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Nandrolone treatment decreases the α_{1B}-adrenoceptor mRNA level in rat kidney, but not the density of α_{1B}-adrenoceptors in cultured MDCK-D1 kidney cells

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Abstract

We have previously shown that treatment of rats with the anabolic androgen steroid nandrolone decreased the density of α_{1B}-adrenoceptors in the rat kidney [Uhlén, S., Lindblom, J., Kindlundh, A., Muhisha, P., Nyberg, F., (2003). Nandrolone treatment decreases the level of rat kidney α_{1B}-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 368, 91–98]. This effect may have been caused either by decreased de novo synthesis of α_{1B}-adrenoceptors or by increased degradation of ars-adrenoceptors. In the present study, we show that treatment of rats with nandrolone decreases the level of mRNA for the α_{1B}-adrenoceptor in the kidneys, implying decreased synthesis of α_{1B}-adrenoceptors. On the other hand, nandrolone did not decrease the density of α_{1B}-adrenoceptors in Madin-Darby Canine Kidney (MDCK) cells, even though the sub-cell line tested, MDCK-D1, expresses both the androgen receptor and the α_{1B}-adrenoceptor. It is concluded that the regulation of α_{1B}-adrenoceptor expression by anabolic androgenic steroids is intricate and cell-type specific.

Keywords: Kidney; α_{1B}-adrenoceptor; Nandrolone; MDCK-D1

1. Introduction

Anabolic androgenic steroids, such as testosterone and synthetic derivatives thereof (Hoberman and Yesalis, 1995), are often abused for their performance improving and cosmetic effects (Kindlundh et al., 1998). However, anabolic androgenic steroid abuse is associated with serious health risks, for example augmented increase in blood pressure during exercise (Grace et al., 2003). The androgen receptor is believed to mediate a hypertensive effect in male spontaneously hypertensive rats (SHR) (Reckelhoff et al., 1999), possibly by stimulating the renin–angiotensin system and/or by increasing proximal tubular reabsorption (Reckelhoff and Fortepiani, 2004; Reckelhoff and Granger, 1999). The influence of anabolic androgenic steroids on renal function is of interest, since the kidney is one of the most important organs for the long-term regulation of blood pressure, adjusting body fluid volume via a feed back system (Guyton, 1991). Using radioligand binding, we previously found that treatment of rats with nandrolone led to a marked, about 50%, decrease in the density of α_{1B}-adrenoceptors in the kidney (Uhlén et al., 2003). The main function of kidney α_{1B}-adrenoceptors is to increase the resorption of Na^+-ions in the tubuli (DiBona and Kopp, 1997), inducing antidiuresis, and thereby an increase in plasma volume and blood pressure (Elhawary and Pang, 1994).

In the present study we use quantitative real-time polymerase chain reaction (qPCR) to test the hypothesis that nandrolone down-regulates renal α_{1B}-adrenoceptors at the gene transcription/mRNA level in the male rat. In an effort to obtain easily interpretable data from isolated cells, we also investigated the effect of nandrolone on the density of α_{1B}-adrenoceptors in Madin-Darby Canine Kidney (MDCK) cells, i.e. in a cell model of Na^+-resorbing distal tubule/proximal collecting duct

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cells (Madin and Darby, 1958; Taub and Saier, 1979). Specifically, the sub-cell line MDCK-D1, which expresses α_{1B}-adrenoceptors (Meier et al., 1983) was used.

2. Materials and methods

2.1. Chemicals

[7-Methoxy-3H]-prazosin (84 Ci/mmol) was from Amersham Biosciences. BMY7378 (8-[2-[4-(2-methoxypheny)-l-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione), metitepine and 5-methyl-urapidil were from Research Biochemicals Inc., Natick, MA, U.S.A. Nandrolone decanoate (Deca-Durabol®) was from Organon, Oss, Netherlands. All other chemicals were from Sigma-Aldrich and were of analytical quality.

2.2. Treatment of rats with nandrolone and tissue processing

16 outbred male Sprague–Dawley rats (B&K, Sollentuna, Sweden) with an initial body weight of 275±2.4 g were housed in groups of four in air-conditioned rooms (12 h dark:12 h light cycle) at 22–23 °C and a humidity of 55%. The animals had free access to water and R36 food pellets (Labfor, Lactamin, Vadstena, Sweden). The animals received daily intramuscular injections of 15 mg/kg nandrolone decanoate (n=8; Deca-Durabol, Organon, Oss, Netherlands) or vehicle, i.e. peanut oil (n=8; Apoteket AB, Umeå, Sweden) for 14 days, 2 h after lights on. The volume of injection was 0.1 ml. On day 15, the rats were killed by decapitation, and cross-sections of the kidney were rapidly dissected, immersed in RNAlater solution (Ambion) and kept in room temperature for approximately 1 h to allow the solution to infiltrate the tissue, then stored at −80 °C until further processed. The animal experiment was approved by the local ethical committee in Uppsala, adhering to the guidelines of the Swedish National Board of Agriculture. RNA isolation and cDNA synthesis was performed as described previously (Lindblom et al., 2005).

2.3. Quantification of renal adrenoceptor and androgen receptor mRNA by qPCR

Relative levels of mRNA were determined by qPCR. All PCR primers were designed using DNA Star software (Lasergene) and based on sequences downloaded for individual rat mRNAs. Primers for the rat house keeping genes β-actin, cyclophilin, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), histone H3, ribosomal protein L19, succinate dehydrogenase complex subunit A, β-tubulin and the genes of interest α_{1A}-adrenoceptor, α_{1B}-adrenoceptor, α_{2A}-adrenoceptor, α_{2B}-adrenoceptor, and androgen receptor, were 18–21 nucleotides in length with melting points between 55 and 60 °C and formed products in the range of 70–100 base pairs (Table 1).

qPCR was performed in a 25-μ1 final reaction volume of 20 mM Tris–HC1 (pH 8.4), 50 mM KCl, 4 mM $MgCI_2$, 0.2 mM dNTP, SYBR Green (1:50,000), using an iCycler real-time detection instrument (Bio-Rad Laboratories). Template concentration was 1 ng/μl, and the concentration of each primer was 0.8 pmol/μl. Taq DNA polymerase (Invitrogen) was used at 0.02 U/μl. For each primer pair, the assay included individual samples in duplicates, a standard curve of four serial dilution points of a mixture of cDNA and a no-template control. Annealing temperature was 62 °C and 50 cycles were performed. Melting point curves were included after the thermocycling in order to confirm that only one product with the expected melting point was formed. iCycler IQ 3.0 (Bio-Rad Laboratories) was used for the analysis of real-time PCR data. Using the geNorm method described by Vandesompele et al. (2002), we first evaluated the stability of the house-keeping genes in order to calculate normalisation factors to which the genes of interest were normalized. After this, the relative expression levels were further normalised to the mean of the control levels. Statistics were performed using Student's t-test, and the criterion of statistical significance was set at $p<0.05$.

Table 1
Primer sequences used in the quantitative real-time PCR assays

Transcript	Species	Accession no.	Forward primer	Reverse primer
β-actin	Rat	NM 031144	cat tgc cgc atc ctc ttc ct	aac cgc tca ttg ccg ata gtg
Cyclophilin	Rat	MI9533	gag cgt ttt ggg tee agg aat	att gcc cgc aag tca aag aaa
GAPDH	Rat	X02231	aca tgc cgc ctg gag a2a cct	gcc cag gat gcc ctt tag tgg
Histone H3	Rat	NM 053985	att cgc aag ctc ccc ttt tag	tgg aag cgc agg tct gtt ttg
Ribosomal protein L19	Rat	NM 031103	tcg cca atg cca act ctc gtc	agc ccg gga atg gac agt ca
SDCA	Rat	NM 130428	ggg agt gcc gtg gtg tca ttg	ttc gee cat age ccc tag tag
β-Tubulin	Rat	NM 173102	cgg aag gag gcg gag agc	agg gtg ccc atg cca gag c
α_{1A}-Adrenoceptor	Rat	NM 017191	cag aag gcg gcg gag tca	gcg tct tgg tag ctt tct tct
α_{1B}-Adrenoceptor	Rat	M60655	gac cac gga ggg agc aaa g	cca tag cca ggg agg aga ttg
α_{2A}-Adrenoceptor	Rat	NM 012739	tgg ccc tcg acg tgc tct tt	cga tgg cct gcg tga tgg a
α_{1B}-Adrenoceptor	Rat	AF366899	gac ggc gca act tcc ctc ta	ggt gee tag cgt ccc tat a
Androgen receptor	Rat	NM 012502	aag agc tgc gga agg gaa aca	ccg gag acg aca cga tgg ac
GAPDH	Canine	AF197950	tgt ccc cac caa caa tgt	tcc gat gcc tgc ttc act act
Androgen receptor	Canine	AI5038240	ttc ggc cac ctc ctc ttc	tgc act gee gee tee act c

GAPDH=glyceraldehyde-3-phosphate-dehydrogenase, SDCA=succinate dehydrogenase complex subunit A.

2.4. MDCK cell culture and establishment of a sub-cell line expressing the α_{1B}-adrenoceptor

The canine kidney distal tubule/proximal collecting duct MDCK cells (ATCC CCL-34) were obtained from the American Type Culture Collection (ATCC), through LGC Promochem, Boras, Sweden. The MDCK-D1 cell line was a kind gift from Martin C. Michel, Amsterdam, Netherlands.

The MDCK cells were grown at 37 °C, 95% air/5% CO_2, in Dulbecco's Modified Eagle's Medium (Sigma D5796), supplemented with 10% fetal calf serum and 100 U/ml penicillin and 0.1 mg/ml streptomycin, in 9 cm diameter standard tissue culture plastic material. Cells were sub-cultured every 3–4 days, after detachment with 0.5 mg/ml trypsin and 0.2 mg/ml EDTA (Sigma T3924).

Plain cultures of MDCK cells, obtained from ATCC, showed negligible binding of [^{3}H]-prazosin, indicating virtual absence of α_1-adrenoceptors. However, sub-cell lines that express substantial levels of α_{1B}-adrenoceptors can be isolated from these cells. This was first shown by Meier et al. (1983) who developed the MDCK-D1 sub-cell line.

A novel MDCK-RP α_{1B}-expressing sub-cell line was developed by first trypsinizing an early passage of the MDCK cells from ATCC. A dilute suspension was then seeded into a 96-well microtiter plate, so that most of the wells got delivered one single cell. In six of the single cell wells the cultures started to grow into monolayers. These cultures were trypsinized and then expanded serially in 24- and 6-well dishes, and finally in a flask containing 8 ml medium. The six sub-cell lines obtained were then screened for α_1-adrenoceptors with [^{3}H]-prazosin, α_2-adrenoceptor with [^{3}H]-RX821002, dopamine D1 receptors with [^{3}H]-SCH23390, and muscarinic receptors with [^{3}H]-*N*-methyl-scopolamine. One sub-cell line expressed α_1-adrenoceptors, and was named MDCK-RP. None of the six sub-cell lines expressed α_2-adrenoceptors or dopamine D1 receptors, while three sub-cell lines expressed muscarinic receptors (data not shown). In the MDCK-RP cells, the density of α_1-adrenoceptors was about 60 fmol/mg protein (Table 3). In the MDCK-D1 sub-cell line, the density of α_{1B}-adrenoceptors was about 210 fmol/mg membrane protein, which is in line with previous findings (Yang et al., 1998).

In order to investigate if nandrolone influences the density of α_{1B}-adrenoceptors in the MDCK cells, cell cultures were treated with nandrolone. A stock solution of 300 μg/ml of nandrolone decanoate was prepared in 50% DMSO. During treatment, MDCK-D1 and MDCK-RP cells were grown in DMEM medium, for 3, 7, or 14 days with or without nandrolone decanoate (7.5 μg nandrolone/ml culture medium).

2.5. MDCK cell membrane preparations and radioligand binding

Cells were harvested by removing the medium and adding 7 ml/plate of 50 mM Tris/5 mM EDTA, pH 7.5. The cells were then scraped off, and homogenized for 20 s by an Ultra-Turrax (IKA T25, equipped with a 8-mm probe), set at medium speed. The homogenates were centrifuged at low speed (500×*g*) for 5 min, in a Beckman J2-21 centrifuge. The resulting supernatants were centrifuged at 38,000×*g* for 10 min. The pellets were resuspended in 2.8 ml/plate of 50 mM Tris–HCl, 1.5 mM EDTA, pH 7.5. These membrane preparations were again ultra turraxed for 3 s, in order to dissolve precipitated material, and then used immediately in binding experiments. Protein concentrations for membrane preparations were measured according to Lowry et al. (195l), with the inclusion of SDS (Markwell et al., 1978).

Each radioligand binding saturation experiment involved distributing membranes obtained from one 13.5-cm-diameter culture dish, into 24 wells of a microtiter plate at about 400 μg of protein/well, in 150 μl of 33 mM Tris–HCl, 1 mM EDTA, at pH 7.5, with different concentrations of [^{3}H]-prazosin±2 μM metitepine. The incubations lasted for 1 h at room temperature, and then the suspensions were filtered and washed on Whatman GF/C filters, using a Brandel cell harvester. In the competition experiments, 0.4–0.6 nM [^{3}H]-prazosin and varying concentration of competitors were used. All assays were performed in duplicate. Curves were drawn using the BindAid radioligand binding analysis package (Wan System, Umeå, Sweden). The figures were constructed using DeltaGraph® Pro 3.5.

2.6. Detection of androgen receptors in MDCK-D1 cells by qPCR

qPCR was used in order to detect androgen receptor mRNA in MDCK-D1 cells. The cells were harvested and homogenised by sonication in TRIzol reagent (Invitrogen) using a Branson sonifier. Thereafter, RNA was isolated and cDNA synthesized as described previously (Lindblom et al., 2005). Using the protocol described above, the relative levels of androgen receptors were estimated in individual cDNAs (n=4). PCR primers for the canine house-keeping gene GAPDH and the androgen receptor were designed as described above (see also Table 1).

3. Results

3.1. Quantification of mRNA levels in the rat kidney and in MDCK-D1 cells

Using the geNorm method described by Vandesompele et al. (2002), we first calculated the pairwise variation between the house-keeping genes in order to analyze their relative stability. The order of stability was cyclophilin=ribosomal protein L19>succinate dehydrogenase complex subunit A>β-actin>β-tubulin>GAPDH>H3. Normalisation factors derived from the geometric means of the three most stable house-keeping genes (cyclophilin, ribosomal protein L19, and succinate dehydrogenase complex subunit A) were used for the subsequent normalization of the genes of interest. Student's *t*-tests indicated that nandrolone increased the levels of α_{1A}- and

Table 2
Effects of nandrolone decanoate treatment (15 mg/kg daily for 14 days) on normalized transcription levels of the α_{1A}-, α_{1B}-, α_{2A}- and α_{2B}-adrenoceptors and the androgen receptor in the male rat kidney as measured by quantitative PCR

	Control	Nandrolone	*p*-value
α_{1A}-Adrenoceptor	100±4	153±10	0.0001
α_{1B}-Adrenoceptor	100±16	45±4	0.003
α_{2A}-Adrenoceptor	137±9	100±9	0.006
α_{2A}-Adrenoceptor	100±5	100±7	0.48
Androgen receptor	121±12	100±6	0.08

Data are presented as mean±S.E.M.

Table 3
The density of α_{1B}-adrenoceptors in membranes from control respective nandrolone-treated MDCK-D1 and MDCK-RP cells

	B_{max}±S.E.M (fmol/mg protein)	% change	Significance (p value)	pK_d±S.E.M. (^{3}H-prazosin)	(n)
MDCK-D1 control	208±7			10.04±0.11	3
+nandrolone 3 days	262±14	+26	0.324	9.99±0.06	3
MDCK-D1 control	211±3			10.16±0.06	4
+nandrolone 7 days	248±14	+18	0.001	10.09±0.02	4
MDCK-D1 control	339±6			10.16±0.03	4
+nandrolone 14 days	558±14	+65	0.000001	10.10±0.03	4
MDCK-D1 control	56±3			10.17±0.19	3
+nandrolone 3 days	65±14	+15	0.014	10.13±0.23	3

α_{2A}-adrenoceptor mRNA by 53% and 37%, respectively, but reduced the level of α_{1B}-adrenoceptor mRNA by 55% (all $p<0.01$). No effect was observed on α_{2B}-adrenoceptor mRNA ($p=0.48$). A 20% increase in androgen receptor mRNA level was less than significant ($p=0.08$). The results from rat kidney are summarized in Table 2. Using GAPDH as reference, we employed qPCR to detect mRNA for the androgen receptor in MDCK-D1 cells. We found that the androgen receptor is indeed expressed in MDCK-D1 cells, at 0.33±0.05% of the reference gene.

3.2. Evidence that nandrolone does not decrease the density of α_{1B}-adrenoceptors in cultured MDCK-D1 and MDCK-RP cells

In order to investigate whether nandrolone down-regulates α_{1B}-adrenoceptors in cultured kidney cells, the MDCK-RP cells were treated for 3 days, and the MDCK-D1 cells for 3 days, 7 days, or 14 days with nandrolone (7.5 μg/ml culture medium). The [^{3}H]-prazosin saturation experiments showed that the numbers of α_1-adrenoceptors were slightly increased in membranes prepared from nandrolone-treated MDCK cells. After 3 days, the increase in numbers of α_1-adrenoceptors in the nandrolone-treated groups were +26% for the MDCK-D1 sub-cell line and +15% for the MDCK-RP sub-cell line (Table 3; Fig. 1A–B). After 7 days the α_1-adrenoceptor density increased by +18% in the nandrolone-treated MDCK-D1 cells, and after 14 days by 65% (for significance, see Table 3).

3.3. Verification that only the α_{1B}-subtype of α_1-adrenoceptors is present in nandrolone-treated MDCK-D1 and MDCK and RP cells

In order to identify the subtype of α_1-adrenoceptors in both control and 3 days nandrolone-treated MDCK-D1 cells, we performed competition experiments for [^{3}H]-prazosin with the α_{1A}-selective substance 5-methyl-urapidil, the α_{1D}-selective

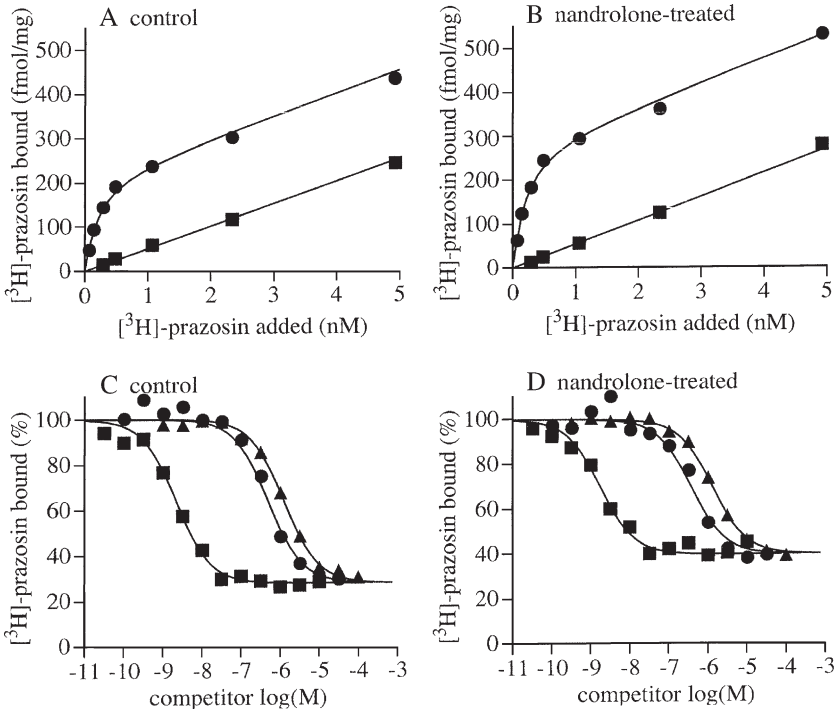

Fig. 1. (A and B) Saturation curves for [^{3}H]-prazosin. Shown are total binding (●) and binding in the presence of 2 μM of metitepine (■). (C and D) Competition curves for the substances metitepine (■), 5-methyl-urapidil (●), and BMY7378 (▲). The experiments were performed on α_{1B}-adrenoceptors in membranes obtained from control (A, C) or 3 days nandrolone-treated MDCK-D 1 cells (B, D).

Table 4
Affinity-constants ($pK_i \pm$S.E.M.; $n=3$) of 5-methyl-urapidil (5-MU), BMY7378 and metitepine, competing for 0.6 nM [^{3}H]-prazosin at MDCK-D1 membranes obtained from control cells or cells treated for 3 days with nandrolone

	pK_i 5-MU	pK_i BMY7378	pK_i metitepine
MDCK-D1	7.43±0.11	7.02±0.06	9.74±0.06
+nandrolone	7.45±0.11	6.95±0.06	9.79±0.07
MDCK-RP	7.37±0.04		
+nandrolone	7.47±0.05		

5-MU was also tested at MDCK-RP cell membranes.

substance BMY7378, and the potent non-selective substance metitepine. As can be seen in Table 4 and in Fig. 1C–D all the tested substances had similar affinities in membranes from control versus nandrolone-treated cells. The low affinity of the α_{1A}-selective 5-methyl-urapidil (pK_i about 7.4) rules out the presence of α_{1A}-adrenoceptors in the MDCK-D1, and also MDCK-RP, cells in both control and nandrolone-treated cells. The low affinity of the α_{1D}-selective BMY7378 (pK_i about 7.0) rules out the presence of the α_{1D}-subtype (Zhong and Minneman, 1999) in the MDCK-D1 cells. Metitepine, which has high affinity for α_{1A}- and α_{1B}-adrenoceptors (Mugisha et al., 2002), showed high affinity, indicating that the site labeled by [^{3}H]-prazosin in the MDCK-D1 cells represents α_{1B}-adrenoceptors and not a non-specific binding site.

4. Discussion

In our previous study (Uhlén et al., 2003) we showed that chronic nandrolone treatment reduced renal α_{1B}-adrenoceptor [^{3}H]-prazosin binding by approximately 50%. In the present study, using qPCR, we show that the same treatment reduced α_{1B}-adrenoceptor mRNA by 55%, indicating an inhibitory effect of nandrolone on the transcription of the α_{1B}-gene in the kidney (Table 2). Interestingly, significant up-regulation of the α_{1A}- and α_{2A}-adrenoceptor mRNA were also observed. In our previous study (Uhlén et al., 2003) nandrolone caused a slight increase both in α_{1A}- and α_{2A}-adrenoceptor binding, but this was far from significant. Thus, the effect of nandrolone on renal α_{1A}- and α_{2A}-adrenoceptors was more visible at the mRNA level as compared to protein level. The lack of any effect of nandrolone treatment on α_{2B}-adrenoceptors is in line with our previous results, but is somewhat surprising, as this receptor subtype is believed to be positively regulated by endogenous androgens (Gong et al., 1995).

The MDCK cell line, derived from distal tubule/proximal collecting duct cells from a cocker spaniel in 1958, is an often used, established line of differentiated kidney epithelial cells (see Taub and Saier, 1979). Subsequently, a clonal sub-cell line expressing α_{1B}-adrenoceptors, MDCK-D1, was established (Meier et al., 1983). In the present study we confirm the results of Meier et al. (1983), that the MDCK cell culture (in our case obtained from ATCC in 2003) developed into different sub-cell lines in culture, thereof one (MDCK-RP) expressed the α_{1B}-adrenoceptor. Using qPCR, we detected androgen receptor mRNA in MDCK-D1 cells at a level of approximately 0.3% of the house-keeping gene GAPDH. This is comparable to the level previously found in the rat hypothalamus (Lindblom et al., 2005). The apparent co-expression of α_{1B}-adrenoceptors and androgen receptors in MDCK-D1 cells motivated the study of the effect of nandrolone on the density of α_{1B}-adrenoceptors in the MDCK-D1 cells, thereby investigating whether nandrolone affects the expression of α_{1B}-adrenoceptors directly in isolated cells. However, nandrolone did not decrease the density of α_{1B}-adrenoceptors in the MDCK-D1 or MDCK-RP cells. On the contrary, nandrolone treatment induced an increase in the density of α_{1B}-adrenoceptors in the cultured MDCK-D1 and MDCK-RP cells (Table 3). This indicates that the nandrolone-induced decrease in the density of kidney α_{1B}-adrenoceptors in vivo (Uhlén et al., 2003), as well as the down-regulatory effect of nandrolone on kidney α_{1B}-mRNA observed in the present study, does not have a corresponding functional counterpart in the MDCK cell model.

The rat kidney expresses both α_{1A}- and α_{1B}-adrenoceptors (Mugisha et al., 2002). One possible reason why we could not detect a decrease of [^{3}H]-prazosin binding in the nandrolone-treated MDCK cells could be if a population of the α_{1A}-subtype got up-regulated while the α_{1B}-subtype got down-regulated. Therefore, verification that only the α_{1B}-subtype of α_1 adrenoceptors is present in nandrolone-treated MDCK-D1 and MDCK-RP cells was warranted. The competition experiments with the subtype-selective compounds 5-methyl-urapidil and BMY7378 resulted in virtually identical monophasic curves in membranes from versus nandrolone-treated cells (Fig. 1C–D), and as discussed in Results, the affinities (Table 4) confirmed that solely the α_{1B}-adrenoceptor subtype was present in the MDCK cells.

The molecular mechanisms underlying the nandrolone-induced down-regulation of the density of α_{1B}-adrenoceptors in the kidney is not understood. It has been shown previously that androgens induce the transcription of a number of genes in the mouse kidney (Asadi et al., 1994). Our results from the rat kidney in vivo, that nandrolone specifically down-regulates the level of α_{1B}-mRNA, favour the conclusion that anabolic androgenic steroids in some specific kidney cells containing α_{1B}-adrenoceptors decrease the transcription of the α_{1B}-adrenoceptor gene. The down-regulatory effect of nandrolone on α_{1B}-mRNA in the kidney might be mediated by a direct effect of a nandrolone/receptor complex on the gene for the α_{1B}-adrenoceptor, or on the genes for some other transcription factors which in turn would down-regulate the expression of the α_{1B}-adrenoceptor (see Whitfield et al., 1999; Verrijdt et al., 2003). The promoter region of the α_{1B}-adrenoceptor gene is complex (see Gao and Kunos, 1994; Zuscik et al., 1999). Regarding transcriptional regulation of the α_{1B}-adrenoceptor gene, it has been shown that the same transcriptional regulator, nuclear factor 1, may act either as a positive or as a negative regulator of the α_{1B}-adrenoceptor gene, depending on cell-type-specific cofactors (Gao and Kunos, 1999). This complexity was suggested to underlie the tissue-specific regulation of the α_{1B}-adrenoceptor gene.

Screening for kidney cells in which anabolic androgenic steroids down-regulate α_{1B}-adrenoceptors is hampered by the fact that even though α_1-adrenoceptors are present in the

kidney, and there are plenty of kidney cell lines (e.g. human HEK293, human HK-2, monkey COS, canine MDCK, porcine LLC-PKl, murine M-1, rat NRK, rat IMCD, oppossum OK), almost none of these kidney cell lines seems to express substantial amounts of endogenous α_1-adrenoceptors.

The nandrolone-induced decrease of the density of presumably resorption-stimulatory α_{1B}-adrenoceptors in the kidney (Uhlén et al., 2003) is the opposite effect to the expected, considering that anabolic steroids augments blood pressure. A possibility is that α_{1B}-adrenoceptors in the tubuli indeed are down-regulated, but that nandrolone in the kidney, or elsewhere in the body, induces a more dominant hypertensive effect by other mechanisms, for example by stimulating the renin–angiotensin system or endothelin release (Reckelhoff and Fortepiani, 2004). This latter hypothesis includes the possibility that α_{1B}-adrenoceptors would be down-regulated as a counter-regulatory response to nandrolone-induced hypertension.

In summary, in the present study we show that nandrolone decreases the level of mRNA for the α_{1B}-adrenoceptor in some kidney cells (native rat kidneys) but not in others (canine MDCK-D1 cells). In our previous study we found that nandrolone decreased the density of α_{1B}-adrenoceptors in some tissues (rat kidney) but not in others (rat spleen) (Uhlén et al., 2003). Altogether, these results indicate that regulation of the expression of the α_{1B}-adrenoceptor by anabolic androgenic steroids is tissue and cell-type specific.

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