PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 461–467 (1987)

Effect of 3,3′-Iminodiproprionitrile (IDPN) on Corticosteroidogenesis of Isolated Adrenocortical Cells[1] (42502)

ROCCO V. CARSIA,[2] LESLIE A. SCHWARZ, AND HELEN WEBER

Department of Animal Sciences, Rutgers—The State University of New Jersey, New Brunswick, New Jersey 08903

Abstract. The neurotoxic agent, 3,3′-iminodiproprionitrile (IDPN), is a disrupter of neurofilament- and intermediate filament-organelle association. In the present study, the effect of IDPN on corticosteroidogenesis was investigated using isolated rat (having few intermediate filaments) and domestic fowl (having abundant intermediate filaments) adrenocortical cells. Cells were incubated with or without steroidogenic agents and precursors and with or without various concentrations of IDPN for 2 hr. IDPN had similar inhibitory potencies (as indicated by the half-maximal inhibitor concentrations (ID_{50} values)) with both rat and domestic fowl cells despite their grossly different intermediate filament content. However, the average ID_{50} values of IDPN varied with the different steroidogenic agents and precursors used. The average IDPN ID_{50} values for maximal ACTH- and 8-bromo-cyclic AMP (8-Br-cAMP)-induced corticosterone production were equivalent (49.7 and 45.7 m*M*, respectively). However, the IDPN ID_{50} values for maximal ACTH-induced cAMP production, maximal 25-hydroxycholesterol- and pregnenolone-supported corticosterone production, and maximal ACTH- and 8-Br-cAMP-induced protein synthesis varied from 3.7 to 5.4 times the average ID_{50} values for maximal ACTH- and 8-Br-cAMP-induced corticosterone production. Thus, the inhibitory action of IDPN was not closely linked to the inhibition of ACTH–transmembrane signaling via cAMP, protein synthesis, and steroidogenic enzyme activity. The data suggest that IDPN inhibited corticosteroidogenesis at a step after cAMP but before cholesterol side-chain cleavage and that the inhibition was not dependent on the presence of intermediate filaments.

Ample evidence suggest that cytoskeletal elements are involved in corticosteroidogenesis. Microfilament disrupting agents such as cytochalasins (1–4) and actin antibody (5) have been shown to block corticosteroidogenesis. The use of these microfilament disrupting agents has provided evidence suggesting that microfilaments mediate the interaction of cytoplasmic cholesterol with mitochondria for subsequent cholesterol side-chain cleavage, the rate-limiting step of steroidogenesis (2, 3). In contrast, there have been conflicting reports of the action of microtubule disrupting agents on corticosteroidogenesis. Thus, the role of microtubules in corticosteroidogenesis remains controversial (6–9).

Recent work using agents that disrupt the association of neurofilaments and intermediate filaments with microtubules and other organelles suggests that intermediate filaments may also play a role in corticosteroidogenesis (10). One of these agents, 3,3′-iminodiproprionitrile (IDPN), like other cytoskeletal disrupting agents, exhibited a bimodal effect on corticosteroidogenesis by Y-1 mouse adrenal tumor cells; at low and high concentrations, respectively, it stimulated and inhibited ACTH-induced steroidogenesis (10). However, details of the effect of IDPN on the corticosteroidogenic pathway have not been reported. Moreover, there have been no reports of work with normal adrenocortical cells, freshly isolated from the animal, and incubated for brief periods. Freshly isolated adrenocortical cells exhibit steroidogenic responses to ACTH similar to the adrenal gland *in vivo* (11). However, adrenocortical cells in suspension lack an extensive cytoskeletal arrangement as exhibited by normal (12) and tumorous (1–10) adrenocortical cells in long-term culture.

Accordingly, in the present study we evaluated the acute influence of IDPN on the

[1] Supported by Rutgers University Research Council Grant 1984–1985 and the New Jersey Agricultural Experiment Station (NJ 06109).

[2] To whom reprint requests should be addressed at the Department of Animal Sciences, Rutgers—The State University, Bartlett Hall, P.O. Box 231, New Brunswick, NJ 08903.

0037-9727/87 $1.50

function of isolated rat and domestic fowl adrenocortical cells. Rat adrenocortical cells have few intermediate filaments, whereas domestic fowl cells have an abundance of intermediate filaments (13). However, adrenocortical tissue and cells from both species secrete predominantly corticosterone (80–90%) (11, 13–15), thus providing a common end product glucocorticoid for evaluation and comparison. Thus, the effect of IDPN on various parts of the corticosteroidogenic pathway could be evaluated in cells that differ widely in their intermediate filament content and that secrete a common end product glucocorticoid.

Materials and Methods. *Adrenocortical cell isolation and incubation.* Adrenocortical cells were prepared from the adrenal glands of sexually mature male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) and sexually mature White Leghorn roosters (Avian Services, Frenchtown, NJ) as described in detail previously (16). The basic medium for tissue dissociation and cell incubation was Krebs–Ringer Hepes (*n*-2-hydroxyethylpiperazineethanesulfonic acid) buffer (24.2 m*M* Hepes, 118.5 m*M* NaCl, 4.75 m*M* KCl, 2.54 m*M* $CaCl_2$, 1.20 m*M* KH_2PO_4, 1.20 m*M* $MgSO_4$, 11.1 m*M* glucose, pH 7.5). In brief, adrenal glands were trimmed free of adhering connective tissue and diced into small pieces. The tissue fragments were dissociated using mechanical agitation and the basic medium containing 0.2% collagenase (Type II, Sigma Chemical Co., St. Louis, MO). Cells were then placed on a 40% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) continuous density gradient and centrifuged (400*g* for 15 min at 4°C) to separate adrenocortical cells from broken cells, cell fragments, and other adrenal cell types. Enriched adrenocortical cell fractions (87–93% adrenocortical cells) were washed free of Percoll and resuspended in the basic medium containing 0.25% bovine serum albumin (incubation medium; Type V, Sigma Chemical Co.). Additions to the incubation medium were various concentrations of IDPN (3,3′-iminodipropionitrile, Eastman Kodak Co., Rochester, NY) and maximal steroidogenic concentrations of ACTH (ACTH-(1-24), Cortrosyn, Organon, Inc., West Orange, NJ), 8-Br-cAMP, pregnenolone (Sigma Chemical Co.), and 25-hydroxycholesterol (Steraloids Inc., Wilton, NH). The total incubation volumes were 250–500 μl (90% of the incubation volume was cell suspension, 10%, a solution containing a steroidogenic agent or precursor). Prior to the addition of these agents to the cell suspensions, ACTH was dissolved in a solution of 0.9% NaCl, pH adjusted to 2.6 with 1.0 *N* HCl, containing 0.1% bovine serum albumin, whereas IDPN, 8-Br-cAMP, 25-hydroxycholesterol, and pregnenolone were made up in the incubation medium. Initial dilutions of the precursor steroids were carried out in absolute ethanol. However, the final maximal concentration of ethanol (0.04%) did not affect corticosteroidogenesis. Incubations were carried out at 37°C (rat cells) or 40°C (domestic fowl cells) for 2 hr in a shaking water bath. After incubation, cell suspensions were frozen at −20°C until radioimmunoassay for corticosterone and determination of cAMP. In each experiment at least 92% of the cells were viable after incubation as indicated by trypan blue dye exclusion.

Determination of corticosterone and cAMP. Corticosterone, the major glucocorticoid secreted by rat (11) and domestic fowl (13–15) adrenocortical tissue and cells, was measured by a modification of the radioimmunoassay procedure of Roy *et al.* (17) using specific antibody (Miles Research Products, Elkhart, IN). cAMP produced by rat adrenocortical cells was measured using a protein-binding kit (Diagnostic Products Corp., Los Angeles, CA). In our experiments as little as 0.1 ng corticosterone/ml and 0.5 pmole cAMP/ml could be detected. Radioimmunoassay of aliquots of the same pooled cell suspensions (performed with each assay) showed intrassay coefficients of variation of 6.3 and 8.2%, respectively, and interassay coefficients of variation of 9.2 and 12.3%, respectively.

Determination of protein synthesis. The influence of IDPN on maximal ACTH- and cAMP-induced protein synthesis by isolated rat and domestic fowl adrenocortical cells was determined using a modification of the procedure of Schulster *et al.* (18) as described in detail previously (19). In brief, the incorporation of L-[3,4,5(*N*)-^{3}H]leucine (152.0 Ci/mmole; New England Nuclear, Boston, MA) into trichloroacetic acid-precipitable proteins

was measured. Within- and between-experiment coefficients of variation were 7.6 and 11.2%, respectively.

Analysis of data. The effects of IDPN on some parameters of the responses of isolated adrenocortical cells to steroidogenic agents were determined. The average within- and between-experiment coefficients of variation of cell incubation (calculated from basal corticosterone values after 2 hr of incubation) were 9.7 and 15.2%, respectively. Data comprising the sigmoidal inhibitory dose–response curves shown in this report were analyzed using a four-parameter logistic equation model (20) that is available as a computer program (ALLFIT, Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Nashville, TN). Half-maximal inhibitory concentrations (ED_{50}) were calculated and statistically analyzed using this program.

Other data were statistically analyzed by analysis of variance (21). Data are expressed as the means ± SEM; means were deemed significantly different at $P \leq 0.05$.

Results. *Effect of IDPN on corticosterone production.* To gain information on the cellular loci of possible inhibition of corticosteroidogenesis by IDPN, isolated rat and domestic fowl adrenocortical cells were incubated with various concentrations of IDPN and with or without maximal steroidogenic concentrations of ACTH (0.1 ng/ml; Fig. 1), 8-Br-cAMP (1 m*M;* Fig. 2), and the steroidogenic precursors, 25-hydroxycholesterol (10 μM; Fig. 3) and pregnenolone (10 μM; data not shown). IDPN had little effect on basal corticosterone production (Figs. 1 and 3). On the other hand, regardless of steroidogenic agent or precursor, IDPN inhibited maximal corticosterone production in a concentration-dependent manner (Figs. 1–3). In addition, with the exception of maximal 25-hydroxycholesterol-supported corticosterone production (Fig. 3), IDPN inhibited rat and domestic fowl corticosterone production with equivalent potencies as indicated by the equivalent ID_{50} values (Table I). Thus, by and large, IDPN inhibited different loci of rat and domestic fowl corticosteroidogenic pathways in a similar manner.

In contrast to the similar IDPN ID_{50} values for rat and domestic fowl corticosterone production, some IDPN ID_{50} values were different for the different steroidogenic agents and precursors used. For example, maximal ACTH- and 8-Br-cAMP-induced corticosterone pro-

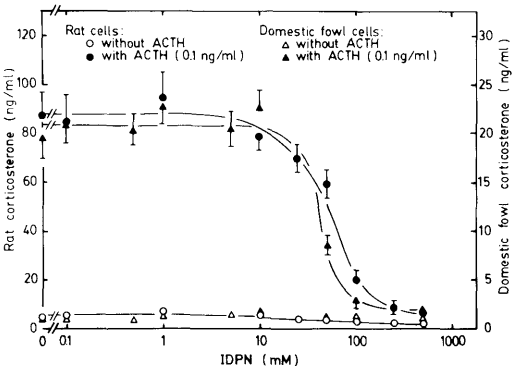

FIG. 1. IDPN inhibition of ACTH-induced corticosterone production by isolated rat and domestic fowl adrenocortical cells. Rat (2.5×10^4 cells/ml) and domestic fowl (5×10^4 cells/ml) cells were incubated with various concentrations of IDPN and with or without a maximal steroidogenic concentration of ACTH (0.1 ng/ml) for 2 hr. Each symbol represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

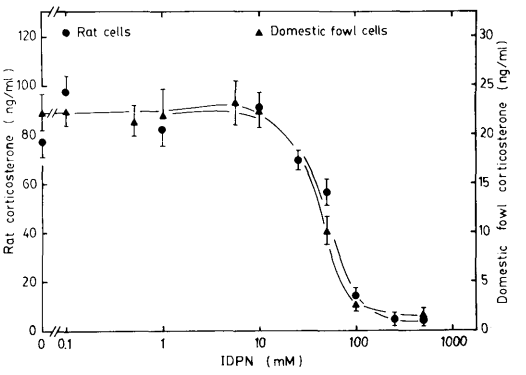

FIG. 2. IDPN inhibition of 8-Br-cAMP-induced corticosterone production by isolated rat and domestic fowl adrenocortical cells. Rat (2.5×10^4 cells/ml) and domestic fowl (5×10^4 cells/ml) cells were incubated with various concentrations of IDPN and with a maximal steroidogenic concentration of 8-Br-cAMP (1 m*M*) for 2 hr. Each symbol represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

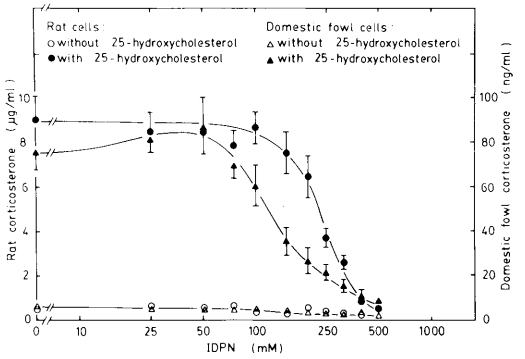

FIG. 3. IDPN inhibition of 25-hydroxycholesterol-supported corticosterone production by isolated rat and domestic fowl adrenocortical cells. Rat (2×10^5 cells/ml) and domestic fowl (2×10^5 cells/ml) cells were incubated with various concentrations of IDPN and with or without a maximal steroidogenic concentration of 25-hydroxycholesterol (10 μM) from 2 hr. Each symbol represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

duction were inhibited with equivalent potencies (49.7 and 45.7 mM, respectively; data for rat and domestic fowl cells pooled and averaged). However, the IDPN ID_{50} values for maximal corticosterone production supported by 25-hydroxycholesterol and pregnenolone were 183.1 and 165.7 mM, respectively (data for rat and domestic fowl cells pooled and averaged) or about 3.7 times the ID_{50} values for maximal ACTH- and 8-Br-cAMP-induced corticosterone production. The data suggest that IDPN inhibition of ACTH- and 8-Br-cAMP-induced corticosteroidogenesis was not closely linked to inhibition of conversion of precursors to corticosterone.

Effect of IDPN on ACTH-induced cAMP production. The preceding studies suggested that IDPN might act early in the pathway of events after ACTH and cAMP but before cholesterol side-chain cleavage. To support this postulate, we measured the influence of IDPN on maximal ACTH-induced cAMP production by isolated rat adrenocortical cells. Isolated domestic fowl cells were not used because the amount of cAMP produced by fowl cells was below the detection sensitivity of the assay. In the absence of ACTH, IDPN slightly stimulated cAMP production (Fig. 4). In the presence of ACTH, IDPN inhibited cAMP production. However, the ID_{50} value was 287.3 mM or 5.4 times greater than the ID_{50} value for ACTH-induced corticosterone production (Table I). Thus, IDPN inhibition of ACTH-induced corticosterone production was not closely linked to the inhibition of cAMP production.

Effect of IDPN on ACTH- and 8-Br-cAMP-induced protein synthesis. Agents known to inhibit protein synthesis will also inhibit corticosteroidogenesis induced by ACTH and 8-Br-cAMP (18, 22). To learn if the inhibition of corticosteroidogenesis observed after addition of IDPN was related to an effect on protein synthesis, we measured the influence of IDPN on leucine incorporation into proteins in cells maximally stimulated with ACTH and 8-Br-cAMP (Fig. 5). Within a species IDPN

TABLE I. HALF-MAXIMAL INHIBITORY CONCENTRATION (ID_{50})[a] OF IDPN FOR ADRENOCORTICAL CELL FUNCTION

Additions to cell incubation	Cell function	Adrenocortical cell	
		Rat	Domestic fowl
ACTH	Corticosterone production	53.2 ± 7.4	46.2 ± 6.8
ACTH	cAMP production	287.3 ± 42.3*	—
ACTH	Protein synthesis	173.2 ± 23.2*	232.7 ± 24.9*
8-Br-cAMP	Corticosterone production	47.3 ± 6.1	44.0 ± 8.7
8-Br-cAMP	Protein synthesis	178.4 ± 18.2*	226.9 ± 23.5*
25-hydroxycholesterol	Corticosterone production	239.7 ± 46.7*	126.4 ± 23.6*
Pregnenolone	Corticosterone production	132.1 ± 31.9*	199.2 ± 57.2*

[a] Each value is the mean ± SEM of ID_{50} values from three experiments.
* ID_{50} values significantly different from corresponding ID_{50} values for ACTH-induced corticosterone production.

inhibited ACTH- and 8-Br-cAMP-induced protein synthesis with equivalent potencies (Table I). However, there were slight species differences: The average ID_{50} values for ACTH- and 8-Br-cAMP-induced protein synthesis were 175.8 and 229.8 m*M* for rat and fowl cells, respectively. Nevertheless, these values were 3.5–4.1 times greater than the ID_{50} value for maximal ACTH-induced corticosterone production. Thus, IDPN inhibition of ACTH- and 8-Br-cAMP-induced corticosterone production was not closely linked to the inhibition of protein synthesis.

Kinetics of IDPN inhibition. The kinetics and reversibility of IDPN inhibition of corticosteroidogenesis were examined to learn whether the observed effects of IDPN were due to toxic effects on the cells (Fig. 6). ACTH-induced corticosterone production was terminated abruptly by IDPN (500 m*M*). However, ACTH-induced corticosterone was gradually restored after cells treated with IDPN were washed and then resuspended in fresh medium with ACTH but without IDPN. Thus, IDPN, at the highest concentration used in these experiments, was not toxic to cells.

Discussion. The present study shows that IDPN, a neurotoxic compound that specifically disrupts the association of neurofilaments and intermediate filaments with other organelles, acutely inhibited corticosteroidogenesis by isolated rat and domestic fowl adrenocortical cells. Although IDPN had little effect on basal corticosterone production (Figs. 1 and 3), it inhibited corticosterone production maximally induced by ACTH and 8-Br-cAMP (Figs. 1 and 2) and that maximally supported by 25-hydroxycholesterol (Fig. 3) and pregnenolone (data not shown) in a dose-dependent manner. Our results in part support the results of earlier work with Y-1 mouse adrenal tumor cells (10), but differ from the results with tumor cells in that in the present study IDPN failed to augment ACTH-induced steroidogenesis.

Data presented here suggest that IDPN inhibited corticosteroidogenesis by affecting a factor(s) in the steroidogenic pathway after ACTH–transmembrane signaling via cAMP but before cholesterol side-chain cleavage. Both maximal ACTH- and 8-Br-cAMP-induced corticosterone production by rat and domestic fowl adrenocortical cells were inhibited by IDPN with the same efficacy (average ID_{50} values of 49.7 and 45.7 m*M*, respectively;

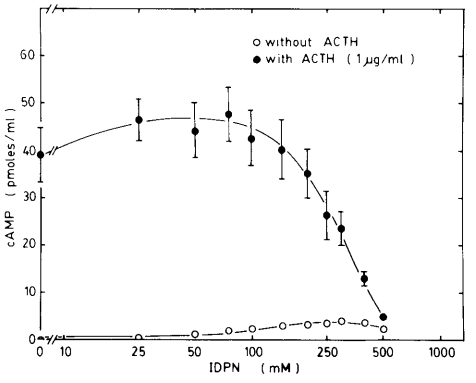

FIG. 4. IDPN inhibition of ACTH-induced cAMP production by isolated rat adrenocortical cells (2×10^5 cells/ml). Cells were incubated with various concentrations of IDPN and with or without a maximal cAMP-inducible concentration of ACTH (1 μg/ml) for 2 hr. Each symbol represents the mean of cAMP values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

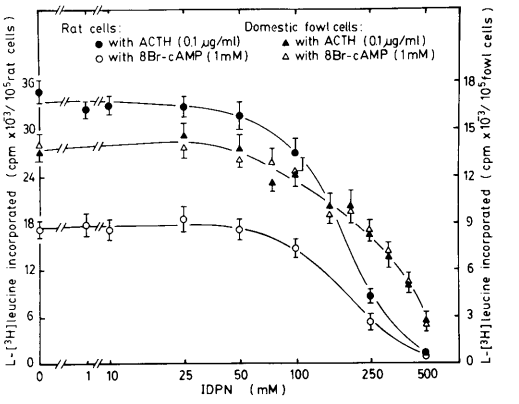

FIG. 5. IDPN inhibition of ACTH- and 8-Br-cAMP-induced protein synthesis by isolated rat and domestic fowl adrenocortical cells. Rat (1×10^5 cells/ml) and domestic fowl (4.5×10^5 cells/ml) adrenocortical cells were incubated with various concentrations of IDPN and with a maximal steroidogenic concentration of ACTH (0.1 μg/ml) or 8-Br-cAMP (1 m*M*) for 2 hr. Each symbol represents the mean of L-[^{3}H]leucine incorporation values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

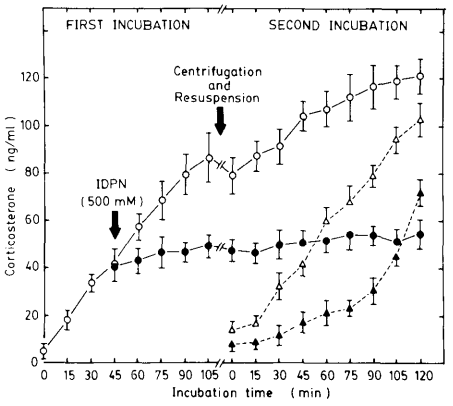

FIG. 6. IDPN inhibition of ACTH-induced corticosterone production by isolated rat adrenocortical cells and reversal of the inhibition after removal of IDPN. During the first incubation, cells (1×10^5 cells/ml) were incubated with a maximal steroidogenic concentration of ACTH (10 ng/ml, open circles); IDPN was added at 45 min (500 m*M*, closed circles). At 105 min, the cells were centrifuged (100*g* for 15 min) and resuspended in various media for the second incubation. Cells treated with IDPN during the first incubation were resuspended in the original medium (closed circles) or fresh medium containing additional ACTH (10 ng/ml, closed triangles). Similarly, untreated cells during the first incubation were resuspended in original medium (open circles) or fresh medium containing additional ACTH (10 ng/ml, open triangles). Each symbol represents the mean of corticosterone values from six cell incubations (three cell incubations from each of two experiments). SEM are represented by bars when they are larger than the symbols.

Table I). These calculated ID_{50} values are lower than those estimated from the results with Y-1 tumor cells (about 80 m*M*) (10). In contrast, much higher IDPN ID_{50} values (three to five times) were obtained for maximal ACTH-induced cAMP production (287.3 m*M*), maximal ACTH- and 8-Br-cAMP-induced protein synthesis (202.5 and 197.5 m*M*, respectively), and maximal corticosterone production supported by 25-hydroxycholesterol (183.1 m*M*) and pregnenolone (165.7 m*M*) (average ID_{50} values for both rat and fowl cells). Thus, IDPN inhibition of ACTH-induced corticosterone production was not closely linked to inhibition of ACTH–transmembrane signaling via cAMP, protein synthesis, and the function of steroidogenic enzymes. Perhaps the lower IDPN ID_{50} values (about 48 m*M*) represent the specific interaction of IDPN with an integral factor(s) in the steroidogenic pathway, whereas the higher IDPN ID_{50} values (greater than 150 m*M*) represent more general interactions of IDPN with other cellular proteins.

Three observations argue against the possibility that the effects of IDPN were toxic to the cells. First, cells were viable after incubation with IDPN as indicated by trypan blue dye exclusion (see Materials and Methods). Second, low concentrations of IDPN that inhibited ACTH- and 8-Br-cAMP-induced corticosterone production had no detectable effect on protein synthesis. Third, although maximal ACTH-induced corticosterone production was abruptly stopped by the highest concentration of IDPN used (500 m*M*), it was gradually restored after the inhibited cells were washed and resuspended in fresh medium with ACTH but without IDPN (Fig. 6).

The steroidogenic factor(s) with which IDPN interacts to inhibit corticosteroidogenesis is unknown. However, work with neural tissues suggests that microtubule-associated proteins (MAPs) might be candidates for further investigation. For example, there is evidence that IDPN interferes with the function of MAPs, resulting in the disruption of the association of microtubules and neurofilaments (23, 24). In addition, MAPs associate with both actin filaments and microtubules (25), two cytoskeletal elements implicated in steroidogenesis. However, the presence of ultrastructurally identifiable intermediate filaments may not be a prerequisite for this postulated involvement of MAPs in corticosteroidogenesis. In the present study, IDPN had an equivalent or very similar efficacy of inhibition of corticosteroidogenesis of isolated rat and domestic fowl adrenocortical cells despite their grossly different intermediate filament content (13). Furthermore, results of recent work are consistent with the postulate that nonfilamentous or globular forms of cytoskeletal proteins, such as tubulin (9) and possibly actin (26), play a pivotal role in steroidogenesis. Perhaps MAPs may have a similar nonfilamentous function in corticosteroidogenesis. The present study presents two types of normal adrenocortical cells that differ markedly in their intermediate filament content to test this hypothesis.

We thank Dr. Robert G. Nagele for helpful discussions of the data of this manuscript. In addition, we thank Ted Kreitzman for expert technical assistance and Ms. C. Uckele for the preparation of this manuscript.

1. Mrotek JJ, Hall PF. The influence of cytochalasin B on the response of adrenal tumor cells to ACTH and cyclic AMP. Biochem Biophys Res Commun **64**:891–986, 1975.
2. Mrotek J, Hall PF. Response of adrenal tumor cells to adrenocorticotropin: Site of inhibition by cytochalasin B. Biochemistry **16**:3177–3181, 1977.
3. Mrotek J, Hall PF, Lacko I. Action of ACTH, cAMP and cytochalasin B on steroid production by Y-1 mouse adrenal tumor cells in culture. Experentia **38**: 1107–1110, 1982.
4. Kowal J, Mattson P. Effects of cytochalasin B on unstimulated and adrenocorticotropin-stimulated adrenocortical tumor cells *in vitro*. Endocrinology (Baltimore) **111**:1632–1646, 1982.
5. Hall P, Charpponnier M, Nakamura M, Gabbinani G. The role of microfilaments in the response of adrenal tumor cells to ACTH. J Biol Chem **254**:9080–9084, 1979.
6. Temple R, Wolff J. Stimulation of steroid secretion by antimicrotubular agents. J Biol Chem **248**:2691–2698, 1973.
7. Ray P, Strott CA. Stimulation of steroid synthesis by normal rat adrenocortical cells in response to antimicrotubular agents. Endocrinology (Baltimore) **103**: 1281–1288, 1978.
8. Mrotek JJ, Hall PF. The action of ACTH on adrenal tumor cells is not inhibited by anti-tubular agents. Gen Pharmacol **9**:269–273, 1978.
9. Clark MA, Shay JW. The role of tubulin in the steroidogenic response of murine adrenal and rat Leydig cells. Endocrinology (Baltimore) **109**:2261–2263, 1981.
10. Lee HS, Mrotek JJ. The effect of intermediate filament inhibitors on steroidogenesis and cytoskeleton in Y-1 mouse adrenal tumor cells. Cell Biol Int Rep **8**: 463–482, 1984.
11. Sayers G. Bioassay of ACTH using isolated cortex cells. Ann NY Acad Sci **297**:220–241, 1977.
12. Rainey WE, Hornsby PJ, Shay JW. Morphological correlates of adrenocorticotropin-stimulated steroidogenesis in cultured adrenocortical cells: Differences between bovine and human adrenal cells. Endocrinology (Baltimore) **113**:48–54, 1983.
13. Carsia RV, Scanes CG, Malamed S. Isolated adrenocortical cells of the domestic fowl (*Gallus domesticus*): Steroidogenic and ultrastructural properties. J Steroid Biochem **22**:273–279, 1985.
14. DeRoos R. Effect of mammalian corticotropin and progesterone on corticoid production by chicken adrenal tissue *in vitro*. Gen Comp Endocrinol **13**:455–459, 1969.
15. Nakamura T, Tanabe Y. *In vitro* corticosteroidogenesis by the adrenal gland of the chicken (*Gallus domesticus*). Gen Comp Endocrinol **21**:99–107, 1973.
16. Carsia RV, Scanes CG, Malamed S. Loss of sensitivity to ACTH of adrenocortical cells isolated from maturing domestic fowl. Proc Soc Exp Biol Med **179**: 279–282, 1985.
17. Roy K Jr, Garza R, Maroulis G, Abraham GE. Radioimmunoassay of plasma corticosterone. Anal Lett **7**:109–118, 1974.
18. Schulster D, Richardson MC, Palfreyman JW. The role of protein synthesis in adrenocorticotropin action: Effects of cycloheximide and puromycin on the steroidogenic response of isolated adrenocortical cells. Mol Cell Endocrinol **2**:17–29, 1974.
19. Carsia RV, Moyle WR, Wolff DJ, Malamed S. Acute inhibition of corticosteroidogenesis by inhibitors of calmodulin action. Endocrinology (Baltimore) **111**: 1456–1461, 1982.
20. De Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay, and physiological dose-response curves. Amer J Physiol **235**:E97–E102, 1978.
21. Snedecor GW, Cochran WG. Statistical Methods, Ames, Iowa: State Univ Press, 6th ed., pp285–289, 1967.
22. Koritz SB, Bhargava G, Schwartz E. ACTH action on adrenal steroidogenesis. Ann NY Acad Sci **297**: 329–335, 1977.
23. Papasozomenos SCh, Binder LI, Bender PK, Payne MR. Microtubule associated protein 2 within axons of spinal motor neurons: Associations with microtubules and neurofilaments in normal and β-β^1-iminodiproprionitrile-treated axons. J Cell Biol **100**:74–85, 1985.
24. Parhad I, Griffin JW, Hoffman PN, Koves JF. Selective interruption of axonal transport of neurofilament proteins in the visual system by β,β^1-iminodiproprionitrile (IDPN) intoxication. Brain Res **363**:315–324, 1986.
25. Griffiths LM, Pollard TD. Evidence for actin filament–microtubule interaction mediated by microtubule-associated proteins. J Cell Biol **78**:958–965, 1978.
26. Osawa S, Betz G, Hall PF. Role of actin in the responses of adrenal cells to ACTH and cyclic AMP: Inhibition by DNase I. J Cell Biol **99**:1335–1342, 1984.

Received September 5, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted December 16, 1986.