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HETEROGENEITY OF 11β-HYDROXYSTEROID DEHYDROGENASE IN RAT TISSUES

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Summary—Using specific antisera to purified rat liver 11β-hydroxysteroid dehydrogenase (11-HSD), we showed that the antigen is widely distributed in rat organs. Enzyme activity and immunoreactivity generally corresponded. Highest by both criteria were liver, testis, kidney and lung. In some tissues (epididymis, pancreas and duodenum) activity was found, but antigen corresponding to 11-HSD at a M_w of 34 kDa was absent. It is suggested that these tissues have alternate enzyme forms. The 11-HSD of brain and liver were compared. Brain enzyme may control selective binding of aldosterone to Type I receptors in the hippocampus and other regions. Rat brain 11-HSD resembled that of liver or kidney in most characteristics. It differed in (a) its steroid specificity: cortisol was a good substrate for liver 11-HSD, and a poor substrate for brain enzyme; (b) stability of 11-oxoreductase (11-OR) component. Brain 11-OR was not readily inactivated; 11-OR from other tissues lost activity rapidly and spontaneously. The variations in properties of 11-HSD in specific tissues may reflect aspects of its various specific functions.

Most mammalian tissues are capable of converting 11β-hydroxysteroids to 11-oxosteroids in a reversible process catalyzed by 11β-hydroxysteroid dehydrogenase (11-HSD). Recent evidence supports the suggestion that the enzyme mediates corticosteroid–receptor interaction, and through this process, cell specific responses [1, 2]. The varied expression of 11-HSD in different tissues is the consequence of the level of enzyme, of variations in the proportions of 11β-hydrogenase (11-DH) and 11-oxoreductase (11-OR) components of 11-HSD, or of available cofactor. The components of the 11-HSD system are shown in Fig. 1. The view that 11-DH and 11-OR are separate yet interdependent entities provides a convenient explanation for the frequently encountered observation that 11-DH is dominant in some tissues, 11-OR is dominant in others, and that these relationships are known to shift in response to changing physiological circumstances [3].

Support for the two enzyme hypothesis was sought by purifying the enzymes. Rat liver corticosteroid 11-DH was obtained as an electrophoretically homogenous protein [4]. All attempts to detect 11-OR activity in the purified enzyme failed. Possible explanations for this failure were instability of liver 11-OR [5], or separation of the dehydrogenase from the reductase. However, isolation of 11-DH was not followed by the isolation of 11-OR from rat liver. Because of this, the two enzyme hypothesis could not be rigorously established. Alternative hypotheses were: (a) a single polypeptide containing independent 11-DH and 11-OR sites; and (b) a single active site whose behavior as dehydrogenase or reductase depends on its conformational characteristics (Fig. 2).

To test these alternatives, cDNA corresponding to rat liver 11-DH was used to construct a vaccinia virus recombinant carrying a 1.2 kb *Eco* RI fragment which incorporated a nearly full length cDNA [6]. This was expressed in osteosarcoma cells. A protein, M_w 34 kDa, was generated that contained both 11-DH and 11-OR activities. The properties of the recombinant enzyme closely resembled the properties of the rat liver enzyme. Both were membrane bound, and were extracted in soluble form by detergents. Both were glycoproteins of 34 kDa M_w. The 11-OR of the liver microsomes and of the recombinant enzyme were unstable and rapidly inactivated. Both versions of 11-DH were inhibited by glycyrrhetinic acid (K_I for 11-DH = 2 nM) [7]. Both formed an immunoprecipitin with antibody to rat liver 11-DH.

The evidence we obtained was contrary to the two enzyme hypothesis. The data appeared rather to be most consistent with a single

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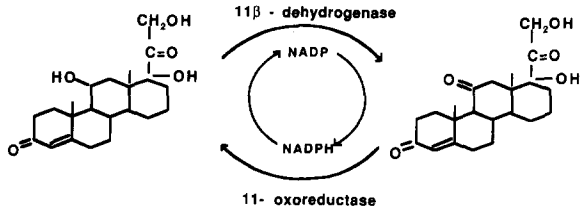

Fig. 1. Component elements of the 11-HSD reaction.

enzyme catalyzing both oxidation and reduction, though the alternative single enzyme models described above could not be distinguished. In the following exposition, a single enzyme model is assumed.

Monospecific polyclonal antibodies generated against homogeneous rat liver 11-DH were used to screen tissues for antibody reacting components corresponding in molecular weight after SDS electrophoresis to the 34 kDa rat liver antigen [8]. Since the levels of 11-HSD activity in most tissues were low, we anticipated that the intensities of the 34 kDa bands corresponding

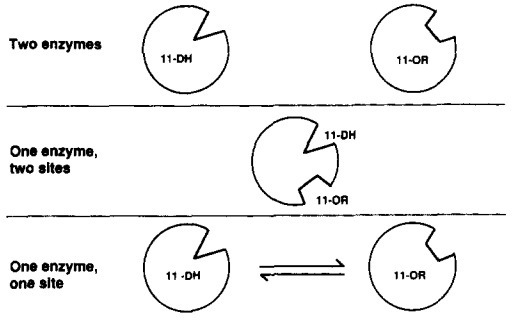

Fig. 2. Three models illustrating how 11-DH and 11-OR activities may be expressed by 11-HSD.

Table 1. 11-DH activity in microsomes of rat tissues

Tissue	Specific activity (nmol/min mg protein)
Liver	4.7 ± 1.0
Kidney	2.1 ± 0.6
Testis	1.9 ± 0.6
Lung	0.36 ± 0.1
Brain	0.034 ± 0.002
Heart	0.022 ± 0.009
Epididymis	0.028 ± 0.022
Prostate	0.007 ± 0.003
Stomach	0.013 ± 0.002
Duodenum	0.021 ± 0.001
Jejunum	0.016 ± 0.008
Caecum	0.010 ± 0.003
Colon	0.040 ± 0.020

Values shown are means ± SD for three determinations.

in mobility to 11-HSD would be low. Consequently, screening of tissues was performed after loading the gels with high levels of protein. Detection of 11-DH antigen was done under low stringency conditions in order to maximize the likelihood of detecting a 34 kDa antibody binding protein. There was a general correspondence between enzyme activity and immunoreactivity. An indication of relative activity levels in a number of tissues is shown in Table 1. In adult rats, highest levels of enzyme by either kinetic or immunological criteria were in the liver, testis, kidney and lung. Figure 3 shows that many enzymatically active tissues had 34 kDa immunoreactive proteins, but several did not. A possible interpretation, consistent with data reported from other laboratories, is that enzymatically active tissues that reveal no 34 kDa protein on Western blots have other forms of 11-HSD that are structurally unique. Some tissues showed more than one antibody

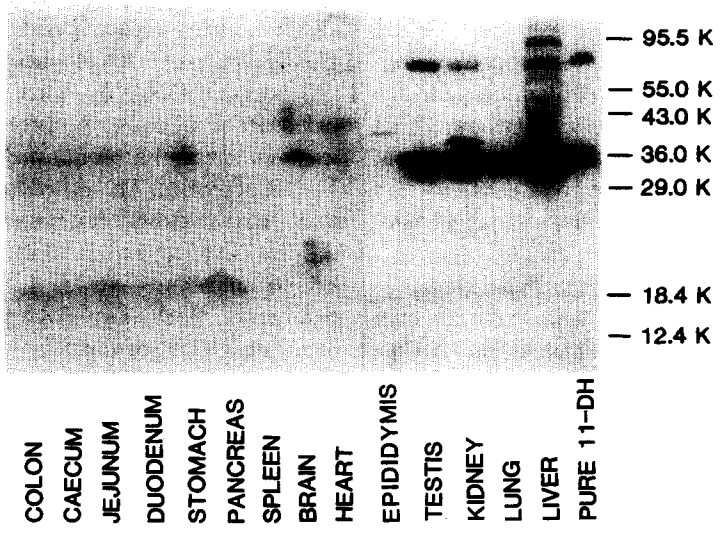

Fig. 3. Western blot analysis of rat tissues with rat liver 11-DH antiserum. Gels were overloaded with detergent (Triton DF-18) extracts of tissues in order to detect low level 34 kDa antigen. All lanes contained equal levels of protein.

staining species. It is likely that some of these are artifacts of overloading, but it is not readily obvious from the extent or complexity of antibody reactivity how the various cross reacting components are related to enzyme activity.

Brain 11-HSD is interesting both from a physiological standpoint, and as an illustration of subtle organ specific differences in enzyme properties. Activity of 11-HSD in rat brain, though low, is significant because its presence in specific regions is essential for selective expression of mineralocorticoid and glucocorticoid dependent activity. Brain responds to both steroid classes through Type I mineralocorticoid receptors. Type II glucocorticoid receptors specifically utilize glucocorticoids [9]. In the hippocampus, specific aldosterone dependent responses have been demonstrated, including suppression of salt appetite [10, 11] and alteration of blood pressure [12].

The problems associated with access of aldosterone to Type I receptors are similar to those encountered in the kidney [13, 14]. As in the kidney, there are many more molecules of corticosterone than aldosterone per unit volume capable of binding to Type I receptors. Since these receptors bind gluco- and mineralocorticoids equally well, the mineralocorticoid is put at a great disadvantage. This is overcome in the kidney by the action of 11-DH which converts corticosterone to an innocuous metabolite (11-dehydrocorticosterone), which is unable to compete with aldosterone for the Type I receptor. Those brain regions rich in Type I receptors should also have high concentrations of 11-DH to inactivate corticosterone, if the kidney model is applicable. There is, however, evidence that the brain utilizes both corticosterone and aldosterone, that both steroids may be needed concurrently for optimal function, and that each steroid, through interacting with the Type I receptor, performs different functions. It would therefore be expected that the level of 11-DH be lower than in the kidney, in order that corticosterone is not completely inactivated. It would also be anticipated that, if corticosterone is to be maintained at an optimal level, fine control requires the counterbalance of 11-OR activity.

The activity of 11-HSD is detected throughout the brain, with levels of activity that are characteristic of each region [15]. Activities are highest in the hippocampus and cerebral cortex (Fig. 4). *In situ* hybridization with 11-DH cDNA, and immunohistochemical staining are strong, consistent with the high activity

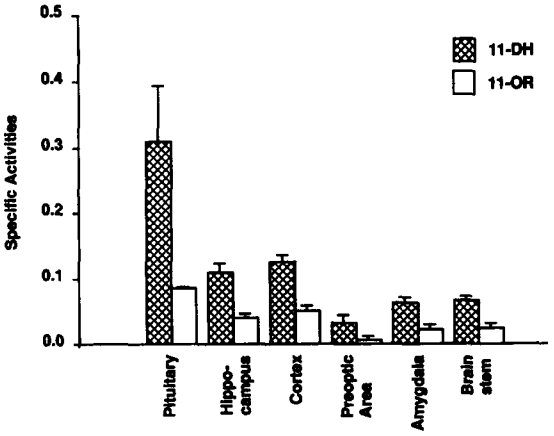

Fig. 4. Comparison of 11-DH and 11-OR activities in brain regions and pituitary.

levels [16]. With one of three 11-DH antisera (antiserum 56–125), all regions of the brain display a strong 26 kDa antibody reacting protein and a weak or undetectable 34 kDa protein. Specific staining intensities of the 26 kDa protein correlate well with the 11-DH activities and with regional brain distribution (Fig. 5). It is not, however, an aberrant form of 11-HSD. When separated from 34 kDa on a Sepharose-NADP affinity column, the 26 kDa protein was devoid of activity. Only the 34 kDa component was enzymatically active. Brain enzyme was similar to liver or kidney enzyme in most ways: (a) each immunoreacted with 11-DH antibody; (b) the M_w of each was 34 kDa; (c) each was inhibited by 2 nM glycyrrhetinic acid; (d) each was a glycoprotein; (e) each was associated with a particulate fraction; and (f) crude particulate homogenates catalyzed both 11-oxidation and -reduction. The brain enzyme differed from liver and kidney enzyme in its substrate specificity.

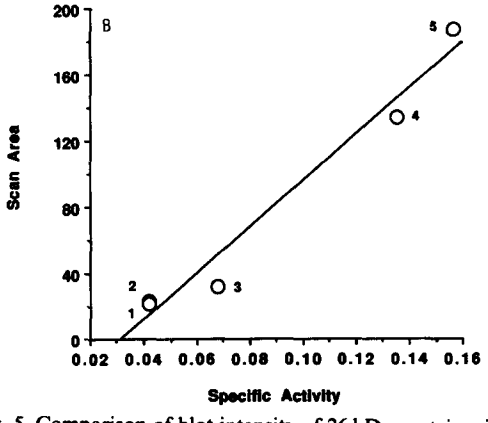

Fig. 5. Comparison of blot intensity of 26 kDa protein with antiserum 25–165 and 11-DH activity in various brain regions. 1, brain stem; 2, pre-optic area; 3, amygdala; 4, hippocampus; 5, cerebral cortex.

In the latter tissues both corticosterone and cortisol were good substrates (corticosterone/cortisol = 10). In brain, cortisol was a poor substrate, while corticosterone was efficiently oxidized. Unlike liver and kidney, brain oxidoreductase was not inactivated during purification. The oxidation–reduction ratio (O/R = 3) of crude brain preparations was not altered in purified 34 kDa protein. Thus, in contrast with other sources, brain 11-OR was stable.

The basis for the differences in properties of 11-HSD from different tissues is not apparent. That the enzyme is predominantly oxidative in some organs, while primarily reductive in others has long been known [3]. That organs evolve during ontogeny from an environment primarily supporting 11-oxoreduction to one supporting 11-dehydrogenation is also well known [17].

We have recently presented evidence that the oligosaccharide component of 11-HSD influences the relative expression of 11-oxidation or -reduction [18]. It may also determine enzyme stability. Whether posttranslational modification or changing environmental conditions are responsible for the tissue specific diversity of 11-HSD is being actively investigated in our laboratories. It is clear that unraveling the complexities of this versatile enzyme will remain a continuing challenge.

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