Genet. Res., Camb. (1987), **49**, *pp.* 61–67 *With 2 text-figures Printed in Great Britain*

A new regulatory gene in the histidine decarboxylase gene complex determines the responsiveness of the mouse kidney enzyme to testosterone

RICHARD J. MIDDLETON[1],† S. A. M. MARTIN[2],‡ AND GRAHAME BULFIELD[1]*

[1] *Gene Expression Group, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian, EH25 9PS, U.K.*

[2] *Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JN, U.K.*

(*Received 21 July 1986 and in revised form 24 October 1986*)

Summary

The level of histidine decarboxylase in mouse kidney normally differs between the sexes with females higher than males. In a strain derived from feral Danish mice (DAN), however, both males and females have the same, high, HDC activity due to the males being insensitive to repression by testosterone. Genetic analysis indicates that this insensitivity is caused by a variant allele of a new gene in the histidine decarboxylase gene complex, *Hdc-a*; the *Hdc-a*^{b} allele in C57BL/10 confers high sensitivity to testosterone whereas the *Hdc-a*^{w} allele in the DAN strain confers low sensitivity. In addition, the DAN strain has a novel haplotype for the other three known elements of [*Hdc*]: the allele *Hdc-s*^{d} of the structural gene, the *Hdc-c*^{d} allele of the gene determining enzyme concentration, and the oestrogen-inducible allele *Hdc-e*^{b}.

Introduction

Mouse kidney histidine decarboxylase (HDC; EC 4.1.1.22) is a valuable system for the analysis of mammalian gene regulation. In the kidney, HDC levels are modulated by three hormones: they are inducible by thyroxine and oestrogen and repressible by androgens (Rosengren, 1962; Kahlson & Rosengren, 1968; Grahn *et al.* 1973; Bulfield & Nahum, 1978). In most strains female mice have considerably higher kidney HDC levels than males, owing mainly to the differences in levels of circulating sex hormones (Martin *et al.* 1984). Considerable genetic variation exists in both constitutive enzyme levels and the response of HDC to hormone administration (Martin *et al.* 1984; Martin & Middleton, unpublished).

A gene complex, [*Hdc*], comprising both structural and regulatory elements has been identified; *Hdc-s*, the structural gene for the enzyme, has been mapped to chromosome 2 of the mouse (Martin & Bulfield, 1984*a*). Cosegregating with the structural gene is a regulatory gene, *Hdc-c*, which determines the kidney enzyme concentration (Martin *et al.* 1984). A second regulatory gene modulates the response of HDC to oestrogen (*Hdc-e*; Martin & Bulfield, 1984*b*) and is also part of [*Hdc*]. There are several haplotypes comprising different combinations of alleles of the different components in the gene complex (Martin & Bulfield, 1986), suggesting that the components are genetically independent entities.

In this paper we describe a fourth component of the gene complex, *Hdc-a*, which controls the repression of HDC levels by testosterone. Alleles of *Hdc-a* differ between the inbred strain C57BL/10 and a strain DAN derived from feral mice; the *Hdc-a*^{b} allele in C57BL/10 confers high sensitivity to testosterone whereas *Hdc-a*^{w} allele in the DAN strain confers low sensitivity.

Materials and Methods

(i) *Animals*

The inbred strains of mice C57BL/10ScSn (abbreviated to C57BL/10 throughout) and DBA/2 were obtained from Bantin and Kingman Ltd, Grimston, Hull, U.K. The DAN strain (non-inbred) was derived from a small population of *Mus musculus musculus* (Mus 2) trapped in Denmark; the original stock were a gift from Dr J. P. Hjorth, University of Aarhus, Denmark. The progenitors were segregating for high

* Corresponding author.

† Present address: Department of Biochemistry, University of Birmingham, Birmingham, B15 2TT, U.K.

‡ Present address: Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, U.K.

and low HDC activity in both sexes and were made true-breeding for the high activity phenotype. The HDC linkage testing stock, HLS, was derived from the HT linkage testing stock (the gift of Dr M. Lyon, MRC Radiobiology Unit, Harwell, U.K.). This strain is homozygous for three chromosomes 2 markers *pa* (*pallid*), *a* (*non-agouti*), and *bp* (*brachypody*) and has low (C57BL/10-like) male HDC activity.

(ii) *Hormone implants*

Animals of 10–12 weeks of age were implanted subcutaneously with hormone pellets as described previously (Martin & Bulfield, 1984*a*). Testosterone treated mice were killed after 7 days of implantation, those receiving thyroxine after 10 days and those receiving oestradiol after 14 days, to obtain the maximum effect of each hormone.

(iii) *Enzyme assays*

HDC activity in kidney homogenates was measured by the release of $^{14}CO_2$ from L-[carboxyl-^{14}C]histidine (Amersham International, Amersham, U.K.) as previously described (Martin *et al.* 1984) and enzyme activity is expressed as nmol histidine utilized/min/g wet weight of kidney at 30 °C.

Ornithine decarboxylase (ODC) activity in kidney homogenates was measured by the release of $^{14}CO_2$ from L-[carboxyl-^{14}C]ornithine (Amersham International, Amersham, U.K.). Fresh kidneys were homogenized in 5 x v/w of 0·1 M phosphate buffer pH 6·8 at 4 °C. Homogenates were spun at 1000 g_{av} for 15 min at 4 °C and the supernatant used for ODC activity determinations. The reaction mixture contained 100 μl of supernatant and 150 μl of a substrate mixture containing a final assay concentration of 0·1 mM pyridoxal-5′-phosphate (cofactor; freshly prepared), 2·0 mM ornithine and 10^5 cpm [^{14}C]ornithine in 0·1 M phosphate buffer pH 6·8. After 1 h with gentle shaking at 30 °C, 250 μl of 10% TCA was added and the $^{14}CO_2$ released trapped and counted as described for the HDC assay (Martin *et al.* 1984). ODC enzyme activities are expressed as nmol ornithine utilized/min/g wet weight of kidney.

(iv) *Thermal stability*

Supernatants of kidney homogenates were incubated in a water-bath at 55·0 ± 0·1 °C for varying lengths of time and the residual HDC activity determined (Martin & Bulfield, 1984*a*).

(v) *Immunoprecipitation*

Rabbit anti-foetal rat HDC antiserum (the gift of Dr T. Watanabe, Osaka Medical School, Japan) was used to titrate the concentration of HDC in kidneys. Three pairs of kidneys from each group were pooled and homogenized in 5 x (v/w) 0·1 M phosphate pH 6·8 containing 0·1% sodium azide and homogenates centrifuged at 1000 g_{av} for 15 min at 4 °C and the supernatants used for the assay. Varying amounts of homogenates were added to 1·5 ml Sarstedt centrifuge tubes and made up to 200 μl with 0·1 M phosphate buffer containing 0·1% azide, and 5 μl of antiserum was added to each tube. A control set of tubes was prepared with 5 μl of buffer instead of antiserum. All the tubes were then mixed for 30 min on a gently rotating platform at room temperature and placed at 4 °C overnight. Ten μl of 200 mg/ml *Staphylococcus aureus* protein A (Sigma U.K.) in 0·1 M phosphate buffer was added to each tube and they were shaken on ice for 30 min on a rotating platform. All the tubes were spun at 11000 g_{av} in an MSE microcentaur centrifuge for 5 min and the residual HDC activity in the homogenate assayed. Activity was plotted against increasing volume of supernatant for the constant volume of antiserum, and HDC concentration was determined by regression analysis as the point at which the antibody was saturated.

Results

(i) *The HDC phenotype of DAN mice*

In most strains of mice kidney HDC levels are higher in females than males; for example, C57BL/10 and DBA/2 mice have low and high HDC activity respectively with males a quarter to a tenth of the activity of females. Mice from the feral derived DAN strain differ; both sexes have high (DBA/2 female-

Table 1. *Effect of hormone implantation on histidine decarboxylase activity*[a] *in C57BL/10, DBA/2 and DAN mice*

Strain	Sex	Control	*n*	Testosterone	*n*	Thyroxine	*n*	Oestradiol	*n*
C57BL/10	Female	0·45 ± 0·09	10	0·04 ± 0·01	3	21·8 ± 0·57	10	0·59 ± 0·20	3
	Male	0·03 ± 0·01	4	0·01 ± 0·01	3	0·04 ± 0·01	3	0·03 ± 0·01	3
DBA/2	Female	9·87 ± 0·81	10	0·36 ± 0·02	4	27·04 ± 1·09	3	3·44 ± 0·13	3
	Male	2·05 ± 0·31	10	0·22 ± 0·04	6	2·31 ± 0·42	3	1·64 ± 0·12	6
DAN	Female	7·91 ± 0·71	21	1·59 ± 0·13	8	31·2 ± 2·19	4	8·24 ± 3·42	3
	Male	7·12 ± 0·49	22	1·37 ± 0·06	8	29·9 ± 2·42	3	8·46 ± 0·61	3

[a] Mean ± S.E. expressed as nmol/min/g tissue at 30 °C.

https://doi.org/10.1017/S0016672300026744 Published online by Cambridge University Press

like) HDC activity (Table 1). The DAN phenotype is not due to low male testosterone levels, nor do the males show any significant differences in testes size or fertility when compared with other common strains (R. J. Middleton, unpublished).

(ii) *Effect of testosterone, thyroxine and oestradiol on histidine decarboxylase activity in C57BL/10, DBA/2 and DAN mice*

In C57BL/10 and DBA/2 there are sex differences in HDC levels in untreated animals and sex differences in response to hormones. On the other hand in DAN animals, not only are HDC levels the same in untreated males and females but both sexes respond identically to all hormone treatments (Table 1). Furthermore, testosterone implants repress HDC levels less in DAN males and females than in either sex of the DBA/2 or C57BL/10 strains, illustrating the insensitivity of DAN animals to testosterone. It can also be seen that HDC in DAN males is induced by thyroxine whilst the enzyme in C57BL/10 and DBA/2 males is not. The lack of HDC induction by thyroxine in C57BL/10 and DBA/2 males is probably due to interaction of the hormones with the repression caused by circulating testosterone dominant (Martin *et al.* 1984); the induction of HDC by thyroxine in DAN males also suggests that these animals are insensitive to testosterone.

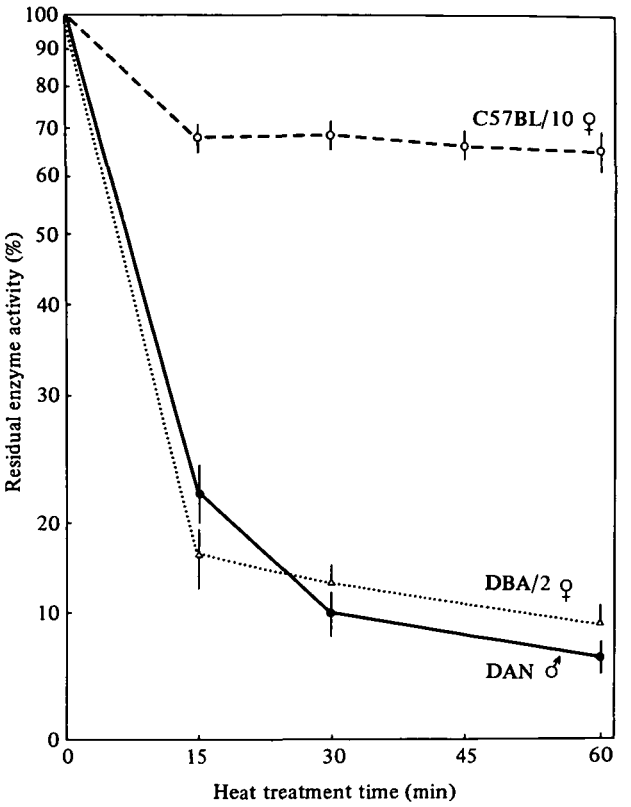

Fig. 1. Heat stability of HDC at 55 °C in three strains of mice, C57BL/10, DBA/2 and DAN. Each point represents the mean and standard error of three determinations.

(iii) *Effect of testosterone on ornithine decarboxylase activity in the three strains*

To investigate whether the insensitivity to testosterone in the DAN strain is a general feature of insensitivity of the kidney to testosterone, another androgen sensitive kidney enzyme was examined. Ornithine decarboxylase (ODC, EC 4.1.1.17) in the mouse kidney is induced by androgens (Pajunen *et al.* 1982) and the activity of this enzyme in untreated and testosterone implanted animals of the three strains is shown in Table 2. The data confirm a previously reported difference between C57BL/6 and DBA/2 in induction of ODC by testosterone (Bullock, 1983); this difference is due to polygenic variation rather than a single gene difference (R. J. Middleton, unpublished data). The DAN strain shows a similar sex difference in ODC levels and responds similarly to C57BL/10 and DBA/2 animals. This indicates that the insensitivity to testosterone of HDC in DAN mice cannot be a general feature of all androgen sensitive genes in the mouse kidney and is probably specific to [*Hdc*].

(iv) *Are the activity differences in the DAN strain due to an alteration in the structure or in the concentration of kidney HDC?*

The high male HDC activity phenotype of DAN animals could be due to a structural difference in the HDC molecule or to differences in HDC concentration. Therefore the thermostability of the DAN HDC was examined as an indicator of structural differ-

Table 2. *Effect of testosterone on ornithine decarboxylase activity*[a] *in C57BL/10, DBA/2 and DAN mice*

Strain	Sex	Control	*n*	Testosterone	*n*
C57BL/10	Female	0·81 ± 0·04	5	85·61 ± 4·78	3
	Male	8·50 ± 1·38	7	78·13 ± 3·23	10
DBA/2	Female	1·02 ± 0·06	3	139·31 ± 15·69	3
	Male	39·18 ± 8·82	7	125·85 ± 20·00	9
DAN	Female	0·55 ± 0·06	3	120·28 ± 6·16	3
	Male	21·76 ± 10·51	5	147·29 ± 5·20	6

[a] Mean ± S.E. expressed as nmol/min/g tissue at 30 °C.

https://doi.org/10.1017/S0016672300026744 Published online by Cambridge University Press

Table 3. *Titration of histidine decarboxylase concentration in the kidneys of DBA/2 and DAN mice*

Strain	Sex	Control HDC activity[a]	Intercept[b]	HDC specific[c] activity
DAN	Male	4·91 ± 0·74	61·8 ± 5·7	1·52
DBA/2	Female	5·43 ± 0·82	51·7 ± 3·1	1·40

[a] Mean ± S.E. of three experiments without added antiserum, expressed as nmol of histidine utilized/min/g wet weight kidney (see Materials and methods).
[b] Intercept, in μl of kidney homogenate, calculated from regression of the amount of homogenate added against HDC activity remaining after incubation with a fixed amount of antiserum; the line crosses the X-axis at the point where the antibody becomes saturated with antigen (see Materials and methods).
[c] Specific activity of HDC molecules is calculated by multiplying the Control activity by the Intercept and dividing by the assay volume (200 μl).

Table 4. *Effect of testosterone on histidine decarboxylase activity[a] in C57BL/10, DAN and (C57BL/10 × DAN) F_1 males*

Strain	Control	*n*	Testosterone	*n*
C57BL/10	0·03 ± 0·01	4	0·004 ± 0·01	3
DAN	7·12 ± 0·49	22	1·37 ± 0·06	8
F_1 (observed)	3·57 ± 0·17	10	0·92 ± 0·08	10
F_1 (expected)[b]	3·58		0·69	

[a] Mean ± S.E. expressed as nmol/min/g tissue at 30 °C.
[b] Calculated on the basis of a codominant mode of inheritance.
For the control group $\chi^2 < 0{\cdot}001$ (N.S); for the testosterone implanted group $\chi^2 < 0{\cdot}076$ (N.S).

ences and immunotitration was carried out to detect differences in enzyme concentration.

It is already known that there is a thermal stability difference in HDC between C57BL/10 and DBA/2 (Martin & Bulfield, 1984*a*); the HDC stability in DAN mice is indistinguishable from the enzyme in DBA/2 mice (Fig. 1) and this is consistent with both strains having the same allele at the structural gene, *Hdc-s*.

Kidney HDC concentration was measured in both the DBA/2 and DAN strains by immunotitration with a fixed amount of anti-HDC antiserum, and calculated from the regression of enzyme concentration against the residual enzyme activity and calculating the intercept on the X-axis (see Materials and methods). A comparison of DAN male mice with DBA/2 female mice, which have similar measurable HDC activities, shows that they also both have similar HDC concentration (Table 3). The high DAN male HDC levels are therefore due to an increase in enzyme concentration. The specific activities of the enzymes can be estimated from these data by multiplying the control activity by the intercept; these also do not differ significantly between the DAN males and DBA/2 females and this is consistent with the HDC molecules being structurally similar, as was concluded from the thermal stability data. These results also imply that the DAN strain has the DBA/2-like *d* allele of the *Hdc-c* gene which regulates enzyme concentration (Martin *et al.* 1984).

(v) *Segregation analysis*

C57BL/10 males have low HDC activity which is repressed to basal levels by testosterone administration; DAN males have high enzyme activity, which is repressed by testosterone but is less sensitive to the hormone. Heterozygous (C57BL/10 × DAN) F_1, males have intermediate levels of HDC activity which are testosterone-repressible to levels intermediate between the two parental strains (Table 4). These results are consistent with additive modes of inheritance for both the difference in constitutive phenotype and the response of HDC to testosterone.

Codominant monogenic inheritance of the untreated phenotypes is confirmed by the distribution of phenotypes observed in the male progeny of a back-cross of the F_1 to C57BL/10 (Fig. 2). HDC activities in F_1 animals are intermediate between those of the parental strains and backcross animals show a bimodal distribution of HDC activities about the F_1 and the C57BL/10 values.

To test for the cosegregation of the high male HDC activity and the low-testosterone-sensitivity phenotypes, backcross males were unilaterally nephrectomized and HDC activity assayed on the removed

https://doi.org/10.1017/S0016672300026744 Published online by Cambridge University Press

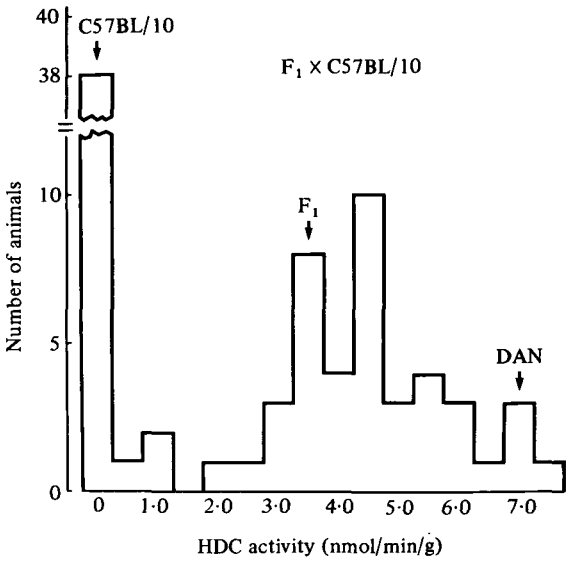

Fig. 2. Segregation analysis of progeny from a backcross of (C57BL/10 × DAN) F_1 to C57BL/10. Each square represents the HDC activity of an individual male. Arrows indicate the mean male HDC activity of the parental strains and their F_1.

kidney. The animals were then implanted with testosterone for a further 7 days, sacrificed and the HDC activity estimated on the remaining kidney. Out of the seven animals which could be unambiguously scored, there were no recombinants between the phenotype of high untreated HDC levels and the phenotype of low sensitivity to testosterone (Table 5).

The data are therefore consistent with segregation of codominant alleles of a single gene which differ between C57BL/10 and DAN and determine the sensitivity of HDC to repression by testosterone. We designate the gene determining testosterone sensitivity of HDC *Hdc-a* in accordance with the rules of the Committee for Standardised Genetic Nomenclature for Mice (Lyon, 1981). The allele $Hdc\text{-}a^b$ (C57BL/10) determines sensitivity of kidney HDC to testosterone and the allele $Hdc\text{-}a^w$ (DAN) determines relative insensitivity to testosterone.

(vi) *Mapping of* Hdc-a

A gene complex, [*Hdc*], comprising a structural gene for kidney HDC and elements regulating its expression has been mapped to chromosome 2 of the mouse (Martin *et al.* 1984, Martin & Bulfield, 1984*a*,*b*, 1986). The HDC linkage testing stock, HLS, contains three chromosome 2 recessive markers, *pallid* (*pa*), *non-agouti* (*a*) and *brachypody* (*bp*) and was used to test for linkage of *Hdc-a* with [*Hdc*]. HLS males have low C57BL/10-like HDC activity and (HLS × DAN) F_1 heterozygous males have intermediate levels ($2{\cdot}19 \pm 0{\cdot}13$ nmol/min/g), indicating a codominant mode of inheritance. Forty seven of the 61 male progeny of a backcross of the F_1 to HLS were of the parental phenotype for the three chromosome 2 markers and *Hdc-a*, whic places *Hdc-a* on chromosome 2 (Table 6); analysis of the 14 recombinants mapped *Hdc-a* relative to *pa* and *bp* with map distances of $2{\cdot}3 \pm 4{\cdot}5$ cM and $23{\cdot}3 \pm 10{\cdot}3$ cM (95% confidence limits), respectively (Table 6). This is consistent with the linkage data for the HDC gene complex and places *Hdc-a* within or very close to [*Hdc*]. The [*Hdc*] gene complex from DAN mice has been made congenic on C57BL/10 (8 backcross generations; R. J. Middleton, unpublished) confirming tight linkage of *Hdc-a* with the other components of [*Hdc*].

Table 5. *Effect of testosterone on histidine decarboxylase activity*[a] *in backcrossed male progeny which had been unilaterally nephrectomized*

Animal No.	HDC activity before implantation	HDC activity after testosterone implantation	Testosterone sensitivity[b]
1	6·35	1·46	Low
2	5·59	1·29	Low
3	4·93	0·84	Low
4	4·43	1·37	Low
5	3·59	0·95	Low
6	2·65	1·60	Low
7	2·04	1·26	Low
8	0·02	0·00	—
9	0·00	0·00	—
10	0·01	0·00	—
11	0·00	0·00	—
12	0·00	0·00	—

[a] HDC activity, expressed as nmol/min/g tissue at 30 °C, of male individuals from a (C57BL/10 × DAN) F_1 × C57BL/10 backcross.
[b] The sensitivity of each individual was assessed by examining the activity after implantation, those in the range of F_1 (Table 4) scored as low, those in range of C57BL/10 are impossible to score due to the low levels of HDC before implantation.

https://doi.org/10.1017/S0016672300026744 Published online by Cambridge University Press

Table 6. *Alleles transmitted by (DAN × HLS) F_1 females backcrossed to HLS males*[a]

Region of recombination	Locus *pa*	Locus *Hdc-a*[b]	Locus *bp*	Number of progeny[c]
Parental	*pa*	*b*	*bp*	22
	+	*w*	+	25
(*pa*, *Hdc-a*) *bp*	*pa*	*b*	+	6
	+	*w*	*bp*	6
pa (*Hdc-a*, *bp*)	*pa*	*w*	+	0
	+	*b*	*bp*	1
Hdc-a (*pa*, *bp*)	*pa*	*w*	*bp*	1
	+	*b*	+	0
				61

[a] The HLS stock has low HDC activity in males and its chromosome 2 genotype is presumed to be *pa Hdc-a*^{b}*bp*/*pa Hdc-a*^{b}*bp*. The DAN genotype is + *Hdc-a*^{w} +/+ *Hdc-a*^{w} +.

[b] Individuals with activities less than 1·00 nmol/min/gm are designated *Hdc-a*^{b}/*Hdc-a*^{b} and individuals of activities 2·00 nmol/min/gm scored as *Hdc-a*^{w}/*Hdc-a*^{b}. There were no progeny which could not be classified unambiguously to either of the groups.

[c] Only male progeny scored.

(vii) *The DAN haplotype of the histidine decarboxylase gene complex.*

DAN represents a fourth haplotype, W, of [*Hdc*] and comprises the alleles *Hdc-s*^{d} (structural locus, Martin & Bulfield, 1984*a*), *Hdc-c*^{d} (kidney enzyme concentration, Martin *et al.* 1984), *Hdc-e*^{b} (response to oestrogen, Martin & Bulfield, 1984*b*, 1986) and *Hdc-a*^{w} (this paper).

Discussion

The HDC gene complex on chromosome 2 in the mouse consists of a structural gene, *Hdc-s*, and two regulatory genes, *Hdc-c* (controlling enzyme concentration; Martin *et al.* 1984) and *Hdc-e* (conferring response to oestrogens; Martin & Bulfield, 1984*b*). We report the discovery of a third regulatory gene, *Hdc-a*, in a feral derived strain of mice (DAN) that determines the response of [*Hdc*] to testosterone and other androgens (R. J. Middleton, unpublished data). Two alleles of *Hdc-a* have been identified: *Hdc-a*^{b}, the allele in C57BL/10 and in most other strains, is responsible for high sensitivity of HDC to testosterone and for the low constitutive male HDC activity phenotype; the *Hdc-a*^{b} allele also prevents induction of HDC by oestrogens or thyroxine in male mice. The other allele, *Hdc-a*^{w}, in the DAN strain, confers low sensitivity to testosterone and the phenotype of high HDC activity in DAN males; furthermore male mice with the W haplotype of [*Hdc*] respond to induction by oestradiol and thyroxine (Table 1). Since the kidneys of DAN mice respond normally to testosterone, as shown by the induction of ODC activity, it is likely that the insensitivity to testosterone conferred by the *Hdc-a*^{w} allele is specific to HDC and does not have pleiotropic effects.

The genetic evidence indicates that the testosterone insensitivity is due to a single gene and that it maps at or near the structural locus. It is therefore part of [*Hdc*] on chromosome 2 and, as the two known alleles are codominant, this implies that *Hdc-a* is *cis*-acting on the structural gene. The presence of this third regulatory gene at or near the structural gene will make the analysis of the molecular architecture of the HDC gene complex of great interest.

In addition to the *Hdc-a*^{w} allele, the other elements of the gene complex in the DAN strain are of interest; the DAN strain is a new haplotype of [*Hdc*]. The thermal stability data suggest that the DAN strain has the *Hdc-s*^{d} structural gene in common with the DBA/2; this is confirmed by the specific activity of the HDC molecules in these two strains being similar, as measured by the immunoprecipitation of the enzymes. The immunological data show that DAN strain animals and DBA/2 females have similar numbers of active molecules of HDC and therefore both have the *d* allele of the *Hdc-c* gene (Martin *et al.* 1984).

These two elements of the DAN gene complex are DBA/2-like, but part of the complex is more like C57BL/10 since HDC is oestradiol inducible, whilst DBA/2 is oestradiol repressible (Martin & Bulfield, 1984*b*). Thus both the C57BL/10 and DAN strains have the *b* allele at the *Hdc-e* locus. Therefore the DAN strain has a novel haplotype: *Hdc-s*^{d}, *Hdc-e*^{b}, *Hdc-c*^{d}, *Hdc-a*^{w}, reinforcing the conclusion that there are at least four discrete recombinable genes in the gene complex (Martin & Bulfield, 1986) and the discovery of this new haplotype will be important in the molecular analysis of [*Hdc*] (see also Pfister *et al.* 1982). It is not possible to decide whether the DAN haplotype has arisen by recombination or by mutation and this will be revealed by molecular analysis.

We would like to thank Kathleen Williamson for technical assistance. This work was supported by an M.R.C. project grant.

References

Bulfield, G. & Nahum, A. (1978). Effect of the mouse mutants *Testicular feminisation* and *Sex reversal* on hormone-mediated induction and repression of enzymes. *Biochemical Genetics* **16**, 743–750.

Bullock, L. P. (1983). Androgen and progestin stimulation of ornithine decarboxylase activity in the mouse kidney. *Endocrinology* **112**, 1903–1909.

Grahn, B., Henningson, S., Kahlson, G. & Rosengren, E. (1973). Alterations in activities of ornithine and histidine decarboxylases provoked by testosterone in mice. *British Journal of Pharmacology* **48**, 113–120.

Kahlson, G. & Rosengren, E. (1968). New approaches to the physiology of histamine. *Physiology Reviews* **48**, 155–196.

Lyon, M. F. (1981). Rules and guidelines for gene nomenclature. In *Genetic Variants and Strains of the Laboratory Mouse* (ed. M. C. Green), pp. 107. New York, Stuttgart: Gustav Fischer-Verlag.

Martin, S. A. M. & Bulfield, G. (1984*a*). A structural gene (*Hdc-s*) for mouse histidine decarboxylase. *Biochemical Genetics* **22**, 645–655.

Martin, S. A. M. & Bulfield, G. (1984*b*). A regulatory locus, *Hdc-e*, determines the response of mouse kidney histidine decarboxylase to estrogen. *Biochemical Genetics* **22**, 1037–1046.

Martin, S. A. M. & Bulfield, G. (1986). Genetic analysis of a new haplotype of the histidine decarboxylase gene complex in C57BL/6 mice. *Genetical Research* **47**, 131–134.

Martin, S. A. M., Taylor, B. A., Watanabe, T. & Bulfield, G. (1984). Histidine decarboxylase phenotypes of inbred mouse strains: a regulatory locus (*Hdc*) determines kidney enzyme concentration. *Biochemical Genetics* **22**, 305–322.

Pajunen, A. E., Isomaa, V. V., Janne, O. A. & Bardin, C. W. (1982). Androgenic regulation of ornithine decarboxylase in mouse kidney and its relationship to changes in cytosol and nuclear androgen receptor changes. *Journal of Biological Chemistry* **257**, 8190–8198.

Pfister, K., Paigen, K., Watson, G. & Chapman, V. (1982). Expression of β-glucuronidase haplotypes in prototype and congenic mouse strains. *Biochemical Genetics* **20**, 519–536.

Rosengren, E. (1962). Formation of histamine in the pregnant mouse. *Experientia* **18**, 176–177.

https://doi.org/10.1017/S0016672300026744 Published online by Cambridge University Press