Several distinct enzymes catalyze 20α-hydroxysteroid dehydrogenase activity in mouse liver and kidney

Yorishige Imamura [a,*], Misa Ohtaguro [b], Hideaki Shimada [b]

[a] *Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862-0973, Japan*

[b] *Faculty of Education, Kumamoto University, 2-40-1, Kurokami, Kumamoto 860-8555, Japan*

*Correspondence author.

Yorishige Imamura

Graduate School of Pharmaceutical Sciences, Kumamoto University,

5-1, Oe-honmachi, Kumamoto 862-0973, Japan

Fax: +81-96-371-4151

E-mail address: yorishig@gpo.kumamoto-u.ac.jp (Y. Imamura)

Abstract

The effects of flavonoids and quinones on NADPH- and NADH-dependent 20α-hydroxysteroid dehydrogenase (20α-HSD) activities were examined in cytosolic fractions from the liver and kidney of mice. Judging from the data for the inhibition of NADPH- and NADH-dependent 20α-HSD activities by flavonoids and quinones, enzyme catalyzing renal NADPH-dependent 20α-HSD activity was found to be distinct from enzyme(s) catalyzing hepatic NADPH- and NADH-dependent 20α-HSD activities. Sulfobromophthalein (SBP) had little ability to inhibit hepatic NADPH-dependent 20α-HSD activity and bromophenol blue (BPB) exhibited a weak activation against the enzyme activity, whereas SBP and BPB were potent and moderate inhibitors, respectively, of hepatic NADH-dependent 20α-HSD activity. Thus, enzyme catalyzing hepatic NADPH-dependent 20α-HSD activity appeared to be distinct from enzyme catalyzing hepatic NADH-dependent 20α-HSD activity. The data for the pH profiles of hepatic NADPH- and NADH-dependent 20α-HSD activities also led us to the conclusion. Based on these results, we propose the possibility that several distinct enzymes catalyze NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice.

Keywords: 20α-Hydroxysteroid dehydrogenase activity; Mouse liver; Mouse kidney; Cofactor requirement; Inhibitors; Activators

1. Introduction

Aldo-keto reductases (AKRs) constitute a large family of enzymes that catalyze the NAD(P)(H)-dependent oxidoreduction of a variety of substrates, including sex steroids [1, 2]. 20α-Hydroxysteroid dehydrogenase (20α-HSD) belongs to the AKR superfamily and catalyzes the stereoselective reduction of progesterone to its inactive metabolite 20α-hydroxy-4-pregnen-3-one [1, 3]. This enzyme has been originally purified from rat ovary and characterized [4-6]. Furthermore, genes encoding human, rabbit, rat, and mouse 20α-HSDs have been cloned [*AKR1C1* (human), *Akr1c5* (rabbit), *Akr1c8* (rat), *Akr1c18* (mouse)] [7-12]. Progesterone is necessary for maintaining mammalian pregnancy and its conversion into 20α-hydroxy-4-pregnen-3-one is associated with the termination of pregnancy. On the other hand, progesterone at high levels has adverse effects on the development of fetuses [13, 14]. Thus, 20α-HSD probably plays a role in regulating the amount of progesterone and protects the fetuses from the cytotoxic effects of progesterone.

It has been reported that in mice, 20α-HSD activity or its mRNA expression is widely found in non-reproductive tissues [15-19]. Our previous paper [20] has also demonstrated that 20α-HSD activity is detectable in cytosolic fractions from the liver, kidney and lung of mice. Interestingly, both NADPH and NADH were effective as the cofactors for 20α-HSD activity in mouse liver cytosol, whereas 20α-HSD activity in mouse kidney cytosol required only NADPH as the cofactor [20]. These findings suggest the existence of several distinct enzymes catalyzing 20α-HSD activity in non-reproductive tissues other than the ovary. Such a well-known example is mouse 17β-HSD type 5 (Akr1c6), which possesses a low 20α-HSD activity [17, 21].

Akr1c12 expressed highly in mouse liver and gastrointestinal tract may also catalyze 20α-HSD activity, since this enzyme has the ability to reduce progesterone [18, 22]. Furthermore, 20α-HSD activity in human placenta is catalyzed by 17β-HSD type 1 or type 2 belonging to short-chain dehydrogenase/reductase (SDR) enzymes [3]. However, information on multifunctional enzymes that catalyze 20α-HSD activity in tissues such as the liver and kidney has been very limited.

The purpose of the present study was to elucidate the inhibition or activation by flavonoids, quinones and phthalein analogs such as phenolphthalein (PP) of enzymes that catalyze NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice. Furthermore, pH profiles of these 20α-HSD activities were compared with each other.

2. Materials and methods

2.1. Materials

Progesterone and 20α-hydroxy-4-pregnen-3-one were purchased from Sigma (St. Louis, MO). Flavonoids were obtained from the following sources: apigenin and naringenin (Aldrich, Milwaukee, WI); quercetin (Wako Pure Chemicals, Tokyo, Japan); fisetin (Tokyo Kasei, Tokyo, Japan); daidzein (Sigma). Quinones were from the following sources: 9,10-phenanthrenequinone (9,10-PQ) (Sigma); 1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ), 9,10-anthraquinone (9,10-AQ), aceanthrenequinone (AAQ) and acenaphthenequinone (ANQ) (Aldrich). Phthalein analogs were from the following sources: phenolphthalein (PP) (Wako Pure Chemicals); sulfobromophthalein (SBP), phenolsulfonphthalein (PSP) and bromophenol blue

(3,3',5,5'-tetrabromophenolsulfonphthalein, BPB) (Aldrich). NADPH, NADH, NADP and NAD were purchased from Oriental Yeast (Tokyo, Japan). All other chemicals were of reagent grade.

2.2. Animals

Male ddY mice at 8 weeks of age were purchased from Japan SLC (Shizuoka, Japan). The animals had free access to a diet of standard laboratory chow and water. All animal experiments were undertaken in compliance with the guideline principles and procedures of Kumamoto University for the care and use of laboratory animals.

2.3. Preparation of cytosolic fraction

The animals were slightly anesthetized and killed by decapitation. The liver and kidney were quickly excised, and homogenized in a Potter-Elvehjem homogenizer with three volumes of 10 mM sodium-potassium phosphate buffer containing 1.15% KCl (pH 7.4). All subsequent procedures were performed at 3–5˚C. The homogenates were centrifuged at 10,000g for 20 min and the resulting supernatants were centrifuged at 105,000g for 60 min to obtain the cytosolic fraction. The cytosolic fractions from the liver and kidney of male mice were used as enzyme preparations.

2.4. Assay of 20α-HSD activity

The reaction mixture for the reduction activity consisted of progesterone (0.1 mM), cofactor (0.5 mM), enzyme preparation (cytosolic fraction) and 100 mM sodium-potassium phosphate buffer (pH 7.4) in final volume of 2.0 ml. In the case of determination of the optimal pH, 100 mM sodium-potassium phosphate buffers at

5.0–9.0 were used. The mixture was incubated at 37 ˚C for 30 min under aerobic condition. The reaction was stopped by addition of 0.5 ml of 1.0 N HCl to the mixture. The reduction product (20α-hydroxy-4-pregnen-3-one) of progesterone was determined by high-performance liquid chromatography (HPLC) according to a slightly modified method of Swinney et al [23]. HPLC was carried out using a Shimadzu LC-10AD HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a Tosoh ODS-80Ts column (Tosoh, Tokyo, Japan) and a JASCO 875-UV monitor (240 nm) (JASCO, Tokyo, Japan). Mixture of water-acetonitrile-methanol-tetrahydrofuran (44:28:17:11, v/v) was used as a mobile phase at a flow rate of 0.6 ml/min. Protein concentration was determined by the method of Lowry et al. [24] with bovine serum albumin as the standard.

2.5. Inhibition experiments

Inhibitors (flavonoids, quinones and phthalein analogs) were dissolved in dimethyl sulfoxide (DMSO) or methanol, and then added to the reaction mixture. The final concentration of DMSO or methanol did not exceed 2 % (v/v), and this concentration did not affect the enzyme reaction. The final concentration of inhibitors was 10 μM for flavonoids and quinones and 50 μM for phthalein analogs.

3. Results

3.1. Cofactor requirement for 20α-HSD activity

The cofactor requirement for 20α-HSD activity was re-examined in cytosolic fractions from the liver and kidney of mice (Fig. 1). As expected, 20α-HSD activity in liver cytosol required NADH in addition to NADPH as the cofactors. In kidney

cytosol, only NADPH was confirmed to be effective as the cofactor for the enzyme activity.

3.2. Inhibition of 20α-HSD activity by flavonoids

Figure 2 shows the inhibitory effects of flavonoids on NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice. The enzyme activities were determined at a physiological pH of 7.4. Flavonoids except daidzein were potent inhibitors of hepatic NADPH-dependent 20α-HSD activity (Fig. 2A). The order of the inhibitory potencies of these flavonoids was fisetin > apigenin > naringenin > quercetin. Furthermore, these four flavonoids were effective inhibitors of hepatic NADH-dependent 20α-HSD activity, although the inhibitory potencies were somewhat lower than those against hepatic NADPH-dependent 20α-HSD activity (Fig. 2B). By contrast, these flavonoids including daidzein had little ability to inhibit renal NADPH-dependent 20α-HSD activity (Fig. 2C).

3.3. Inhibition of 20α-HSD activity by quinones

The inhibitory effects of quinones on NADPH- and NADH-dependent 20α-HSD activities were examined in cytosolic fractions from the liver and kidney of mice. As shown in Figs. 3A and 3B, 9,10-PQ and 1,2-NQ exhibited moderate inhibitions against hepatic NADPH- and NADH-dependent 20α-HSD activities. The inhibitory potency of 9,10-PQ against renal NADPH-dependent 20α-HSD activity was lower than those against hepatic NADPH- and NADH-dependent 20α-HSD activities (Fig. 3C). Quinones other than 9,10-PQ and 1,2-NQ had little ability to inhibit these hepatic and

renal 20α-HSD activities, although 1,4-NQ inhibited weakly hepatic NADH-dependent 20α-HSD activity.

3.4. Inhibition or activation of 20α-HSD activity by phthalein analogs

In an attempt to elucidate the multiplicity of enzymes that catalyze 20α-HSD activity, the effects of phthalein analogs (PP, SBP, PSP and BPB) on NADPH- and NADH-dependent 20α-HSD activities were examined in cytosolic fractions from the liver and kidney of mice. Among the phthalein analogs, PP and PSP were potent inhibitors of hepatic NADPH-dependent 20α-HSD activity (Fig. 4A). These two compounds also inhibited hepatic NADH-dependent 20α-HSD activity (Fig. 4B). Interestingly, although SBP had little ability to inhibit hepatic NADPH-dependent 20α-HSD activity and BPB exhibited a weak activation against the enzyme activity (Fig. 4A), SBP and BPB were potent and moderate inhibitors, respectively, of hepatic NADH-dependent 20α-HSD activity (Fig. 4B). As shown in Fig. 4C, SBP exhibited a potent inhibition against renal NADPH-dependent 20α-HSD activity, whereas PP had little ability to inhibit the enzyme activity. Furthermore, PSP and BPB were found to activate weakly renal NADPH-dependent 20α-HSD activity (Fig. 4C).

3.5. pH profile of 20α-HSD activity

Figure 5 shows the pH profiles of NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice. Hepatic NADPH-dependent 20α-HSD activity showed a broad pH optimum around 6.5 (Fig. 5A). On the other hand, the optimal pH of hepatic NADH-dependent 20α-HSD activity was around 7.4 (Fig. 5B). In the case of renal NADH-dependent 20α-HSD

activity, a pH optimum was observed around 6.0 (Fig. 5C).

4. Discussion

Enzymes belonging to the AKR1C subfamily have been demonstrated to function as various hydroxysteroid dehydrogenases (HSDs) [1, 2, 21]. For example, Akr1c6 and Akr1c18 catalyze 17β-HSD and 20α-HSD activities, respectively, in mouse tissues. In addition to 17β-HSD activity, Akr1c6 exhibits a low 20α-HSD activity [17, 21]. We have recently shown that cofactor requirement for 20α-HSD activity in mouse liver is different from that in mouse kidney [20]. The different cofactor requirements for these 20α-HSD activities were confirmed in the present study. Thus, the multiplicity of enzymes catalyzing 20α-HSD activity was further examined in cytosolic fractions from the liver and kidney of mice.

Most recently, human progesterone metabolizing enzyme AKR1C1 (20α-HSD) has been shown to be potently inhibited by flavonoids like naringenin [25]. Interestingly, human 17β-HSD type 5 also possesses a high 20α-HSD activity [26], and is inhibited by flavonoids such as quercetin and kaempferol [27]. Our previous papers have demonstrated that 20α-HSD activities in mouse liver and rat liver are inhibited by flavonoids [20, 28]. Furthermore, AKR enzymes have been reported to display high catalytic activities for quinones including 9,10-PQ [29-32], suggesting that quinones are useful as substrate inhibitors of AKR enzymes. Thus, the effects of flavonoids and quinones on NADPH- and NADH-dependent 20α-HSD activities were examined in cytosolic fractions from the liver and kidney of mice. Judging from the data for the inhibition of these 20α-HSD activities by flavonoids and quinones, it is concluded that

enzyme catalyzing renal NADPH-dependent 20α-HSD activity is distinct from enzyme(s) catalyzing hepatic NADPH- and NADH-dependent 20α-HSD activities. However, flavonoids showed a similar tendency to inhibit efficiently hepatic NADPH- and NADH-dependent 20α-HSD activities, although the inhibitory potencies of flavonoids against hepatic NADH-dependent 20α-HSD activity were somewhat lower than those against NADPH-dependent 20α-HSD activity. Quinones such as 9,10-PQ and 1,2-NQ also exhibited similar inhibitions against hepatic NADPH- and NADH-dependent 20α-HSD activities.

In order to characterize further enzyme(s) catalyzing hepatic NADPH- and NADH-dependent 20α-HSD activities, we compared the effects of phthalein analogs such as PP on these enzyme activities. It has been reported that phthalein analogs not only inhibit AKRs, but also activate them [33-35]. For example, SBP produces inhibition and activation, respectively, for mouse Akr1c20 and human AKR1C4 (3α-HSD type 1) [34, 35]. Thus, several phthalein analogs (PP, SBP, PSP and BPB) were used as specific inhibitors or activators of enzymes catalyzing 20α-HSD activity. Although SBP had little ability to inhibit hepatic NADPH-dependent 20α-HSD activity and BPB exhibited a weak activation against the enzyme activity, SBP and BPB were potent and moderate inhibitors, respectively, of hepatic NADH-dependent 20α-HSD activity. These results indicate clearly that enzyme catalyzing hepatic NADPH-dependent 20α-HSD activity is distinct from enzyme catalyzing hepatic NADH-dependent 20α-HSD activity. The data for the pH profiles of hepatic NADPH- and NADH-dependent 20α-HSD activities also led us to the conclusion.

Among mouse members of the AKR1C subfamily, Akr1c18 (20α-HSD), Akr1c6 (17β-HSD type 5) and Akr1c12 have the ability to catalyze 20α-HSD activity [17, 21,

22]. Akr1c18 is highly expressed in the ovary, kidney, lung and spleen [18]. On the other hand, Akr1c6 is a liver-specific enzyme [18] and Akr1c12 is highly expressed in the liver and gastrointestinal tract [18, 22]. Furthermore, although Akr1c6 is an NADPH-dependent enzyme [17], Akr1c12 utilizes NADH in addition to NADPH as the cofactor [22]. Based on these results, we propose the possibility that hepatic NADPH-dependent 20α-HSD activity is mainly catalyzed by Akr1c6 rather than by Akr1c12. Unlike hepatic NADPH-dependent 20α-HSD activity, hepatic NADH-dependent 20α-HSD activity is probably catalyzed by only Akr1c12. In the ovary, 20α-HSD plays an important role in the conversion of progesterone into its inactive metabolite. Unexpectedly, Akr1c18 (mouse NADPH-dependent 20α-HSD) is highly expressed not only in the ovary, but also in the kidney as described above [18]. Akr1c18 may be an enzyme catalyzing renal NADPH-dependent 20α-HSD activity. Furthermore, it is possible that SDR enzymes in addition to AKR enzymes are involved in 20α-HSD activity. A possible approach to this problem is to investigate the potencies of specific inhibitors for SDR enzymes.

In conclusion, the present study provides evidence that several distinct enzymes belonging to the AKR1C subfamily catalyze 20α-HSD activity (the stereoselective reduction of progesterone to 20α-hydroxy-4-pregnen-3-one) in cytosolic fractions from the liver and kidney of mice. Further studies are in progress to identify multifunctional enzymes catalyzing 20α-HSD activity in non-reproductive tissues of mice.

References

[1] D. Hyndman, D.R. Bauman, V.V. Heredia, T.M. Penning, The aldo-keto reductase superfamily homepage, Chem. Biol. Interact. 143-144 (2003) 621-631.

[2] Y. Jin, T. M. Penning, Aldo-keto reductases and bioactivation/detoxication, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 263-292.

[3] T.M. Penning, Molecular endocrinology of hydroxysteroid dehydrogenases, Endocr. Rev. 18 (3) (1997) 281-305.

[4] W.G. Wiest, R.B. Wilcox, Purification and properties of rat ovarian 20α-hydroxysteroid dehydrogenase, J. Biol. Chem. 236 (9) (1961) 2425-2428.

[5] R.B. Wilcox, W.G. Wiest, Further studies of rat ovarian 20α-hydroxysteroid dehydrogenase, Steroids 7 (5) (1966) 395-413.

[6] M. Mori, W.G. Wiest, Purification of rat ovary 20α-hydroxysteroid dehydrogenase by affinity chromatography, J. Steroid Biochem. 11 (4) (1979) 1443-1449.

[7] M. Nishizawa, T. Nakajima, K. Yasuda, H. Kanzaki, Y. Sasaguri, K. Watanabe, S. Ito, Close kinship of human 20α-hydroxysteroid dehydrogenase gene with three aldo-keto reductase genes, Genes Cells 5 (2) (2000) 111-125.

[8] Y. Zhang, I. Dufort, P. Rheault, V. Luu-The, Characterization of a human 20α-hydroxysteroid dehydrogenase, J. Mol. Endocrinol. 25 (2) (2000) 221-228.

[9] W.R. Lacy, K.J. Washenick, R.G. Cook, B.S. Dunbar, Molecular cloning and expression of an abundant rabbit ovarian protein with 20α-hydroxysteroid dehydrogenase activity, Mol. Endocrinol. 7 (1) (1993) 58-66.

[10] R. Miura, K. Shiota, K. Noda, S. Yagi, T. Ogawa, M. Takahashi, Molecular cloning of cDNA for rat ovarian 20α-hydroxysteroid dehydrogenase (HSD1), Biochem. J.

299 (Pt 2) (1994) 561-567.

[11] J. Mao, W.R. Duan, C.T. Albarracin, T.G. Parmer, G. Gibori, Isolation and characterization of a rat luteal cDNA encoding 20α-hydroxysteroid dehydrogenase, Biochem. Biophys. Res. Commun. 201 (3) (1994) 1289-1295.

[12] M. Ishida, K.T. Chang, I. Hirabayashi, M. Nishihara, M. Takahashi, Cloning of mouse 20α-hydroxysteroid dehydrogenase cDNA and its mRNA localization during pregnancy, J. Reprod. Dev. 45 (5) (1999) 321-329.

[13] E.A. Petrelli, T.R. Forbes, Toxicity of progesterone to mouse fetuses, Endocrinology 75 (1964) 145-146.

[14] W.S.N. Jayasekara, T. Yonezawa, M. Ishida, K. Yamanouchi, M. Nishihara, Expression and possible role of 20α-hydroxysteroid dehydrogenase in the placenta of the goat, J. Reprod. Dev. 51 (2) (2005) 265-272.

[15] R. Krehbiel, M. Darrach, Studies on mouse liver 21-desoxy- and 21-hydroxy-20α-hydroxysteroid: NADP 20α-oxidoreductase, Can. J. Biochem. 46 (9) (1968) 1075-1080.

[16] R.J. Ertel, F. Ungar, 20α-Hydroxysteroid dehydrogenase and reductive pathways in mouse adrenal glands in vitro, Endocrinology 82 (3) (1968) 527-534.

[17] Y. Deyashiki, K. Ohshima, M. Nakanishi, K. Sato, K. Matsuura, A. Hara, Molecular cloning and characterization of mouse estradiol 17β-dehydrogenase (A-specific), a member of the aldoketoreductase family, J. Biol. Chem. 270 (18) (1995) 10461-10467.

[18] L. Vergnes, J. Phan, A. Stolz, K. Reue, A cluster of eight hydroxysteroid dehydrogenase genes belonging to the aldo-keto reductase supergene family on mouse chromosome 13, J. Lipid Res. 44 (3) (2003) 503-511.

[19] G. Pelletier, V. Luu-The, S. Li, F. Labrie, Localization of 20α-hydroxysteroid dehydrogenase mRNA in mouse brain by in situ hybridization, Mol. Brain Res. 125 (1-2) (2004) 143-146.

[20] H. Shimada, K. Miura, Y. Imamura, Characteristics and inhibition by flavonoids of 20α-hydroxysteroid dehydrogenase activity in mouse tissues, Life Sci. 78 (25) (2006) 2931-2936.

[21] T. Matsunaga, S. Shintani, A. Hara, Multiplicity of mammalian reductases for xenobiotic carbonyl compounds, Drug Metab. Pharmacokinet. 21 (1) (2006) 1-18.

[22] S. Ikeda, E. Okuda-Ashitaka, Y. Masu, T. Suzuki, K. Watanabe, M. Nakao, K. Shingu, S. Ito, Cloning and characterization of two novel aldo-keto reductases (AKR1C12 and AKR1C13) from mouse stomach, FEBS Lett. 459 (3) (1999) 433-437.

[23] D.C. Swinney, D.E. Ryan, P.E. Thomas, W. Levin, Regioselective progesterone hydroxylation catalyzed by Elever rat hepatic cytochrome P-450 isozymes, Biochemistry 26 (22) (1987) 7073-7083.

[24] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1) (1951) 265-275.

[25] P. Brožic, T. Šmuc, S. Gobec, T.L. Rižner, Phytoestrogens as inhibitors of the human progesterone metabolizing enzyme AKR1C1, Mol. Cell. Endocrinol., 259 (1-2) (2006) 30-42.

[26] I. Dufort, P. Rheault, X.-F. Huang, P. Soucy, V. Luu-The, Characteristics of a highly labile human type 5 17β-hydroxysteroid dehydrogenase, Endocrinology 140 (2) (1999) 568-574.

[27] A. Krazeisen, R. Breitling, G. Möller, J. Adamski, Phytoestrogens inhibit human

17β-hydroxysteroid dehydrogenase type 5, Mol. Cell. Endocrinol. 171 (1-2) (2001) 151-162.

[28] S. Shimada, M. Uchida, T. Okawara, S.-I. Abe, Y. Imamura, Inhibitory effects of flavonoids on the reduction of progesterone to 20α-hydroxyprogesterone in rat liver, J. Steroid Biochem. Mol. Biol. 93 (1) (2005) 73-79.

[29] B.P. Schlegel, K. Ratnam, T.M. Penning, Retention of NADPH-linked quinone reductase activity in an aldo-keto reductase following mutation of the catalytic tyrosine, Biochemistry 37 (31) (1998) 11003-11011.

[30] T. O'Connor, L.S. Ireland, D.J. Harrison, J.D. Hayes, Major differences exist the function and tissue-specific expression of human aflatoxin B_1 aldehyde reductase and the principal human aldo-keto reductase AKR1 family members, Biochem. J. 343 (Pt 2) (1999) 487-504.

[31] A. Hinsshelwood, G. McGarivie, E.M. Ellis, Substrate specificity of mouse aldo-keto reductase AKR7A5, Chem. Biol. Interact. 143-144 (2003) 263-269.

[32] S. Ishikura, K. Matsumoto, M. Sanai, K. Horie, T. Matsunaga, K. Tajima, O. El-Kabbani, A. Hara, Molecular cloning of a novel type of rat cytoplasmic 17β-hydroxysteroid dehydrogenase distinct from the type 5 isozyme, J. Biochem. (Tokyo) 139 (6) (2006) 1053-1063.

[33] Y. Higaki, N. Usami, S. Shintani, S. Ishikura, O. El-Kabbani, A. Hara, Selective and potent inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1) that metabolizes neurosteroids derived from progesterone, Chem. Biol. Interact. 143-144 (2003) 503-513.

[34] K. Matsumoto, S. Endo, S. Ishikura, T. Matsunaga, K. Tajima, O. El-Kabbani, A. Hara, Enzymatic properties of a member (AKR1C20) of the aldo-keto reductase

family, Biol. Pharm. Bull. 29 (3) (2006) 539-542.

[35] K. Matsuura, Y. Tamada, Y. Deyashiki, Y. Miyabe, M. Nakanishi, I. Ohya, A. Hara, Activation of human liver 3α-hydroxysteroid dehydrogenase by sulphobromophthalein, Biochem. J. 313 (Pt 1) (1996) 179-184.

Figure legends:

Fig. 1. Cofactor requirement for 20α-HSD activities in cytosolic fractions from the liver and kidney of mice. (A) liver; (B) kidney. Progesterone (0.1 mM) was used as the substrate and NADPH, NADH, NADP or NAD (0.5 mM) was used as the cofactor. Each bar represents the mean ± S.D. of three to five experiments.

Fig. 2. Inhibition of NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice by flavonoids. (A) hepatic NADPH-dependent 20α-HSD activity; (B) hepatic NADH-dependent 20α-HSD activity; (C) renal NADPH-dependent 20α-HSD activity. Progesterone (0.1 mM) was used as the substrate and NADPH or NADH (0.5 mM) was used as the cofactor. The concentration of flavonoids was 10 μM. Each bar represents the mean ± S.D. of three experiments.

Fig. 3. Inhibition of NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice by quinones. (A) hepatic NADPH-dependent 20α-HSD activity; (B) hepatic NADH-dependent 20α-HSD activity; (C) renal NADPH-dependent 20α-HSD activity. Progesterone (0.1 mM) was used as the substrate and NADPH or NADH (0.5 mM) was used as the cofactor. The concentration of quinones was 10 μM. Each bar represents the mean ± S.D. of three to five experiments.

Fig. 4. Inhibition or activation of NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice by phthalein analogs. (A) hepatic NADPH-dependent 20α-HSD activity; (B) hepatic NADH-dependent 20α-HSD activity; (C) renal NADPH-dependent 20α-HSD activity. Progesterone (0.1 mM) was used as the substrate and NADPH or NADH (0.5 mM) was used as the cofactor. The concentration of phethalein analogs was 50 μM. Each bar represents the mean ± S.D. of three to seven experiments.

Fig. 5. pH profiles of NADPH- and NADH-dependent 20α-HSD activities in cytosolic fractions from the liver and kidney of mice. (A) hepatic NADPH-dependent 20α-HSD activity; (B) hepatic NADH-dependent 20α-HSD activity; (C) renal NADPH-dependent 20α-HSD activity. Progesterone (0.1 mM) was used as the substrate and NADPH or NADH (0.5 mM) was used as the cofactor. Each point represents the mean ± S.D. of three to five experiments.