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P-Glycoprotein has negligible effects on estradiol and testosterone in mice

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Abstract

P-Glycoprotein (P-gp) is an important factor at the blood–brain barrier preventing passage of a wide variety of substances into the brain. Several studies have provided evidence that some drugs and certain steroid hormones are substrates or inhibitors of P-gp. However, the situation is unclear with regard to gonadal steroids, which have considerable central nervous effects. In vitro, experiments on the relationship between estradiol and P-gp are equivocal. We used abcb1ab knock-out mice and wild-type mice to determine the uptake of [3H]-17-beta-estradiol and [3H]-testosterone into the cerebrum and other organs after subcutaneous administration. The organ/plasma quotients showed no significant group effects. We concluded that P-gp does not influence the penetration of testosterone and estradiol to a biologically significant extent.

Keywords: Abcb1ab; Estradiol; Testosterone; Knock-out mice; P-Glycoprotein

P-Glycoprotein (P-gp) is a 170-kDa ATP-dependent drug transport protein, located in the apical membrane of endothelial cells. It exports molecules, which attempt to pass through the cell membrane from the outside, protecting cells from toxins. The current model proposes that P-gp intercepts the drug as it moves through the membrane and flips it from the inner leaflet to the outer leaflet into the extracellular space. In mice, P-gp is encoded by the abcb1a and the abcb1b gene, and the overall distribution in mice tissue overlaps well with the single multi-drug resistance gene (ABCB1) in humans. Knock-out mice which lack the abcb1a gene for P-gp have been available since 1994, and in this time have enhanced the understanding of the importance and function of P-gp [8].

High levels of P-gp expression exist at the adrenal gland, the site of glucocorticoid synthesis suggesting that steroids might be substrates of P-gp [10,25]. In fact, dexamethasone was reported to be a substrate of P-gp [20] and our group recently showed that corticosterone, cortisol and aldosterone are regulated by P-gp in vivo; progesterone was influenced by P-gp function to a lesser extent [26].

The more hydrophobic steroid estradiol was reported not to be a substrate for P-gp efflux cell lines [11], but so far, results of in vitro experiments have been equivocal [7,14,17]. Estradiol—like cortisone, corticosterone, testosterone and progesterone—showed inhibitory effects on P-gp in vitro [21]. The relation of estradiol to P-gp in vivo has not been studied extensively. Estrogen and progesterone were reported to induce P-gp expression in the uterus of pregnant mice [1], which is in accordance with in vitro results [16].

Oral estradiol replacement is widely used for treatment of menopausal symptoms. Among the steroids, estrogens have a wide spectrum of biological effects on a variety of physiological functions throughout the body as well as on the course and development of diverse diseases [2,4,12,18,19,24].

Testosterone is a potent inhibitor of P-gp at the cornea [6]. Whether or not testosterone itself is a substrate of P-gp, is unclear. Although differing only slightly from the chemical structure of progesterone, testosterone displayed about twice the P-gp inhibiting activity of progesterone [13]. In a previous study it was claimed that testosterone is known to be a pure inhibitor [7], but valid in vivo evidence is lacking so far.

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Testosterone is essential for all masculine functions including sexual differentiation, development of secondary sex characteristics, spermatogenesis, masculine features of muscle-bone apparatus and male sexual behaviour [5]. Like many other steroids, it has various effects on the central nervous system [27].

The central nervous effects of estradiol and testosterone depend on the access of the neuroactive steroids to the brain. Some steroids are substrates of P-gp and their ability to overcome the blood–brain barrier (BBB) is therefore reduced. We used knock-out mice lacking P-gp in order to investigate whether the penetration of estradiol and testosterone through the BBB is regulated by P-gp.

P-gp (abcb1ab) double knock-out mice, were created by sequential gene targeting in 129/Ola E14 embryonic stem cells and backcrossed seven times (N7) to FVB/N from the C57BL/6 × 129 chimera [23]. FVB/N wild-type mice were received from Taconic (Germantown, USA; FVB/Tac-[KO]Pgy2 N7). A homozygous colony is maintained at the Max Planck Institute of Psychiatry on the N7 FVB/N background through intercrossing of homozygous mice.

All animal experiments were carried out in accordance with the Animal Rights Act of the State of Bavaria, which regulates the use and treatment of experimental animals. The supervising animal care coordinator of the State of Bavaria agreed with all housing and experimental procedures (file reference 209.1/211).

Male abcb1ab knock-out mice ($n = 7$) and FVB/N wild-type mice ($n = 7$) were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 07:00), with food and water ad libitum. The age of animals used was between 23 and 39 weeks. The average weight of abcb1ab knock-out mice was 31.5 ± 2.7 g, for controls 31.7 ± 2.4 g.

Male abcb1ab (−/−) mice ($n = 8$) and FVB/N wild-type mice ($n = 8$) were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 07:00), with food and water ad libitum. The age of all animals used was 14 weeks. The average weight of abcb1ab (−/−) mice was 28.7 g (S.D. ±0.5), for controls 27.7 g (S.D. ±0.4).

[3H]-Testosterone (NEN, Boston, USA, purity: >97%, batch 38, specific activity: 3.55 TBq/mmol; 96.0 Ci/mmol) and [3H]-17-beta-estradiol (NEN, Boston, USA, purity: >97%, batch 3363042, specific activity: 2.59–4.25 TBq/mmol; 70–115 Ci/mmol) were dissolved in 0.9% sodium chloride and 10% ethanol. Either 0.98 ng of [3H]-17-beta-estradiol or 1.7 ng of [3H]-testosterone/g of body weight were administered subcutaneously. The amount of hormones given was calculated using the manufacturer's information on the specific activity. Non-radioactive forms of the hormones were not used.

The total volume injected was 10 μl/g of body weight. Thus approximately 1 g ethanol/kg body weight was administered which is below the known range of intoxication and may increase performance in some mouse strains [22]. Two hours after injection, the mice were deeply anesthetized with halothane and decapitated. Trunk blood was collected in EDTA coated tubes and centrifuged at 4500 rpm for 5 min. Organs with a P-gp containing blood-organ barrier (cerebrum, liver, kidney, adrenal gland and testes) and organs without it (pituitary glands and spleen) were dissected and weighed. Organs and plasma were solved at 40 °C in Biolute S (Zinsser Analytic, Berkshire, UK) for 20 h; 300 μl of each sample was mixed together with 3 ml of Quicksafe A (Zinsser Analytic, Berkshire, UK), acidified with 50 μl of 99.7% acetic acid and shaken. Finally, the radioactivity was measured with a beta-counter. Plasma and organ samples were calibrated using a standard curve.

[3H]-17-beta-Estradiol concentrations in plasma, brain, spleen, liver, kidney, adrenal glands, pituitary glands and testes were not significantly different between abcb1ab knock-out mutants and wild-type controls, 2 h after s.c. injection of 0.98 ng [3H]-17-beta-estradiol/g of body weight (Table 1).

Analysis of variance revealed no significant group effect on absolute [3H]-17-beta-estradiol organ concentrations [Wilks multivariate test of significance; effect of group. $F(5, 8) = 0.97$,

Table 1
[3H]-17-beta-estradiol presented as mean/S.E.M. of plasma (pg/ml) and of organs (pg/g), of organ/plasma and of organ/spleen ratios in abcb1ab −/− and wild type mice 2 h after s.c. injection of 0.98 ng [3H]-17-beta-estradiol/g bodyweight

[3H]-17-Beta-estradiol in plasma and organs $F(5, 8) = 0.97$, significance of $F = 0.539$

	abcb1ab (−/−)		Wild type		Ratio	Significance
	Mean	S.E.M.	Mean	S.E.M.		
Plasma	571	72	615	69	0.9	n.s.
Cerebrum	418	22	466	16	0.9	n.s.
Spleen	429	41	452	18	0.9	n.s.
Adrenal gl.	521	62	524	19	1.0	n.s.
Kidney	441	12	443	15	1.0	n.s.
Liver	1388	110	1864	140	0.7	n.s.
Testes	611	35	699	23	0.9	n.s.
Pituitary gl.	2052	188	1912	172	1.1	n.s.

[3H]-17-Beta-estradiol organ/plasma ratio $F(6, 7) = 1.19$, significance of $F = 0.424$

	abcb1ab (−/−)		Wild type		Ratio	Significance
	Mean	S.E.M.	Mean	S.E.M.		
Cerebr./pl	0.8	0.1	0.8	0.1	1.0	n.s.
Spleen/pl	0.8	0.1	0.8	0.1	1.0	n.s.
Adren. gl./pl	1.0	0.1	1.0	0.2	1.0	n.s.
Kidney/pl	0.9	0.2	0.8	0.1	1.1	n.s.
Liver/pl	2.8	0.6	3.4	0.6	0.8	n.s.
Testes/pl	1.2	0.1	1.3	0.2	0.9	n.s.
Pituitary/pl	3.9	0.5	3.7	1.0	1.1	n.s.

[3H]-17-Beta-estradiol organ/spleen ratio $F(6, 7) = 1.10$, significance of $F = 0.463$

	abcb1ab (−/−)		Wild type		Ratio	Significance
	Mean	S.E.M.	Mean	S.E.M.		
Plasma/sp	1.0	0.1	1.0	0.0	1.0	n.s.
Cerebr./sp	1.3	0.1	1.3	0.1	1.0	n.s.
Adren. gl./sp	1.2	0.1	1.2	0.1	1.0	n.s.
Kidney/sp	1.1	0.1	1.0	0.0	1.1	n.s.
Liver/sp	3.4	0.4	4.2	0.3	0.8	n.s.
Testes/sp	1.5	0.1	1.6	0.1	0.9	n.s.
Pituitary/sp	4.9	0.5	4.3	0.5	1.1	n.s.

Significant differences have not been found.