Under physiological conditions, the interacting microtubules and intermediate filaments may moderate the microtubule or intermediate filament response to ACTH; IDPN may enhance the effects of ACTH by preventing these moderating interactions. Even though it was postulated in this study that IDPN affected the interaction between membrane proteins and cytoskeletal structures, the evidence obtained using microtubule- and microfilament-perturbing agents has not permitted us to confirm this postulate.

Vanadate had no effect on non-stimulated and ACTH-stimulated steroidogenesis, although it caused the aggregation of cytoskeletal and granular structures in a manner similar to IDPN. The lack of an effect on steroid production may be related to observations that vanadate inhibits intracellular phosphate-dependent functions (Cande and Wolniak, 1978; Cantley et al., 1978) or to its effects on the

membrane attachment of intermediate filaments (Wang and Choppin, 1981). In erythrocyte and PtK₁ cells, vanadate inhibited various phosphate-utilizing functions, including the inhibition of Na⁺,K⁺-ATPase (Cande and Wolniak, 1978; Cantley et al., 1978). Phosphate-containing compounds are involved in many cellular functions, such as steroid production (Klingenberg, 1968; Kowal, 1970) and microtubule assembly (Borisov et al., 1975). Vanadate also appeared to cause the detachment of intermediate filaments from the membrane of BHK21-F cells (Wang and Choppin, 1981), a result consistent with the morphological effects of vanadate occurring in Y-1 cell. Although the evidence obtained using IDPN suggested that loss of association between the membrane and intermediate filaments lead to enhancement of the ACTH activity, the absence of this enhancing effect with vanadate appears to be inconsistent with this postulate. Perhaps these two intermediate filament-perturbing agents act to detach the filaments from different species of membrane proteins. Alternatively, the effects of vanadate on phosphate utilization, especially if microtubule assembly or stimulated steroid production was affected, may complicate the vanadate-filament activities and negate the

effects of membrane detachment. At present the effects of these two different perturbing agents can not be reconciled without further experimentation. Irrespective of the mechanism of action of the two perturbing agents, the preliminary results of the present experiments using IDPN and colchicine yield presumptive evidence that intermediate filament and microtubule interactions near the Y-1 cell plasma membrane may lead to the enhanced steroidogenic responses to ACTH. This study also suggests that both vanadate and IDPN affect the arrangement of cytoskeletal structures in the Y-1 mouse adrenal tumor cell, probably because they affect intermediate filaments.

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