Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain

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Abstract Dehydroepiandrosterone sulfate (DHEAS) is the major circulating steroid in man. Pharmacologically, it exerts marked neuropsychiatric effects. Since no target receptor has been identified, we investigated whether the organic anion transporting polypeptide (OATP), a multispecific steroid carrier, transports DHEAS. Expression of the human liver OATP in *Xenopus laevis* oocytes resulted in high-affinity, partially Na^+-dependent uptake of [^{3}H]DHEAS (K_m: 6.6 μmol/l). DHEAS transport was inhibited by bromosulfophthalein, bile acids, sulfated estrogens and dexamethasone. Northern blot analysis showed widespread expression of OATP in human brain. These data identify OATP as the first known target protein of DHEAS in human liver and brain.

Key words: Dehydroepiandrosterone sulfate; Prasterone; Carrier protein; Psychotropic drug; Gene expression

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfated metabolite DHEAS represent the major circulating steroids secreted by the adrenal cortex in man. In human plasma DHEA concentrations average 10 nmol/l, while DHEAS concentrations reach 10 μmol/l in young adults 20–35 years of age [1]. Apart from serving as precursors for endogenous testosterone and estrogen synthesis [2], these hormones exert significant neuropsychiatric effects. The vogue drug prasterone (DHEA) has attained widespread popularity as an 'anti-aging hormone' in the USA, since the age-related decrease in plasma concentrations of DHEA(S) is held responsible for the decline of certain physiological functions associated with aging. At age 80, DHEA(S) levels are only about 20% those at age 25, unlike the age-independent plasma levels of cortisol [3]. DHEA(S) substitution induces an expressed feeling of remarkable psychological and physical well-being, notably an improved quality of sleep, greater energy and an increased ability to handle stress [4]. Oral administration of DHEA to elderly patients with major depression significantly improves depression ratings and memory performance [5].

After oral administration, DHEA is largely converted to DHEAS in the liver and the plasma levels of the latter increase rapidly [6]. The metabolism of DHEA into potentially active sex steroids occurs in tissues possessing androgen or estrogen receptors, notably in adipose tissue, muscle, prostate, brain and particularly in the liver, from where active DHEA metabolites are released into the circulation and reach their target tissues [1]. DHEAS is strongly bound to albumin in plasma and undergoes renal tubular reabsorption [6]. The precise mode of action of DHEA(S) is unknown, since no selective receptor has been identified. The aim of this study was to identify a specific transport protein which mediates DHEAS transport in human liver and brain.

Of the known membrane proteins and receptors involved in the uptake and metabolism of steroid hormones, the so-called organic anion transporting polypeptide (OATP) – a transmembrane transport protein for anionic steroids with a strong level of expression in the brain [7,8] – was considered a potential candidate for specific cell surface interaction with DHEAS. Both the human OATP as well as its rat homologues oatp-1 [9] and oatp-2 [10] have been shown to function as multispecific amphipathic steroid transporters for the conjugated steroids estrone-3-sulfate [8] and estradiol-17β-D-glucuronide [10,11]. To study whether the human OATP could represent a physiological target protein for DHEAS in human liver, kidney and brain, OATP-mediated DHEAS transport was functionally characterized in the *Xenopus laevis* frog oocyte expression system and OATP expression in human brain determined by Northern blot analysis.

2. Materials and methods

2.1. Materials

7-^{3}H(*N*)]Dehydroepiandrosterone sulfate ([^{3}H]DHEAS) (21.1 Ci/mmol) was obtained from DuPont-New England Nuclear, Bad Homburg, Germany. [α-^{32}P]Deoxycytidine triphosphate ([α-^{32}P]dCTP) (3000 Ci/mmol) was obtained from Amersham International, Buckinghamshire, UK. Dexamethasone, water soluble, was obtained from Sigma, Deisenhofen, Germany.

2.2. Animals

Mature *Xenopus laevis* females were purchased from Nasco, Fort Atkinson, WI, USA, and kept under standard conditions as described [12].

2.3. Expression of human OATP in Xenopus laevis oocytes

The cDNA coding for the human OATP [7] was ligated directionally into the plasmid pSPORT1 (Gibco BRL, Gaithersburg, MD, USA). Plasmid DNA was prepared with the Qiagen miniprep system (Qiagen, Hilden, Germany) and linearized with *Not*I. Capped complementary RNA was transcribed in vitro with T7 RNA polymerase (Promega Corp., Madison, WI, USA) [13]. *Xenopus laevis* oocytes were prepared as previously described [14]. After an overnight incubation at 18°C, healthy oocytes were microinjected with 10 ng of human OATP complementary RNA and were subsequently cultured

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Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHEA(S), the sum of DHEA and DHEAS; OATP, human organic anion transporting polypeptide; oatp, rat organic anion transporting polypeptide; BSP, bromosulfophthalein

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for 72 h in modified Barth's solution [14] to allow expression of the OATP.

2.4. DHEAS uptake in oocytes

Uptake of [^{3}H]DHEAS was measured in an NaCl medium, consisting of (in mmol/l) 100 NaCl, 2 KCl, 1 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES adjusted to pH 7.5 with Tris. H_2O-injected oocytes were used as controls. Fifteen oocytes were prewashed in the uptake medium and incubated at 25°C in 100 μl of the same medium containing [^{3}H]DHEAS at the indicated concentrations. Uptakes were stopped by the addition of 3 ml ice-cold uptake buffer and oocytes were washed with 3×10 ml of ice-cold uptake buffer. Each oocyte was dissolved in 500 μl of 10% SDS and 5 ml scintillation fluid (Ultima Gold, Canberra Packard, Frankfurt/Main, Germany), and the oocyte-associated radioactivity was counted in a Packard Tri-Carb 2100 liquid scintillation analyzer (Packard Instrument Co., Frankfurt/Main, Germany).

2.5. Northern blot analysis

A Northern blot containing 2 μg messenger RNA from eight different human brain regions was purchased from Clontech Laboratories Inc., Palo Alto, CA, USA. The blot was hybridized with a [α-^{32}P]dCTP-labeled human OATP-cDNA fragment as described previously [7] and was subsequently exposed to autoradiography film for 6 h.

2.6. Statistical analysis

Values are given as the mean ± 1 S.D. The level of significance of DHEAS uptake in the *cis*-inhibition studies was determined using the unpaired two-tailed Student's *t*-test [15]. Statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Characterization of OATP-mediated [^{3}H]DHEAS uptake in oocytes

To establish whether DHEAS is a substrate of the organic anion transporting polypeptide, oocytes injected with OATP-cRNA or water were incubated with 10 μmol/l [^{3}H]DHEAS in an NaCl medium. As shown in Fig. 1, there was clear stimulation of DHEAS uptake in oocytes injected with OATP-cRNA compared with water-injected control oocytes (2.00 ± 0.47 pmol/oocyte vs. 0.26 ± 0.06 pmol/oocyte, respectively, after 30 min). In a Na^+-free medium, in which NaCl had been replaced by 100 mmol/l choline chloride, DHEAS uptake amounted to 0.60 ± 0.02 vs. 0.19 ± 0.02 pmol/oocyte/30 min in OATP-expressing and water-injected oocytes respectively, indicating partial Na^+-dependence of DHEAS uptake.

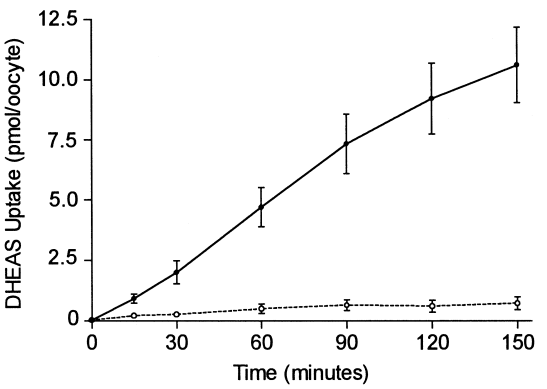

Fig. 1. Transport of DHEAS by the human OATP. *Xenopus laevis* oocytes were injected with 10 ng OATP-cRNA (●) or with an identical volume of H_2O (○). After 3 days in culture, uptake of (10 μmol/l) [^{3}H]DHEAS was measured in an NaCl medium. Data represent the mean ± 1 S.D. of 15 independent oocyte uptake measurements.

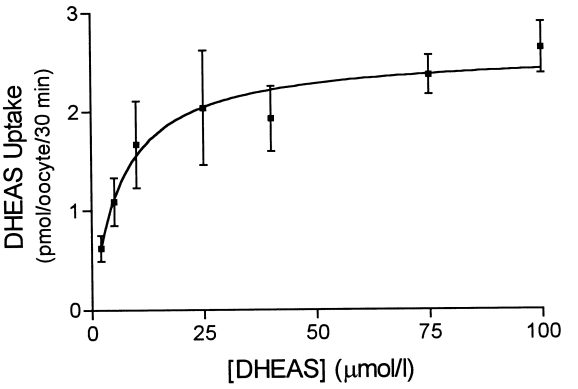

Fig. 2. Kinetics of DHEAS uptake in OATP-cRNA-injected oocytes. Oocytes were injected with 10 ng OATP-cRNA or with an identical volume of H_2O. After 3 days in culture, uptake of [^{3}H]DHEAS was measured in an NaCl medium in the presence of increasing substrate concentrations. Uptake was measured over 30 min, since separate experiments showed linear uptake during this time period at low (0.5 μmol/l) and high (100 μmol/l) substrate concentrations. Non-specific diffusion into H_2O-injected oocytes was subtracted from all uptake values. Individual data points represent the mean ± 1 S.D. of 12–15 oocyte uptake measurements. The curve was fitted by non-linear regression analysis.

To analyze the kinetics of OATP-mediated DHEAS transport, oocytes were incubated with [^{3}H]DHEAS at increasing substrate concentrations over a period of 30 min. As shown in Fig. 2, DHEAS uptake was saturable and of high affinity, with an apparent K_m of 6.6 ± 1.3 μmol/l and a V_{max} of 2.6 ± 0.1 pmol/oocyte/30 min. This K_m value is in the range of DHEAS concentrations in human plasma and central nervous system [16], indicating that OATP could be the physiological transport system for this steroid.

To further characterize OATP-mediated DHEAS transport, the *cis*-inhibitory effect of a variety of conjugated steroids and OATP substrates on DHEAS uptake was examined. OATP-expressing and water-injected *Xenopus laevis* oocytes were incubated with 5 μmol/l [^{3}H]DHEAS in the presence of 100 μmol/l of the respective inhibitor substances. As shown in Fig. 3, OATP-mediated DHEAS uptake was significantly inhibited by the cholephilic organic anion bromosulfophthalein (BSP) (67.5 ± 16.4% inhibition) and by the bile acids taurochenodeoxycholate (75.2 ± 23.9%) and tauroursodeoxycholate (85.7 ± 24.1%). Strong inhibition was also observed in the presence of the OATP substrate estrone-3-sulfate (82.3 ± 17.2%), its 17-hydroxylated derivative estradiol-3-sulfate (92.9 ± 22.0%) and the glucocorticoid dexamethasone (97.3 ± 10.1%).

3.2. Northern blot analysis of OATP expression in human brain

Since OATP had previously been found to be strongly abundant in human brain [7], its distribution in eight different brain regions was investigated by Northern blot analysis. Fig. 4 shows the characteristic pattern of multiple OATP transcripts in all human brain regions tested, with a major hybridization signal at 7.8 kbp and additional cross-hybridizing messenger RNAs of approximately 2.7 kbp, 3.3 kbp and 4.4 kbp. The original size of the cloned human liver OATP cDNA corresponds to the 2.7 kbp band. These data indicate abundant expression and widespread distribution of OATP in the human brain, explaining the marked neuropsychiatric effects of DHEA(S) on the central nervous system.

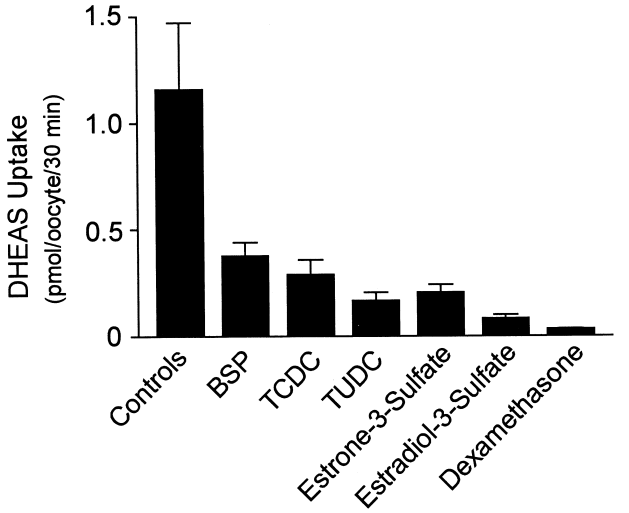

Fig. 3. Effects of organic anions and steroids on DHEAS uptake in OATP-cRNA-injected oocytes. Oocytes were injected with 10 ng OATP-cRNA or with an identical volume of H_2O. After 3 days in culture, uptake of 5 μmol/l [^{3}H]DHEAS was measured in an NaCl medium in the absence (controls) or presence of 100 μmol/l of the indicated inhibitor substances. Non-specific diffusion into H_2O-injected oocytes was subtracted from all uptake values. BSP, bromosulphophthalein; TCDC, taurochenodeoxycholate; TUDC, tauroursodeoxycholate. Individual data points represent the mean ± 1 S.D. of 12–15 oocyte uptake measurements. All uptake values in the presence of inhibitor substances were significantly different ($P < 0.001$) from controls.

4. Discussion

The present study identifies the organic anion transporting polypeptide, a multispecific amphipathic steroid transporter, as the first known target protein of the sex hormone precursor and established psychotropic steroid DHEAS. In the *Xenopus laevis* oocyte expression system, injection of OATP-cRNA significantly stimulated uptake of DHEAS compared to water-injected control oocytes (Fig. 1). Kinetic analysis revealed a high affinity of OATP-mediated DHEAS transport, with an apparent K_m of 6.6 μmol/l and a V_{max} of 2.6 pmol/oocyte/30 min (Fig. 2). This K_m value is in the range of DHEAS concentrations in plasma and brain [16], indicating that OATP could be the physiological DHEAS transport system. OATP-mediated DHEAS uptake was inhibited by the OATP substrates BSP, taurochenodeoxycholate, tauroursodeoxycholate, sulfated estrone and estradiol, as well as by the structurally related glucocorticoid dexamethasone (Fig. 3). Finally Northern blot analysis of OATP expression in the brain showed that multiple transcripts are present in all brain regions tested. This could explain the pronounced neuropsychiatric effects exerted by DHEAS.

The rationale for studying DHEAS transport via OATP originated from the observation that several tissues involved in DHEAS uptake and metabolism also express the steroid transporter OATP. DHEAS is largely formed from DHEA in the liver [6], from where it is effluxed across the sinusoidal hepatocyte membrane back into the circulation. OATP is a bidirectional transport system [17], which functions as an anion exchanger to facilitate the transport of organic anions such as conjugated steroids across the basolateral hepatocyte membrane. Similar anion exchange mechanisms exist in proximal tubular kidney cells, which also express OATP on their apical surface [18]. Apart from mediating tubular reabsorption of conjugated steroids such as DHEAS, OATP serves as an apical secretory pathway for numerous other steroids following their conjugation by sulfotransferases within the tubule cell. In the rat brain, the OATP homologue oatp-1 has been localized to the apical surface of choroid plexus epithelial cells [19], which is in contact with the cerebrospinal fluid. Since the choroid plexus epithelium is known to share many functional properties with hepatocytes, such as active transport of organic anions [19], oatp-1 is likely to be the mediator of vectorial organic anion transport between choroid plexus and cerebrospinal fluid.

The Northern blot data presented in this study indicate that in the human brain, a major site of DHEAS action and metabolism, there is widespread distribution and abundant expression of OATP. Although DHEAS hardly crosses the blood-brain barrier [20], de novo synthesis of neurosteroids by oligodendroglial cells leads to cerebral accumulation of DHEAS [16,21]. The function of OATP in the brain could be to mediate the release of DHEAS into the extracellular space following its synthesis in glial cells. DHEAS action in the central nervous system appears to occur via binding to synaptosomal membranes [16]. DHEAS is a negative non-competitive modulator of the $GABA_A$ receptor [16] and an agonist of the σ_1 receptor, modulating *N*-methyl-D-aspartate-mediated glutamatergic neurotransmission [22]. Because DHEAS concentrations in the brain are subject to physiological variations, this neurosteroid could play an important role in regulating neuronal excitability in the central nervous system.

An active basolateral transport system for DHEAS has been characterized in rat hepatocytes [23]. Interestingly, DHEAS uptake into rat hepatocytes was saturable, partially Na^+-dependent and inhibited by bile acids. Conversely, the uptake of [^{3}H]cholate was strongly inhibited by DHEAS. These results indicated that DHEAS is a substrate of the multispecific hepatocellular bile acid uptake system [23]. Since OATP is the only known multispecific hepatocellular bile acid transporter [24] and possesses all the functional characteristics of the DHEAS uptake system described in rat hepatocytes, it

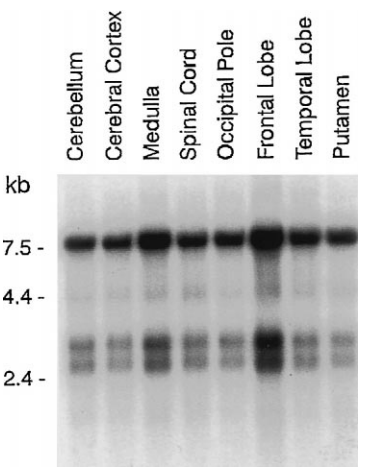

Fig. 4. Northern blot analysis of OATP expression in human brain. The Northern blot contained 2 μg of poly(A)$^+$ RNA from each human brain region. After hybridization with a ^{32}P-labeled OATP-cDNA fragment (see Section 2), the blot was exposed to autoradiography film for 6 h.

probably represents the major DHEAS transport system of human liver.

In summary, OATP mediates high-affinity transmembrane transport of DHEAS and thus represents the first known target protein of this major circulating steroid in man. The hepatocellular and renal tubular transport as well as the numerous psychotropic effects of the neuroactive steroid DHEAS could be attributable to the expression of OATP in these tissues.

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