Cross-talk between steroid-receptor-mediated and cell-membrane-receptor-mediated signalling pathways results in the *in vivo* modulation of c-Met and ornithine decarboxylase gene expression in mouse kidney

Magdalena DUDKOWSKA¹, Agnieszka STACHURSKA, Wanda CHMURZYŃSKA, Barbara GRZELAKOWSKA-SZTABERT and Małgorzata MANTEUFFEL-CYMBOROWSKA

Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warsaw, Poland

The cross-talk *in vivo* between two signalling pathways activated by testosterone via intracellular androgen receptor, and induced by damage to renal tubules evoked by anti-folate [*N*¹⁰-propargyl-5,8-dideazafolic acid (CB 3717)] or folate is reported. We show that CB 3717/folate induces the expression of the hepatocyte growth factor (HGF)/c-Met signalling system in injured kidneys in which a significant, but transient, elevation of the HGF mRNA level occurs. It is followed by a severalfold increase in the c-Met transmembrane receptor message that persists for up to 24 h. The c-Met expression is also positively controlled by testosterone, which induces a significant increase in its mRNA level that is abolished by an anti-androgen, casodex. However, when testosterone and anti-folate/folate are administered sequentially, a substantial (3.5–4.0-fold) decrease in the increase of c-Met expression caused by CB 3717/folate alone

occurs. Similarly, testosterone-induced ornithine decarboxylase (ODC) mRNA level and activity are decreased 2.8–7.7-fold when the androgen is applied together with CB 3717. Antagonism between these pathways is also visible under physiological conditions in the kidneys of male mice in which, owing to elevated endogenous testosterone levels, neither the ODC activity nor the mRNA level is induced by anti-folate/folate, whereas the c-Met message response to these drugs is significantly decreased. Our results document a substantial negative regulation of c-Met and ODC gene expression as a result of the cross-talk between testosterone-activated and HGF-activated pathways and suggest a sex-differentiated response to injury of mouse kidneys.

Key words: androgen receptor, anti-folate, folate, hepatocyte growth factor, testosterone.

INTRODUCTION

The metabolic state and physiological response of a cell or tissue are controlled by a diverse set of extracellular signals such as growth factors, neurotransmitters, peptide hormones and lipophilic hormones acting via their cell-membrane and nuclear receptors. The binding of signalling substances to their receptors activates specific signal transduction pathways that, in turn, modulate the transcription of a target gene or gene network in a temporally defined sequence in a cell-specific or tissue-specific fashion. It has become widely recognized that diverse signalling pathways do not act independently but intersect and interact at different levels, being subject to mutual cross-modulation.

The cross-talk between signal transduction pathways triggered at the plasma membrane and intracellularly seems to be especially interesting. The mechanism of signal transduction by nuclear receptors is far more complex than it seemed a few years ago. Most nuclear receptors are not only ligand-dependent transcription factors activated exclusively by their lipophilic ligands (the classic model), as was generally accepted for years, but they can be also alternatively activated in the absence of their cognate ligands by a variety of extracellular signals from the cell surface [1]. Hydrophilic signalling substances can also positively or negatively modulate the transcription of target genes induced by activated nuclear receptors that can thus be considered the integrators of distinct signalling pathways [2,3].

Previously we have documented the cross-talk between the androgen and catecholamine signalling pathways in hypertrophic kidney [4] as well as the interconnection between the cat-

echolamine-activated and anti-folate-activated pathways in hyperplastic kidney [5]. The modulation of ornithine decarboxylase (ODC) activity and its transcript level in catecholamine-depleted kidneys pointed to synergistic interactions between the signalling pathways examined.

The significant increase in expression of the gene for ODC, encoding a key enzyme in polyamine biosynthesis, is a characteristic feature of androgen-induced and anti-folate/folate-induced hypertrophic and hyperplastic mouse kidney respectively, the although signalling pathways involved in ODC induction differ considerably in both experimental models. In contrast with the well-established androgen receptor (AR)-mediated induction of ODC by testosterone in mouse kidney, the signalling pathway activated by anti-folate [*N*¹⁰-propargyl-5,8-dideazafolic acid (CB 3717)] has not been studied in this organ until now. It is known that CB 3717 and folate, when applied at a high dose, induce renal damage owing to their precipitation at physiological pH in renal tubules [6,7]. This acute renal injury is followed by tubule epithelium repair and regeneration. The observed proliferative response evoked by anti-folate [8] and folate [9] is well correlated with ODC activity induced to the same degree and with identical kinetics by both drugs [10].

Although multiple growth factors participate in such a complex process as the regeneration of an injured organ, hepatocyte growth factor (HGF) and its c-Met tyrosine kinase receptor seem to have a pivotal role in renal regeneration after acute injury [11]. HGF, which is identical with scatter factor, is a pleiotropic mesenchymal-derived growth factor implicated in the control of several biological processes such as embryogenesis, cell growth,

Abbreviations used: AP-1, activator protein 1; AR, androgen receptor; CB 3717, *N*¹⁰-propargyl-5,8-dideazafolic acid; c-Met, receptor of HGF; HGF, hepatocyte growth factor; ODC, ornithine decarboxylase.

¹ To whom correspondence should be addressed (e-mail magdap@nencki.gov.pl).

wound healing and organ regeneration [12,13]. In the injured kidney, HGF, via its cell-membrane c-Met tyrosine kinase receptor, acts as morphogen and mitogen to reconstruct the renal tubule epithelium and to accelerate recovery from acute renal failure [9,14,15]. In contrast with HGF, c-Met is induced exclusively in the damaged organ [9,15] and, as recently postulated, has an important role in the specific targeting of HGF to the injured tissue [9].

Here we present results on the up-regulation *in vivo* of the expression of c-Met receptor and its ligand HGF in hyperplastic kidney injured with anti-folate and with folate. Furthermore, we document cross-talk between the testosterone-activated and anti-folate/folate-activated pathways in both female and male mouse kidneys, in which testosterone is of exogenous and endogenous origin respectively.

MATERIALS AND METHODS

Experimental

Swiss mice of both sexes (2.5–3 months old) were injected intraperitoneally for 24 h (or for the duration indicated) with a quinazoline anti-folate, CB 3717, or folate (300 mg/kg or 250 mg/kg respectively), dissolved in PBS with the addition of a few drops of 1 M NaOH to obtain a final pH of 9–9.5 (controls received PBS only). Female mice were also treated with testosterone (5 days, 125 mg/kg subcutaneously) dissolved in soybean oil (controls received soybean oil only) or with testosterone and either CB 3717 or folate. The experimental details are given in the legends to the appropriate figures and tables. An anti-androgen, casodex (40 mg/kg subcutaneously), dissolved in soybean oil, was injected 1 h before testosterone or 1 day before CB 3717 administration, unless indicated otherwise. The mice were killed by cervical dislocation; the kidneys were removed, weighed, cut into several pieces and used for enzyme and RNA determinations.

Enzyme activity and Northern blot analysis

The kidney pieces were immediately homogenized in an appropriate buffer and processed for ODC activity determination as described previously [16] or were frozen at -70°C and processed later for Northern blot analysis. Total RNA was isolated, subjected to gel electrophoresis and blotted on nylon membranes; the levels of chosen mRNA species were then estimated as described [4]. The probes used were *Eco*RI linearized pODC 934 plasmid containing a 1.4 kb fragment of mouse ODC cDNA, a 1.4 kb *Eco*RI insert of pRBC1 plasmid coding fragment of rat HGF cDNA [17] and a *Bst*XI insert of pRmet2 plasmid coding an 842 bp fragment of rat c-Met cDNA [18]. ODC, HGF and c-Met mRNA levels were quantified by densitometric scanning of the autoradiograms with a Molecular Dynamics Personal Laser Densitometer (ImageQuant software) and corrected for total RNA loaded on the gels as determined by ethidium bromide staining of 28 S rRNA. The protein concentration was estimated by the method of Lowry [19]. Data were analysed statistically by the Mann–Whitney test.

Chemicals

All chemicals purchased from commercial sources were of analytical grade. Testosterone was obtained from Polfa (Jelenia Góra, Poland). Rediprime (Random Primer Labeling), Hybond-N, Hyperfilm-MP, [α - ^{32}P]dCTP (110 TBq/mmol) and D,L-[1- ^{14}C]ornithine dihydrochloride (2.15 GBq/mmol) were obtained from Amersham (Little Chalfont, Bucks., U.K.). CB 3717 and casodex were generously provided by Zeneca Pharmaceuticals

(Alderley Park, Macclesfield, Cheshire, U.K.). Probes for HGF, c-Met and ODC were kindly provided by Dr T. Nakamura (Osaka University Medical School, Osaka, Japan), Dr Y. Liu (Brown University School of Medicine, Providence, RI, U.S.A.) and Dr L. Kaczmarek (Nencki Institute of Experimental Biology, Warsaw, Poland) respectively.

RESULTS

HGF and its receptor (c-Met) mRNA species are induced in mouse kidney injured with CB 3717 and with folate

The level of renal HGF mRNA was examined to determine whether the HGF signalling pathway is activated at the cell surface by anti-folate concomitantly with the induction of ODC gene expression and the subsequent regeneration of CB 3717-injured, hyperplastic kidney [8]. HGF mRNA, hardly detectable in the control kidneys, increased after anti-folate injury (Figure 1). It was up-regulated 7-fold 3–6 h after injury; at 24 h an up to 2-fold augmentation of HGF transcript level could be still observed. A similar pattern of HGF mRNA induction was also found in the folate-injured kidneys (results not shown).

Both drugs examined, CB 3717 and folate, that evoked renal damage similarly increased c-Met mRNA level (Table 1). The

Figure 1 Time-course of CB 3717-induced renal HGF mRNA level as demonstrated by Northern blot analysis

Each lane represents RNA from an individual mouse; each experimental group consisted of three to five mice.

Table 1 Comparison of the effect of CB 3717 and folate on renal c-Met mRNA level in female mice

Results are expressed as means $\pm$ S.D.; the numbers of mice are given in parentheses. The data were processed by the Mann–Whitney test; *P* values were calculated compared with the appropriate control; *P'* compares folate-treated mice with CB 3717-treated mice. Abbreviation: n.s., not significant.

Time (h)	Relative values of c-Met mRNA				
	CB 3717	<i>P</i>	Folate	<i>P</i>	<i>P'</i>
0	1.00 $\pm$ 0.36 (10)	—	1.00 $\pm$ 0.41 (9)	—	—
1	2.55 $\pm$ 0.85 (4)	<0.01	2.04 (2)	—	—
3	3.14 $\pm$ 1.32 (3)	<0.01	4.08 $\pm$ 2.36 (3)	<0.01	n.s.
6	2.24 $\pm$ 0.29 (3)	<0.01	7.28 $\pm$ 4.52 (3)	<0.01	0.05
24	6.87 $\pm$ 3.78 (15)	<0.0001	7.33 $\pm$ 2.65 (6)	<0.001	n.s.

Figure 2 Effect of CB 3717/folate and/or testosterone on renal c-Met mRNA level in female mice (A, B) and in male mice (C) as demonstrated by Northern blot analysis

Testosterone was injected for 5 days, folate and CB 3717 for 1 day, and casodex 1 h before testosterone administration. In (B), mice pretreated with testosterone for 4 days were injected with folate for a further 1 day. Each lane represents RNA from an individual mouse. Relative values of c-Met mRNA compared with control (means $\pm$ S.D.), the number of mice in each experimental group (in parentheses) and *P* values (compared with control or as indicated) are shown beneath the appropriate lanes.

Table 2 Down-regulation of renal ODC activity as a result of cross-talk between pathways induced by testosterone and by CB 3717/folate

Testosterone was injected for 5 days, CB 3717 and folate for the durations indicated. The results are expressed as means $\pm$ S.D.; the numbers of mice are given in parentheses. The data were processed by Mann–Whitney test; *P* values were calculated compared with testosterone-treated mice.

Treatment	ODC activity (%)	<i>P</i>
Testosterone	100.00 $\pm$ 18.79 (13)	–
Testosterone + CB 3717, 1 day	26.73 $\pm$ 9.99 (8)	< 0.0001
Testosterone + CB 3717, 6 days	28.35 $\pm$ 11.17 (8)	< 0.0001
Testosterone + folate, 1 day	13.46 $\pm$ 2.21 (10)	< 0.0001

kinetics of induction of c-Met mRNA was different from that of the HGF message, especially in the folate-injured kidneys. Thus c-Met mRNA increased gradually up to 7-fold during the first 6 h after injury and remained at this elevated level for up to 24 h (Table 1, Figure 2A).

AR-mediated signalling is involved in the up-regulation of c-Met expression

In testosterone-evoked hypertrophic kidney, under conditions of maximal induction of ODC by the androgen (see the Materials and methods section), a distinct, statistically significant 2.2-fold increase in c-Met mRNA was observed (Figure 2B). This testosterone-induced increase in the c-Met transcript was completely abolished by an anti-androgen, casodex, to a level statistically indistinguishable from the control (Figure 2B).

The elevation of the c-Met message level by testosterone was confirmed by the comparison of renal c-Met mRNA in female and male mice (Figure 2C). In males the c-Met RNA content was 3.7-fold higher than in females; this is correlated with the level of endogenous testosterone in both sexes. Collectively, these results

suggest the involvement of the AR signalling pathway in the induction of c-met expression in mouse kidney.

Cross-talk between testosterone-activated and anti-folate/folate-activated signalling pathways results in negative modulation of renal c-Met expression

In female mice the pronounced induction of the c-Met message by anti-folate as well as folate, 7-fold at 24 h (Table 1, Figure 2A), was decreased to less than 2-fold when folate (Figure 2B) or CB 3717 (1.96 ± 0.79 ; *P* < 0.01 compared with CB 3717, *n* = 4) application was preceded by testosterone. It therefore seems that the activation of both AR-mediated and HGF-receptor-mediated pathways by testosterone and by anti-folate/folate respectively results in a 3.5–4.0-fold down-regulation of c-Met expression induced by CB 3717 or folate.

Consistent with these results was a significantly (*P* < 0.01) decreased response of renal c-Met expression to CB 3717 and folate in males in comparison with females. Thus, as Figure 2(C) shows, the c-Met mRNA level in males was less than doubled by treatment with anti-folate or folate. Interestingly, exactly the same degree of induction of c-Met expression was evoked by CB 3717 as well as by folate in the kidneys of testosterone-treated female mice (Figure 2B).

Cross-talk between testosterone-activated and anti-folate/folate-activated signalling pathways results in negative modulation of renal ODC expression in female mice

As shown previously, the negligible (in female mice) renal ODC activity can be induced 870-fold by the androgen testosterone and to a smaller extent (up to 75-fold) by treatment with CB 3717 or folate [8,10,20]. However, when the two inducers testosterone and CB 3717/folate were given together for 5 days or 1 day respectively (at concentrations evoking the highest ODC induction), the ODC activity was decreased to 27% (or 13% for

Figure 3 The comparison of the cross-talk effect between testosterone-induced and CB 3717-induced pathways (A) or testosterone-induced and folate-induced pathways (B) on renal ODC mRNA level in female mice as demonstrated by Northern blot analysis

Testosterone was injected for 5 days, CB 3717 or folate for 1 day. When both compounds were applied, CB 3717 or folate were injected for 1 day to the mice already pretreated for 4 days with testosterone. Each lane represents RNA from an individual mouse. Relative values of ODC mRNA compared with control (means ± S.D.), the number of mice in each experimental group (in parentheses) and *P* values (compared with control or testosterone as indicated) are shown beneath the appropriate lanes.

folate) of the activity induced by the androgen alone (Table 2). This down-regulation of ODC activity did not depend on the sequence of application of the inducers because a similar, statistically highly significant effect was observed when the anti-folate was given first, 1 day before testosterone (that is given for 6 days) (Table 2). Thus the androgen-mediated and anti-folate/folate-mediated pathways are not independent but interact mutually to regulate ODC activity negatively.

The decrease in testosterone-induced ODC activity by anti-folate was less pronounced when both compounds were injected simultaneously for 5 days (results not shown). It became statistically non-significant when ODC induction by testosterone was submaximal (the time of testosterone treatment being shortened from 5 days to 1 day or its dosage being lowered from 125 to 30 mg/kg).

The antagonism between the androgen and anti-folate or folate in the regulation of ODC gene expression was also visible at the level of the ODC transcript (Figure 3). Thus the ODC mRNA content, augmented approx. 10-fold by testosterone, decreased 3.3-fold and 2.3-fold when CB 3717 (Figure 3A) or folate (Figure 3B) respectively was applied subsequently to the androgen treatment. In fact, this low ODC transcript level became similar and statistically non-significant compared with that evoked by anti-folate or folate when given alone.

Effect of the anti-androgen casodex on anti-folate-induced ODC activity

To examine whether and to what extent the AR was involved in the induction of ODC activity by CB 3717, a set of experiments with the use of the anti-androgen casodex was performed. Under all experimental conditions tested, casodex administered once or twice, namely 1 h or 25 h and 1 h or 5 days and 2 days before CB 3717, produced a small (20–25%) but statistically significant ($P < 0.05$) decrease in anti-folate-induced ODC activity. These results contrast with the testosterone-induced ODC activity that

Figure 4 Comparison of renal ODC mRNA level in females and males and the effect of casodex (A) and on renal ODC mRNA induction by folate in males (B) as demonstrated by Northern blot analysis

Each lane represents RNA from an individual mouse; each experimental group consisted of three to six mice. The relative value of ODC mRNA for control males compared with control females was 3.89 ± 1.56 (mean ± S.D.). Other results are shown in Table 3.

is abolished 87% by casodex [5]. Overall this suggests that at least two signalling pathways, one that is AR-independent and a minor one that is AR-dependent, mediate the induction of ODC by CB 3717.

CB 3717 and folate do not induce renal ODC expression in male mice

Owing to the elevated level of endogenous testosterone, male kidneys, as the androgen-responsive organ, were characterized by a 26-fold higher basal level of ODC activity [16] and a nearly

Table 3 Effect of CB 3717 and folate on renal ODC activity and ODC mRNA level in the absence and the presence of the antiandrogen casodex in male mice

CB 3717 (300 mg/kg) and folate (250 mg/kg) were injected for 1 day, casodex (40 mg/kg) for 2 days. The results are expressed as means $\pm$ S.D.; the numbers of mice are given in parentheses. The data were processed by Mann–Whitney test; *P* values were calculated compared with control, *P'* values were compared with casodex-treated mice. Abbreviation: n.s., not significant.

Treatment	ODC activity (nmol/h per mg of protein)	<i>P</i> , <i>P'</i>	ODC mRNA (relative value)	<i>P</i> , <i>P'</i>
Control	10.11 $\pm$ 5.86 (10)	—	1.00 $\pm$ 0.14 (3)	—
CB 3717	10.01 $\pm$ 4.85 (11)	n.s.	(No data)	
Folate	10.57 $\pm$ 4.14 (12)	n.s.	0.82 $\pm$ 0.38 (6)	n.s.
Casodex	0.87 $\pm$ 0.82 (10)	<i>P</i> < 0.01	0.19 $\pm$ 0.05 (5)	<i>P</i> < 0.01
Casodex + folate	9.28 $\pm$ 3.17 (8)	<i>P'</i> < 0.0001	0.69 $\pm$ 0.36 (6)	<i>P'</i> < 0.01

Figure 5 Response of renal ODC expression to folate is negatively correlated with the degree of activation of the testosterone signalling pathway

The response to folate was examined in succeeding experimental groups (columns A–E) with increasing ODC specific activity determined in the absence of folate (nmol/h per mg of protein, means $\pm$ S.D.); columns A, females, 0.22 $\pm$ 0.14; columns B, casodex-treated males, 0.87 $\pm$ 0.82; columns C, males, untreated 10.11 $\pm$ 5.86; columns D, testosterone (30 mg/kg)-treated females, 25.63 $\pm$ 15.70; columns E, testosterone (125 mg/kg)-treated females, 191.17 $\pm$ 35.93. ODC response to folate is expressed as the ratio of the mean ODC activity (upper panel) or the mean ODC mRNA level (lower panel) determined in folate-treated (1 day) compared with folate-untreated mice; each experimental group consisted of 3–12 mice. Symbol: —, not tested.

4-fold increased ODC transcript level than females (Figure 4A). Unlike females, renal ODC expression (neither activity nor mRNA level) was increased by anti-folate or by folate in males (Table 3, Figure 4B). Casodex, used to inactivate the testosterone-mediated pathway, substantially decreased the basal ODC activity (11.6-fold) and ODC mRNA (5.3-fold). In consequence, in male mice pretreated with this anti-androgen before folate, a highly significant increase occurred in renal ODC activity (10.7-fold) and its transcript level (3.6-fold) (Table 3). These results show that under the conditions of an inactivated testosterone signalling pathway, folate can become in males, as in females, a potent inducer of renal ODC expression.

Modulation of ODC gene expression by folate is negatively correlated with the testosterone-induced signalling pathway

The results of the interaction and mutual dependence of the two signalling pathways studied that regulate ODC gene expression are summarized in Figure 5. As shown, ODC expression was differently affected by folate (or anti-folate; results not shown) depending on the activation of the testosterone-mediated pathway. The degree of activation of this pathway was reflected by the ODC activity (up-regulated by testosterone) determined in the absence of folate. Thus, concomitantly with the increasing ODC activity the effect of folate on ODC expression (activity and mRNA level) changed gradually from dozens-fold induction to pronounced inhibition (Figure 5). It therefore seems that the response of ODC to folate is negatively modulated by the testosterone-dependent signalling pathway.

DISCUSSION

The cross-talk between signalling pathways activated by the two external signals, testosterone and HGF, via an AR and a c-Met receptor respectively is the main issue addressed in the present study. This interaction results in the negative modulation of the two genes examined, *ODC* and *c-met*, when investigated *in vivo* with experimental models of testosterone-induced hypertrophic and CB 3717-induced hyperplastic mouse kidney.

The identification of an unknown signalling substance and its receptor, activated in the kidney by treatment with CB 3717, seemed to be the obligatory first step of our study. The effect of CB 3717 on ODC gene expression seems to be an indirect one, because it is known that this anti-folate, similarly to folate, precipitates in the renal tubules and injures tubular epithelial cells, which is followed by their proliferative response [6–8].

A potent pleiotropic cytokine, HGF, and its specific c-Met tyrosine kinase receptor control multiple biological events including organ regeneration after acute injuries [9,11–15]. Although diverse growth-factor–receptor systems participate and act in concert in such a complex process as the regeneration of the injured kidney [21,22], the important role of HGF as a renotropic factor in this process is widely accepted. Recently, in our paper on the participation of catecholamines in the induction of ODC in hyperplastic mouse kidney, we hypothesized that HGF/c-Met signalling might be activated by treatment with CB 3717 and possibly involved in renal ODC induction and in the regeneration of CB 3717-damaged mouse kidney [5]. Here we demonstrate that CB 3717 does indeed significantly enhance the expression of this growth factor and its c-Met receptor. We also document a similar time-course and up-regulation of the transcript level of HGF/c-Met system in the case of mouse kidney injured with CB 3717 and with folate, thus confirming the recent report of Liu et al. [9] on the effect of folate on HGF signalling in rat kidney. The increase in HGF and c-Met receptor expression precedes the rise in DNA synthesis observed in kidneys damaged by CB 3717 [8] and by folate [9].

This documented role of HGF/c-Met signalling in kidney injured with anti-folate or with folate is consistent with the present knowledge on the complex regulation of expression of the genes encoding HGF and c-Met. Several cytokines and growth factors, including interleukin-6, markedly increase HGF and/or c-Met expression *in vitro*. Interleukin-6, which is known to be induced in the injured tissue [23], can stimulate the expression of HGF as well as of c-Met [18,24], in agreement with the presence of the multiple interleukin-6 response elements in the promoter region of genes encoding both HGF and c-Met [23,25]. Moreover, HGF, as one of the most potent inducers of its own receptor [9,24], might be responsible, together with other factors, for the up-regulation of c-Met expression *in vivo* in the injured kidney.

The response of c-Met expression to steroids is less clear. To our knowledge, hormone response elements and androgen response elements in particular were not found among the numerous potential regulatory elements of the mouse *c-met* promoter [25,26]. In prostate the possible links between HGF/c-Met expression and androgen are not explicit [27,28]. When examined *in vitro*, oestrogen and several other steroids by themselves did not significantly alter c-Met expression [24]. So far, *c-met* has not been considered a testosterone-responsive gene induced in androgen-evoked hypertrophic kidney. Our finding that testosterone significantly augments the expression of c-Met in the mouse kidney and that this increase is prevented by the specific anti-androgen casodex are new observations. It certainly deserves further study; however, it should be noted that these results have already provided important confirmation, namely that the renal c-Met transcript level in mouse males with a substantially higher endogenous androgen level is nearly 4-fold higher than in females.

In contrast with *c-met*, various hormone response elements were identified in the mouse *HGF* gene promoter [23]. Their presence implies that HGF expression might be regulated by steroid hormones. Indeed, an induction of HGF transcript level by oestrogen was reported in mouse ovary [29]. One can therefore speculate that the observed positive response of the renal c-Met message to the androgen is not a direct one but occurs via HGF, a known inducer of its receptor, and possibly via other growth factors or cytokines that up-regulate c-Met expression and are responsive to testosterone. Ongoing experiments on the effect of testosterone on HGF expression in CB 3717-injured kidney should answer this question.

Different transcription factors seem to be involved in the induction of expression of the genes encoding c-Met and ODC in the kidneys under conditions of increased HGF expression due to the organ injury. Stimulation of activator protein 1 (AP-1) is a key event in the cell response to various growth factors, including HGF. There are recent reports that HGF activates AP-1 in rat hepatocytes [30] and in cell lines [31,32]. Moreover, in Hepa 1-6 cells, transcriptional activation of c-Met by HGF is mediated through AP-1, which is consistent with the AP-1-binding site found within the *c-met* core promoter [31]. These results allow us to suggest that the up-regulated expression of *c-met*, found in hyperplastic kidney induced by CB 3717 or by folate, might also be due to the activation of the AP-1 pathway.

The involvement of AP-1 seems less probable for ODC. This highly regulated gene, which responds to a large number of stimuli affecting cell growth and differentiation, is considered a typical HGF-inducible gene [32,33]. Despite the presence of a few AP-1-like sites in its promoter [34], regulation by AP-1 of the gene for ODC remains controversial [34–36]. In contrast, it is well established that ODC is transcriptionally regulated by Myc, being one of the first identified Myc target genes [34,37]. The

finding that Myc expression is up-regulated by HGF [33] argues strongly for the involvement of Myc in the modulation of ODC expression in CB 3717/folate-injured kidneys. We therefore suggest that the above Myc-mediated pathway is the major, AR-independent, one, of the two suggested signalling pathways involved in the induction of renal ODC expression by HGF. A minor, AR-dependent, pathway, sensitive to the anti-androgen casodex might be connected with the specifically altered phosphorylation of AR and/or its co-activator(s) by HGF/c-Met downstream activated protein kinases. Similar ligand-independent activation of AR by extracellular signalling molecules including growth factors has been reported [38,39].

In testosterone-evoked hypertrophic kidney the induction of expression of the two studied genes, *c-met* and *ODC*, by testosterone is mediated by the AR-dependent signalling pathway because it has been significantly impaired by an anti-androgen, casodex. In contrast with the induction of c-Met expression by testosterone, which has not been reported previously, the increase in ODC activity and mRNA levels in the mouse kidney is well established. Several-fold elevation of the ODC message by testosterone is connected with the augmented transcription rate of the ODC gene and/or transcript stabilization [40]. ODC regulation at the transcriptional level by testosterone is further indicated by the presence of a putative androgen response element in the murine ODC promoter that confers androgen responsiveness on the reporter gene [41].

The interaction between intracellular signal transduction pathways might result either in an enhanced signal or a decreased one. In contrast with synergistic interactions reported previously [4,5], signalling pathways mediated by AR and c-Met receptor interact antagonistically, resulting in a decreased expression of the genes encoding ODC and c-Met. This cross-talk is independent of the origin of the testosterone, being observed both in the kidney of female mice treated with exogenous testosterone and in that of males with a high level of endogenous androgen. The anti-androgen switches off this negative modulation of expression of the genes encoding ODC and c-Met. One of the consequences of the documented cross-talk could be the delayed regeneration of CB 3717/folate-injured kidney in male mice and of androgen-induced hypertrophic kidneys in females. Whether this important suggestion based on the results of our study is justifiable is at present under investigation.

According to our working hypothesis, cross-talk between androgen-activated and HGF-activated signalling pathways in the mouse kidney can be regarded as an interaction between the transcription factors AR/c-Myc and AR/AP-1 for the genes encoding ODC and c-Met respectively. Such interactions of AR with diverse proteins, including transcription factors, are an important means of positive [42] or negative [43,44] regulation of gene expression and modulation of cell response to extracellular stimuli. Another mechanism for the antagonism between testosterone and HGF/c-Met signalling could involve a negative regulation of AR expression by the latter pathway. However, such a mechanism would be tissue-specific because the androgen and HGF do not show an antagonism but rather a synergy in growth stimulation of prostate epithelial cells [45]. Finally, direct interference between the androgen and anti-folate/folate, although impossible to exclude, seems less likely because of the difference in time and mode of the inducer application. Further studies on androgen-inducible but not HGF-inducible gene(s) should help to elucidate the mechanism of interaction between the two signalling pathways.

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