Product of Side-chain Cleavage of Cholesterol, Isocaproaldehyde, Is an Endogenous Specific Substrate of Mouse Vas Deferens Protein, an Aldose Reductase-like Protein in Adrenocortical Cells*

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Mouse vas deferens protein (MVDP) is an aldose reductase-like protein that is highly expressed in the vas deferens and adrenal glands and whose physiological functions were unknown. We hereby describe the enzymatic characteristics of MVDP and its role in murine adrenocortical Y1 cells. The murine aldose reductase (AR) and MVDP cDNAs were expressed in bacteria to obtain recombinant proteins and to compare their enzymatic activities. Recombinant MVDP was functional and displayed kinetic properties distinct from those of murine AR toward various substrates, a preference for NADH, and insensitivity to AR inhibitors. For MVDP, isocaproaldehyde, a product of side-chain cleavage of cholesterol generated during steroidogenesis, is the best natural substrate identified so far. In Y1 cells, we found that NADH-linked isocaproaldehyde reductase (ICR) activity was much higher than NADPH-linked ICR activity and was not abolished by AR inhibitors. We demonstrate that in Y1 cells, forskolin-induced MVDP expression enhanced NADH-linked ICR activity by 5–6-fold, whereas no variation in ICR-linked NADPH activity was observed in the same experiment. In cells stably transfected with MVDP antisense cDNA, NADH-linked ICR activity was abolished even in the presence of forskolin, and the isocaproaldehyde toxicity was increased compared with that of intact Y1 cells, as measured by isocaproaldehyde LD50. In Y1 cells transfected with MVDP antisense cDNA, forskolin-induced toxicity was abolished by aminoglutethimide. These results indicate that in adrenocortical cells, MVDP is responsible for detoxifying isocaproaldehyde generated by steroidogenesis.

Aldoketoreductases (AKRs) are monomeric oxidoreductases that catalyze the NADPH-dependent conversion of aldehydes and ketones to their corresponding alcohols (1). Among the AKR superfamily, aldose reductase (AR) has been a focus of interest because of its potential role in the development of secondary diabetic complications (2). In agreement with its broad substrate specificity, AR is thought to accomplish varying physiological roles in osmotic homeostasis (3), steroid conversion (4), and detoxification against xenobiotic and endogenous aldehydes (5). To date, the AR superfamily consists of at least 42 members that differ in their primary structure, substrate specificities, and catalytic properties.

Adult mouse vas deferens contains a large amount of a major protein (mouse vas deferens protein (MVDP)) with an apparent molecular mass of 34,500 Da (6). Recently, it has been shown that MVDP expression is not restricted to the vas deferens, and high levels of MVDP mRNA were found in the adrenal glands (7). Androgens have been shown to be the primary regulating factors of MVDP expression in the vas deferens (8); and recent studies suggest that cAMP is a key regulator of adrenal MVDP expression (9). MVDP shares ~70% amino acid identity with human, rabbit, rat, and mouse ARs (10, 11), and MVDP gene structure is similar to that of the human AR gene (12). However, MVDP is more closely related to the mouse fibroblast growth factor-regulated protein FR-1 (13), Chinese hamster ovary reductase (14), the human AR-like gene ALR1 (15), and human small intestine reductase (16). These proteins share ~80–90% sequence identities and form a distinct subgroup within the AKR1B group according to the new nomenclature proposed by Jez et al. (17). The enzymatic nature of MVDP (AKR1B7 in agreement with the proposed nomenclature (17)) has not yet been demonstrated.

In this study, we have compared the enzymatic characteristics and substrate specificity of MVDP with those of mAR, and we have focused on its physiological role in adrenal glands. The data indicate that MVDP acts as a major reductase for isocaproaldehyde formed during steroidogenesis.

EXPERIMENTAL PROCEDURES

Chemicals—Tolrestat was a gift from Wyet-Ayerst, Sorbinil from Pfizer, and Imirestat from Alcon Laboratories. Isocaproaldehyde was prepared according to the method of Matsumura et al. (18). 4-Hydroxynonenal was provided by Interchim. All other chemicals were purchased from Sigma.

Recombinant Protein Production—MVDP and mAR cDNAs were obtained by polymerase chain reaction amplification with MVDP pUC13 (10) and mAR pGEMT (pmAR13) (11) as templates, respectively, and with outside primers containing engineered 5′- BamHI and 3′- EcoRI sites (5′-BamHI primer, 5′-ACGGATCCATGGCCACCTTCGTGGAA-3′; and 3′- EcoRI primer, 3′- ATTGAATTCTCAGTGTTACCATAC-5′). Recombinant MVDP and mAR were expressed in Escherichia coli by inserting their respective cDNAs into the BamHI and EcoRI sites of pET28a (Novagen) to produce N-terminal fusions with six histidine residues. Recombinant MVDP and mAR were produced in BL21 (DE3) pLysS cells upon isopropyl-β-D-thiogalactopyranoside induction and purified by nickel affinity chromatography according to the manufacturer’s instructions (Novagen). For each protein, column fractions were analyzed by SDS-PAGE, and those containing the purified protein were pooled and stored at 4 °C.

Y1 Cell Lines Expressing MVDP Antisense RNA by Stable Transfection—the pCR3 vector (Invitrogen) harboring the neomycin resistance gene was used to express MVDP antisense RNA in murine adrenocor-
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| Substrates                  | mAR/NADPH | MVDP/NADPH | MVDP/NADH |
|-----------------------------|-----------|------------|-----------|
|                             | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) |
| $\alpha$-Glyceraldehyde     | 0.18       | 1.2        | 0.60      | 0.035     | 1.0        | 0.35                  | 0.025     | 1.2        | 0.02                  |
| Glucose                     | 0.042      | 1.4        | 0.03       | 0.042     | 1.2        | 0.03                  | 0.042     | 1.4        | 0.04                  |
| Xylose                      | 0.025      | 1.4        | 0.025     | 0.05      | 1.0        | 0.05                  | 0.025     | 1.4        | 0.03                  |
| Glucuronate                 | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Menadione                   | ND         | ND         | ND       | ND        | ND         | ND                   | ND        | ND         | ND                    |
| 5α-Dihydrocortisol          | ND         | ND         | ND       | ND        | ND         | ND                   | ND        | ND         | ND                    |
| Methylglyoxal               | 0.18       | 0.06       | 0.30      | 0.042     | 1.2        | 0.042                | 0.025     | 1.4        | 0.03                  |
| 4-Hydroxynonenal            | 0.025      | 1.4        | 0.025    | 0.05      | 1.0        | 0.05                  | 0.025     | 1.4        | 0.03                  |
| Isovaleraldehyde            | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Isopropylaldehyde           | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Dioctyl                     | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| NADH                        | 101        | 1.0        | 10.1     | 1.0     | 1.5        | 0.15                 | 0.1       | 0.15       | 1.0                   |
| NADPH                       | 12.3       | 1.0        | 10.1     | 1.0     | 1.5        | 0.15                 | 0.1       | 0.15       | 1.0                   |

a Not detected.

b For determination of the $K_m$ values for NADPH and NADH, isocaproaldehyde was held constant at 150 and 400 μM, respectively.

c NADPH and NADH are able to use NADPH and also NADH, but with less efficiency (24–26).

d MDV shows kinetic properties distinct from those of mAR. As shown in Table I, common substrates for ARR, including aldo sugars, were poor substrates for recombinant MVDP.

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RESULTS

Kinetic Properties of Recombinant MVDP—To compare the enzymatic activities and substrate specificity of MVDP with those of mAR, both cDNAs were expressed in bacteria to obtain recombinant proteins. The kinetic constants of both proteins, determined for various substrates, are summarized in Table I. Similar to native AR purified from tissues, recombinant mAR has the ability to reduce aldo sugars, glyceraldehyde being the best substrate among them. Except for relatively lower $K_m$ values than those measured for mAR (Table I), the $k_{cat}$ values for glucose, values for recombinant mAR were in accordance with those reported for AR isolated from other species (20–23).

By comparison with other compounds, isocaproaldehyde, methylglyoxal, and 4-hydroxynonenal were the best substrates. MVDP showed kinetic properties distinct from those of mAR. As shown in Table I, common substrates for ARR, including aldo sugars, were poor substrates for recombinant MVDP. For all compounds studied, the $k_{cat}$ values were always lower than those measured for mAR (Table I), even tested under various pH conditions (data not shown). Aldose reductases are able to use NADPH and also NADH, but with less efficiency (24–26).

The data presented here are in agreement with previous findings that recombinant MVDP displays RNA secondary antibodies were added at a 1:15,000 dilution for 1 h at room temperature. Peroxidase activity was detected with the enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech).

Forskolin and Isopropanaldehyde Toxicity Measurement—Stably transfected Y1 cellular clones (AS19 or EV4 cells) were plated in 1 ml of fresh medium in 2-cm$^2$ culture wells at a concentration of 5 $\times$ 10$^4$ cells/ml. After a recovery period, cells were cultured under basal conditions or with 10$^{-4}$ M forskolin alone or with 5 $\times$ 10$^{-4}$ M aminoglutethimide for 24 h. When the LD$_{50}$ of isopropanaldehyde was assayed, cells were exposed to increasing concentrations of isopropanaldehyde for 4 h. After treatments, cells were resuspended, and their viability was immediately estimated by 0.2% (w/v) trypan blue exclusion.

Statistics—Results are given as mean ± S.D. Statistical significance of the differences between treatment groups was determined by Student’s t test.

Table I

Kinetic constants for recombinant mAR and MVDP

| Substrates                  | mAR/NADPH | MVDP/NADPH | MVDP/NADH |
|-----------------------------|-----------|------------|-----------|
|                             | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$ mM$^{-1}$) |
| $\alpha$-Glyceraldehyde     | 0.18       | 1.2        | 0.60      | 0.035     | 1.0        | 0.35                  | 0.025     | 1.4        | 0.03                  |
| Glucose                     | 0.042      | 1.4        | 0.03       | 0.042     | 1.2        | 0.03                  | 0.042     | 1.4        | 0.03                  |
| Xylose                      | 0.025      | 1.4        | 0.025     | 0.05      | 1.0        | 0.05                  | 0.025     | 1.4        | 0.03                  |
| Glucuronate                 | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Menadione                   | ND         | ND         | ND       | ND        | ND         | ND                   | ND        | ND         | ND                    |
| 5α-Dihydrocortisol          | ND         | ND         | ND       | ND        | ND         | ND                   | ND        | ND         | ND                    |
| Methylglyoxal               | 0.18       | 0.06       | 0.30      | 0.042     | 1.2        | 0.042                | 0.025     | 1.4        | 0.03                  |
| 4-Hydroxynonenal            | 0.025      | 1.4        | 0.025    | 0.05      | 1.0        | 0.05                  | 0.025     | 1.4        | 0.03                  |
| Isovaleraldehyde            | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Isopropylaldehyde           | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| Dioctyl                     | 0.090      | 0.94       | 0.090    | 0.054     | 0.77       | 0.054                | 0.090     | 0.94       | 0.09                  |
| NADH                        | 101        | 1.0        | 10.1     | 1.0     | 1.5        | 0.15                 | 0.1       | 0.15       | 1.0                   |
| NADPH                       | 12.3       | 1.0        | 10.1     | 1.0     | 1.5        | 0.15                 | 0.1       | 0.15       | 1.0                   |
ious compounds known as inhibitors of AR. Recombinant MVDP was not inhibited by Sorbinil and p-chloromercuribenzoate and was moderately diminished by Imirestat and Tolrestat, differing in this respect from recombinant mAR.

**MVDP Reduces Isocaproaldehyde in Adrenocortical Cells—** Isocaproaldehyde, the best substrate identified for MVDP, is a product of the side-chain cleavage of cholesterol, the first step of steroid biosynthesis. On the basis of Western blot analysis, both MVDP and AR were detected in adrenal glands. By comparison with known amounts of recombinant proteins, the concentrations of MVDP and AR were found to be very similar, ~1% of total soluble proteins (Fig. 1). It has been shown that in murine adrenocortical cells, MVDP expression is up-regulated by forskolin (9). If isocaproaldehyde is reduced by MVDP in adrenocortical cells, it would be predicted that isocaproaldehyde reductase (ICR) activity should be enhanced by the addition of forskolin. However, since AR has been described as a major reductase for isocaproaldehyde in adrenal glands (18), we have developed a test to discriminate between AR and MVDP ICR activities. As shown in Fig. 2, treatment of Y1 cells for 24 h with 10^{-5} M forskolin strongly increased MVDP expression, whereas a 24-h exposure of the cells to hypotonic medium had no effect on MVDP levels. Conversely, AR levels were not affected by forskolin and were enhanced after exposure to hypertonic medium. As shown in Table III, no variation in NADPH-linked ICR activity was observed in cells stimulated by forskolin or exposed to hypotonic medium. When we used NADH instead of NADPH, a strong activity was measured in unstimulated cells. No stimulating effect was observed in cells exposed to hypotonic medium; however, when cells were exposed to forskolin treatment, NADH-linked ICR activity was strongly enhanced (~5-fold), and this effect was not abolished by the presence of Sorbinil and correlated with forskolin-induced MVDP expression. Similar experiments showed that NADH-linked 4-hydroxynonenal reductase activity was higher than NADPH-linked 4-hydroxynonenal reductase activity, but was not increased after forskolin exposure (Table III). Using the same experimental procedures, NADH-linked ICR activity in adult murine adrenal cytosolic extracts was estimated to ~150 μmol/min/mg of protein, and NADPH-linked ICR activity was determined to ~25 μmol/min/mg of protein extract.

**MVDP Antisense cDNA Abolishes NADH-linked ICR Activity in Adrenocortical Cells—** Besides MVDP, the AKR1B subgroup contains proteins such as FR-1 (15) and human small intestine reductase (16), which are expressed in adrenal glands. To better understand the role of MVDP in adrenal glands, Y1 cells were stably transfected with the pCR3 vector containing MVDP antisense cDNA to prevent MVDP expression or with the empty vector as a control. Nine positive clones stably transfected with the pCR3 vector containing antisense cDNA and 12 clones transfected with the empty vector were selected after Southern blot analysis (data not shown). As expected, high levels of MVDP (Fig. 3) and strong NADH-linked ICR activity

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**TABLE II**

**Effect of inhibitors on recombinant mAR and MVDP**

| Inhibitor               | Remaining activity | % |
|-------------------------|--------------------|---|
| Sorbinil (1 μM)         | 15                 | 100|
| Imirestat (10 μM)       | 0.1                | 82 |
| Tolrestat (10 μM)       | 0.1                | 81 |
| p-Chloromercurbenzoate  | (10 μM)            | 187|

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**TABLE III**

**ICR and 4-hydroxynonenal reductase activities in the murine adrenocortical Y1 cell line**

The activities were assayed on Y1 cell cytosolic fractions with 0.12 mM NADPH or 0.216 mM NADH as cofactor in 100 mM phosphate buffer (pH 6.6). Values are the mean of four independent experiments.

| Parameter                        | Control | Forskolin (10^{-5} M) | NaCl (75 mM) |
|----------------------------------|---------|-----------------------|--------------|
| Specific activity (μmol/min/mg protein) |         |                       |              |
| NADH-linked ICR                  | 1070 ± 415 | 5830 ± 318             | 1140 ± 495   |
| NADPH-linked ICR                 | 41.5 ± 12.5 | 25.2 ± 2.9              | 35.2 ± 13    |
| NADPH-linked HNR                 | 1250 ± 206 | 825 ± 138              | 1250 ± 152   |
| NADPH-linked HNR (%)             | 31.5 ± 9 | 37.5 ± 10.8             | 56.8 ± 11    |

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A 4-hydroxynonenal reductase.

(Table IV) were observed after forskolin treatment in Y1 cells transfected with the empty vector (EV2 and EV4 cells). In contrast, MVDP and its mRNA were undetectable in Y1 cells transfected with MVDP antisense cDNA (AS19 cells) cultured either under basal conditions or with forskolin (Fig. 3, A and B).
Fig. 3. Effect of MVDP antisense RNA on MVDP and mAR expression in neomycin-resistant Y1 cellular clones. Cellular clones stably transfected with pCR3-AS encoding MVDP antisense RNA (AS19) or with pCR3-EV (empty vector; EV2 and EV4) were treated with either forskolin (A and B) or NaCl (A). A, 20 μg of cytosolic proteins were subjected to SDS-PAGE and Western blotting as described in the legends to Figs. 1 and 2. B, RNA was isolated, and equivalent amounts of each sample (25 μg) were analyzed by Northern blot hybridization using 32P-labeled MVDP or β-actin cDNA probes. C, 200 μg of cytosolic proteins from AS19 or EV4 cells cultured with or without 10−5 M forskolin, respectively, were subjected to NEphGE (NEphGE) and Western blotting using a highly titrated anti-MVDP polyclonal antiserum. Arrows indicate the position of the MVDP corresponding migration spot. The two other spots correspond to cross-reacting proteins unrelated to MVDP.

As MVDP and mAR display high homology in their nucleotide sequences, the presence of intact mAR protein levels was checked in these clones. As shown in Fig. 3A, mAR levels were not altered by MVDP antisense mRNA. In Y1 cells, in which antisense RNA completely abolishes MVDP expression (AS19 cells), no NADH-linked ICR was detected, indicating that ICR activity in adrenal glands is due mainly to MVDP (Table IV). Because basal NADH-linked ICR activity was totally blocked in AS19 cells, the presence of MVDP in EV4 cells cultured under basal conditions was checked to account for its involvement in NADH-linked ICR activity. Immunodetection using a highly titrated rabbit anti-MVDP antiserum demonstrated the presence of MVDP in EV4 cells cultured under basal conditions, whereas no MVDP expression was detected in AS19 cells cultured in the presence of forskolin (Fig. 3C).

Forskolin and Isocaproaldehyde Toxicity Measurement—Because forskolin is a potent activator of sterioidogenesis in Y1 cells and therefore a potent activator of isocaproaldehyde production, we measured the viability of AS19 cells lacking MVDP ICR activity when treated with forskolin alone or with aminoglutethimide, an inhibitor of the first step of steroid synthesis. The viability of MVDP-nonexpressing AS19 cells was significantly reduced after a 24-h forskolin exposure compared with EV4 cells (Fig. 4), and this effect was totally blocked when aminoglutethimide was simultaneously added to the medium, suggesting that forskolin is not toxic by itself, but works rather by stimulating the endogenous production of isocaproaldehyde through the enhancement of P450sec activity. LD50 was determined in EV4 and AS19 cells by adding increasing amounts of exogenous isocaproaldehyde (Fig. 5). Under our experimental conditions, LD50 was significantly decreased from 4.9 to 3 mM in cells lacking MVDP, demonstrating that MVDP ICR activity is important for detoxifying isocaproaldehyde.

Isocaproaldehyde Reductase Activity in H295R Cell Line—As MVDP was also previously described to be expressed under forskolin control in the human adrenocortical H295R cell line (9), ICR activity was therefore investigated under the same experimental conditions described for Y1 cells (Table V). In H295R cells, as observed in Y1 cells, NADH-linked ICR activity was higher than NADPH-linked activity and was also enhanced by forskolin treatment, suggesting that in human adrenocortical cells, MVDP ICR activity is also required for detoxifying isocaproaldehyde.

**Table IV**

NADH- and NADPH-linked ICR activities in Y1 clones expressing or not MVDP antisense RNA

| Specific activity | Control | Forskolin (10−5 M) | NaCl (75 mM) |
|------------------|---------|-------------------|-------------|
| mU/ml/mg protein |         |                   |             |
| NADH-linked activity in EV2 | 1010 ± 125 | 4540 ± 300 | 925 ± 130 |
| NADH-linked activity in EV4 | 800 ± 75 | 3700 ± 175 | 701 ± 57.5 |
| NADH-linked activity in AS19 | ND a | ND | ND |
| NADPH-linked activity in EV2 | 5.5 ± 1.5 | 6.5 ± 1 | 16.25 ± 1.6 |
| NADPH-linked activity in EV4 | 20.7 ± 3.5 | 19 ± 4 | 33.5 ± 5.5 |
| NADPH-linked activity in AS19 | 18.5 ± 4 | 11.1 ± 5 | 51.5 ± 4.25 |

a Activity not detected.

**DISCUSSION**

Here we demonstrate that recombinant MVDP was active when tested with a variety of common substrates for AR. However, MVDP displayed kinetic properties distinct from those of classical AR. 1) MVDP has a strong preference for NADH; and 2) its enzymatic activity was highly insensitive to most AR inhibitors tested. Based on amino acid sequence identities, MVDP is more homologous to FR-1 (13), CHO reductase (14), ALR1 (15), and human small intestine reductase (16) than to AR (11). The kinetic properties determined for some of these enzymes indicate that they have different enzymatic activities over a range of substrates. This observation contrasts with the apparent conservation of the amino acid residues known to be involved in AR enzymatic activities.
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Isocaproaldehyde reductase activity in the human H295R cell line

The activities were assayed on cytosolic fractions with 0.12 mm NADPH or 0.216 mm NADH as cofactor in 100 mM phosphate buffer (pH 6.6). Values are the mean of three independent experiments.

| Specific activity | Control | Forskolin (10⁻⁵ M) |
|-------------------|---------|-------------------|
| NADH-linked ICR activity | 1500 ± 550 | 3687 ± 192 |
| NADPH-linked ICR activity | 24 ± 6 | 13.87 ± 6.3 |

Fig. 4. Effect of aminoglutethimide on forskolin-induced cell toxicity. Subconfluent EV4 or AS19 cells were treated with forskolin and aminoglutethimide-supplemented medium for 24 h. After treatments, cell viability was estimated by trypan blue exclusion. Values are means ± S.D. of at least 12 experiments. *, significantly different from control ("p < 0.01).

Fig. 5. LD₅₀ of isocaproaldehyde. Subconfluent EV4 and AS19 cells were treated with increasing isocaproaldehyde concentrations for 4 h. After treatments, cell viability was estimated as described in the legend to Fig. 4. Values are means ± S.D. of at least 12 experiments. *, significantly different from values obtained for EV4 cells exposed to the same isocaproaldehyde concentration ("p < 0.01).

TABLE V
Isocaproaldehyde reductase activity in the human H295R cell line

Asn160, Glu163, Tyr209, Ser210, Leu212, Ser214, Lys262, Ser263, Val264, Thr265, Arg268, Glu271, and Asn273 (31) are conserved within the five sequences, except a Glu at position 21 in CHO reductase. It has been shown that deletion of the last 13 amino acid residues at the C-terminal end of AR decreased catalytic effectiveness, suggesting that this region is crucial to proper orientation of substrates in the active pocket site (32). The C-terminal domains were more divergent among mAR, FR-1, CHO reductase, ALR1, human small intestine reductase, and MVDP, suggesting that these enzymes exhibit different substrate specificities in relation to their tissue distribution.

Whereas AR is present in many tested tissues, expression of MVDP, FR-1, CHO reductase, ALR1, and human small intestine reductase is restricted to a limited number of tissues. Some of these proteins have been shown to be induced by different factors, including hyperosmotic stress or an excess of galactose (AR) (3, 33), growth factors (FR-1) (13), chemical factors (CHO reductase) (14), or hormonal factors (MVDP) (8, 9). At present, it is not known whether ALR1 and human small intestine reductase, the human homologs, are also inducible. Then, the differences observed in kinetic properties, pattern of expression, and mechanisms of induction indicate that all these related proteins are probably involved in different physiological functions. The first step of steroidogenesis is the removal of the cholesterol side chain, resulting in the formation of pregnenolone and isocaproaldehyde (4-methylpentanal), which is metabolized to isocaproic acid and isocapryl alcohol (34). 4-Hydroxynonenal is a reactive aldehyde formed via peroxidative damage to polyunsaturated fatty acids in membrane phospholipids (35). On the basis of the catalytic efficiencies obtained with various substrates, isocaproaldehyde and 4-hydroxynonenal seem to be the preferred substrates catalyzed by recombinant MVDP. Since MVDP and mAR were expressed in similar amounts in adrenal glands, both enzymes may be responsible for the reduction of isocaproaldehyde and 4-hydroxynonenal generated by cellular metabolism. However, several lines of evidence suggest that MVDP, rather than AR, is a major reductase for isocaproaldehyde in murine adrenocortical cells. First, NADH-linked ICR activity (ascribed to MVDP) was higher than NADPH-linked ICR activity (attributed to AR). Second, NADH-linked ICR activity was not inhibited by specific AR inhibitors. Third, NADH-linked ICR activity was strongly enhanced by forskolin, which stimulates MVDP expression, but not by hyperosmotic stress inducing AR overexpression. Fourth, in Y1 cells stably transfected with MVDP antisense cDNA, NADH-linked ICR activity induced by forskolin was completely abolished. Our results differ from those of Matsuura et al. (18), who have shown that in human, monkey, dog, and rabbit adrenal glands, AR is a major reductase for isocaproaldehyde; but both NADH- and NADPH-linked ICR activities measured in the experiments of Matsuura et al. (18) are lower than those from our assay in cytosolic extracts. Some of the possible reasons for these dissimilar results include differences concerning species and experimental procedures. ICR activity has been previously measured in frozen adrenal extracts; in this study, we used fresh extracts of intact adrenal glands and fresh extracts of adrenocortical Y1 cells. Strikingly, in vitro kcat/Km values suggest that mAR/NADPH at equal concentration as is found in adrenal glands should be the main isocaproaldehyde reductase. Ex vivo experiments in Y1 cells contrast with this observation, suggesting that when produced in vitro from a bacterial expression system, MVDP is in a less effective state than when produced endogenously. In agreement with the observations of Grimshaw et al. (36) that activated or oxidized forms of human placenta aldose reductase result in an increase in Km, a net decrease in kcat/Km for...
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MVDP, an oxidized form. Similarly, in Y1 cells lacking MVDP, NADH-linked 4-hydroxynonenal reductase activity is strongly reduced (data not shown). The results suggest that one function for MVDP, rather than mAR, in adrenocortical cells may be detoxification for protection against endogenous harmful aldehydes, including isocaproaldehyde and 4-hydroxynonenal. The biosynthesis of steroids is acutely and chronically stimulated by trophic hormones through the intermediary of cAMP (37). In contrast, chronic effects of trophic hormones result from increased transcription of the genes that encode the steroidogenic enzymes (37) as well as MVDP (9), thereby maintaining optimal capacity for both steroid production and reduction of isocaproaldehyde. Immunodetection of MVDP in Leydig cell cultures2 suggests that MVDP plays this role in other steroidogenic cells.

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