A new allele of the testosterone-responsive gene, *Hdc-a*, in the histidine decarboxylase gene complex of the mouse

RICHARD J. MIDDLETON,* KATHLEEN WILLIAMSON AND GRAHAME BULFIELD†

Gene Expression Group, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, UK

Summary

In C57BL/10 and the majority of other strains of mice, females have about 20-fold higher kidney histidine decarboxylase levels than males; in DBA/2 mice, however, HDC in females is only 3- to 4-fold higher than males. The low ratio HDC phenotype of DBA/2 animals is due to decreased sensitivity of the HDC gene complex to repression by testosterone in males. From conventional crosses and by the use of the BXD recombinant inbred lines we conclude that the C57BL/10:DBA/2 difference, in testosterone sensitivity of HDC, is due to an allelic difference in the regulatory gene *Hdc-a* of the HDC gene complex, [*Hdc*], on chromosome 2; DBA/2 contains a third allele of this gene, *Hdc-a^d*.

1. Introduction

Histidine decarboxylase (HDC; EC 4.1.1.22) levels in mouse kidney are regulated by several hormones. In most inbred strains of mice HDC is inducible by oestrogens and thyroxine and repressible by testosterone (Ronsengren, 1962; Kahlson & Rosengren, 1968; Grahn *et al.* 1973; Bulfield & Nahum, 1978). The structural gene for HDC, *Hdc-s*, is on chromosome 2 (Martin & Bulfield, 1984*a*) and this, together with three closely linked regulatory genes, make up the gene complex, [*Hdc*] (Martin *et al.* 1984; Martin & Bulfield, 1984*b*; Middleton *et al.* 1987).

Levels of HDC in females from C57BL/10 and most strains of mice are about 20-fold higher than males; in a few strains, however, this ratio differs (Martin *et al.* 1984). The sex differences in HDC activity could be due to two factors; either altered circulating levels of sex hormones between the strains, or differences in sensitivity of the HDC gene complex to hormones. The DAN feral derived strain, for example, has a female:male ratio in HDC activity of approximately unity due to an allelic difference in a testosterone sensitivity gene, *Hdc-a*, which maps at or near the structural gene (Middleton *et al.* 1987).

In this paper we report the analysis of another phenotype with an altered sex difference in HDC expression in the DBA/2 inbred strain where female

mice have HDC levels three to four times that of males. Allelic differences between the C57BL/10 and DBA/2 strains have already been found in three genes of the HDC gene complex; these are the structural gene *Hdc-s* (Martin & Bulfield, 1984*a*) and two regulatory genes: *Hdc-c*, affecting concentrations of HDC (Martin *et al.* 1984) and *Hdc-e*, which controls oestrogen inducibility (Martin & Bulfield, 1984*b*). In particular, HDC levels in DBA/2 females are repressible by oestrogen whereas the enzyme is inducible in C57BL/10 females due to alleles of the *Hdc-e* gene (Bulfield & Martin, 1984*b*), therefore the sex difference in HDC levels between the strains could not be an oestrogen effect.

We present evidence that the sex difference in HDC levels between C57BL/10 and DBA/2 strains is due to allelic segregation of the fourth gene of the complex, *Hdc-a* (the testosterone sensitivity gene). DBA/2 contains a third allele of this gene, *Hdc-a^d*.

2. Materials and Methods

(i) *Animals and hormone treatment*

The four inbred strains of mice, C57BL/10ScSn (abbreviated to C57BL/10 throughout), C57BL/6, DBA/2, C3H/He, were obtained from Bantin and Kingman Ltd, Grimston, Hull, U.K. The fifth strain, DAN, is a small non-inbred population of *Mus musculus musculus* (Mus 2) (the gift of Dr J. P. Hjorth, University of Aarhus, Denmark) and is true breeding for the high-male/high-female HDC activity pheno-

* Present address: Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, UK.
† Corresponding author.

Table 1. *Levels of plasma testosterone[a] in 10- to 12-week old male mice of the C57BL/10 and DAN strains*

Strain	Plasma testosterone[b]	(n)
DBA/2	7·12±0·74	(7)
C57BL/10	3·00±0·77	(13)
DAN	3·51±0·54	(4)

[a] Mean±S.E. expressed as ng/ml plasma.
[b] Levels in DBA/2 are statistically different from C57BL/10. $F^1_{18} = 12·03$, $P < 0·01$; whereas DAN are not different from C57BL/10, $F^1_{15} = 0·12$ N.S.

type (Middleton, Martin & Bulfield, 1987). Mice from the recombinant inbred strains BXD12, BXD16 and BXD18 were the gift of Dr I. E. Lush, Department of Genetics and Biometry, University College London, U.K. The remaining BXD strains were imported from Dr B. A. Taylor, The Jackson Laboratory, Bar Harbor, Maine, U.S.A. Intact male mice were implanted with testosterone at 12 weeks of age and killed 7 days later (Martin & Bulfield, 1984a).

(ii) *Histidine decarboxylase assay*

Histidine decarboxylase activity was assayed in kidney homogenates by measuring the release of $^{14}CO_2$ from L-[carboxyl-^{14}C]histidine (Amersham International, Amersham, U.K.) as previously described (Martin et al. 1984); enzyme activity is expressed as nanomoles of histidine utilized per minute per gram wet weight of kidney at 30 °C.

(iii) *Plasma testosterone levels*

Plasma was collected from male mice at 09.00–09.30 (1 hour after lights had been switched on) to minimize any cyclical changes in testosterone levels. Animals were anaesthetized with ether and blood collected by cardiac puncture using heparin-treated pasteur pip-

ettes. The blood was centrifuged at 1000 g_{av} for 10 min at 4 °C to separate off the plasma which was stored at −20 °C until analysis. Testosterone concentration in the plasma was estimated using an [^{125}I]testosterone kit from RIA (UK) Ltd, Washington, UK. Validation of the use of the kit on mouse samples was made using stripped mouse plasma as a control.

3. Results

(i) *Levels of testosterone in intact males from the C57BL/10, DBA/2 and DAN strains*

To examine the possibility that the phenotypic differences between the strains (Middleton et al. 1987) could be due to differences in circulating levels of testosterone, these were measured in 10- to 12-week-old male mice. DBA/2 animals have over twofold higher levels of serum testosterone than C57BL/10 or DAN animals (Table 1). Therefore the relatively high levels of HDC in DBA/2 male mice cannot be explained by low circulating levels of testosterone. In fact, the high testosterone levels in DBA/2 male mice correlates well with their high kidney ODC levels (Middleton et al. 1987).

(ii) *Testosterone repression of histidine decarboxylase activity by hormone implants*

When intact male mice are implanted with testosterone pellets, their HDC is reduced to very low levels (S. A. M. Martin, personal communication; Middleton et al. 1987). The repression of HDC by testosterone was therefore examined in a number of strains (Table 2). The strains fall into three clear groups: one where HDC is below detectable levels in implanted animals (C57BL/10, C57BL/6), a second where implanted animals still have appreciable HDC levels (DBA/2, C3H/He) and a third where, even after implantation, high HDC levels are recorded (DAN). The HDC levels in DAN and C57BL/10

Table 2. *Effect of testosterone on histidine decarboxylase activity[a] in males from a number of strains of mice*

Strain	Control	(n)	Testosterone implanted	(n)
C57BL/10	0·03±0·01	(6)	N.D.[c]	(6)
C57BL/6	0·02±0·01	(3)	N.D.	(4)
DBA/2	2·05±0·31	(10)	0·22±0·04	(6)
C3H/He	2·59±0·25	(3)	0·29±0·06	(3)
DAN	7·12±0·49	(22)	1·37±0·06	(8)
(C57BL/6 × DBA/2)F₁				
Observed	1·15±0·25	(3)	0·16±0·05	(3)
Expected[b]	1·03		0·11	

[a] Mean±S.E. expressed as nanomoles histidine/min/g tissue at 30 °C.
[b] Calculated on the basis of a codominant mode of inheritance. The values do not differ significantly from the observed values, for the control group χ^2_1 value is 0·015 (N.S.); testosterone implanted group χ^2_1 value is 0·022 (N.S.).
[c] N.D. indicates HDC levels below the level of detection in the assay system.

Table 3. *Segregation of two different testosterone repression phenotypes of histidine decarboxylase between C57BL/6 and DBA/2 using the BXD recombinant inbred strains*

Strains	HDC activity*	(n)	Repression phenotype	Structural gene, Hdc-s†
C57BL/6J	N.D.§	(4)	B	B
DBA/2J	0·22±0·04	(6)	D	D
BXD1	N.D.	(5)	B	B
BXD2	N.D.	(3)	B	B
BXD5	N.D.	(3)	B	B
BXD8	0·41±0·21	(4)	D	D
BXD12	0·42±0·18	(5)	D	D
BXD16	0·44±0·15	(4)	D	D
BXD18	0·55±0·07	(9)	D	D
BXD24	0·33±0·16	(3)	D	D
BXD25	N.D.	(5)	B	B
BXD29	0·49±0·21	(3)	D	D
BXD32	N.D.	(4)	B	B

* In male kidneys after testosterone treatment.
† Data taken from Martin *et al.* (1984).
§ N.D. indicates HDC levels below the level of detection.

animals agree with the previously published strain difference in repression by testosterone and is due to an allelic difference at a regulatory gene, *Hdc-a*, which controls the sensitivity of [*Hdc*] to testosterone (Middleton *et al.* 1987). It is possible that the DBA/C3H phenotype is due to a new allelic difference at *Hdc-a*, which is intermediate in its sensitivity to testosterone. (C57BL/10 × DBA/2) F_1 heterozygotes have HDC activities intermediate between the parental strains in both testesterone-treated and untreated animals (Table 2) and this is consistent with an allelic difference at the *Hdc-a* gene between C57BL/10 and DBA/2.

(iii) *Segregation of testosterone sensitivity amongst the BXD recombinant inbred lines*

Male mice from 11 BXD recombinant inbred lines were implanted with testosterone and classified for testosterone sensitivity (Table 3). The RIs fell into only two discrete parental classes: C57BL/6J-like with zero activity (six strains) or DBA/2J-like with activity above 0·20 units (five strains) and this is consistent with a single gene difference between C57BL/6J and DBA/2J. A testosterone sensitivity gene, *Hdc-a*, has already been identified as part of the HDC gene complex (Middleton *et al.* 1987) and the relative testosterone insensitivity of DBA/2 mice could be due to a third allele of this gene acting on its own or in interaction with the alleles of the *Hdc-c* enzyme concentration gene (which also segregate between C57BL/6 and DBA/2; Martin *et al.* 1984).

From the strain distribution pattern of phenotypes in the BXDs (Table 3) it is clear that the gene causing the DBA/2-C57BL/6 difference maps close to [*Hdc*] (for the 11 BXD strains analysed the 95% confidence limits are between 0·00 and 12·44 cM). The simplest
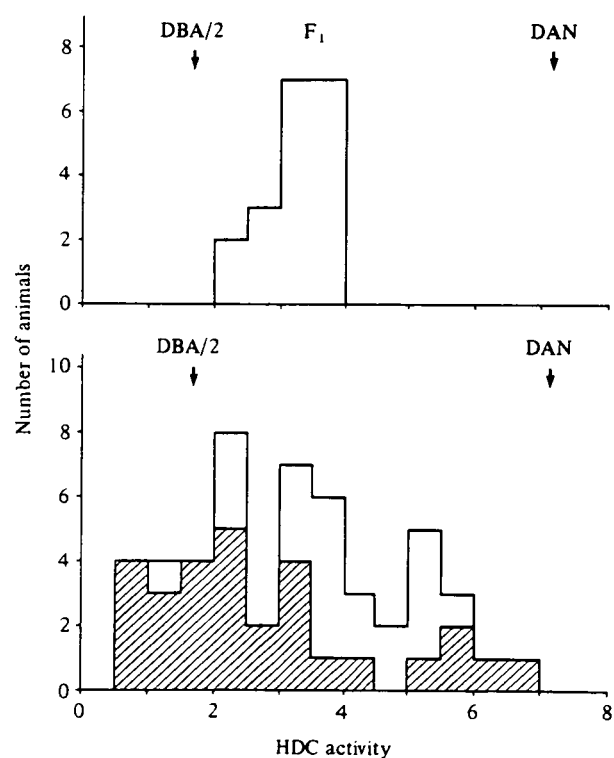

Fig. 1. Segregation analysis: distribution of kidney HDC activities in male animals from the (DBA/2 × DAN)F_1 and a backcross of (DBA/2 × DAN)F_1 with DBA/2. Arrows indicate mean values of parental strains and the F_1. Cross hatching indicates a non-agouti *aa* animal (see text).

explanation of the data from the BXDs is that there is allelic segregation in the testosterone sensitivity gene, *Hdc-a*, within the gene complex, [*Hdc*].

There are already two alleles of this gene: *Hdc-a^w* the testosterone-insensitive allele in the DAN strain and *Hdc-a^b* the testosterone-sensitive allele in the C57BL/10 strain; the allele in the DBA/2 strain is

Table 4. *Distribution of alleles in four strains for all four known genes in the histidine decarboxylase gene complex*[a]

Haplotype	*Hdc-s* allele	Hdc-c allele	Hdc-e allele	Hdc-a allele	Type strain
B.10	b	b	b	b	C57BL/10
D	d	d	d	d	DBA/2
B.6	b	b	d	b	C57BL/6
W	d	d	b	w	DAN

[a] Data on haplotypes from this paper and Martin & Bulfield (1984*a,b*), Martin *et al.* (1984) and Middleton *et al.* (1987).

intermediate in testosterone sensitivity and will be called *Hdc-a*[d].

(iv) *Segregation of testosterone sensitivity in a conventional cross*

DBA/2 mice were mated to DAN mice; these strains have the same alleles at both the *Hdc-s* structural gene and the *Hdc-c* concentration gene but DAN contains the rare *Hdc-a*[w] allele at the testosterone sensitivity gene (Middleton *et al.* 1987). F_1 males had HDC activities approximately intermediate between the two parental strains and these were backcrossed to DBA/2 and HDC activity determined on the progeny. Fifty males were analysed and displayed a bimodal distribution of HDC activities. Although the classes are overlapping, this is consistent with a single gene difference between the two strains, the backcross progeny falling into two groups (Fig. 1).

Also segregating in the cross were the alleles at the *agouti* locus on chromosome 2: nonagouti, *a*, from DBA/2 and A^w from DAN. A contingency chi-square test showed that there was a significant association of the parental DBA-like HDC activity with *a/a* individuals and the high F_1 HDC activity with A^W/a ($\chi^2_1 = 8.57$; $P < 0.005$). This confirms that the difference in testosterone sensitivity between DBA/2 and DAN is due to segregation of a gene on chromosome 2. The *agouti* locus is 16 cM from [*Hdc*] but quantification of the distance of the testosterone-sensitivity gene to *agouti* is not possible because of the partial overlap in phenotypes (Fig. 1) the data are, however, consistent with the gene responsible for the DBA/2 phenotype being at or near [*Hdc*] (Martin *et al.* 1984).

4. Discussion

In most strains of mice females have over 20-fold higher kidney HDC activity than males, whereas in DBA/2 mice the difference is 3- to 4-fold in favour of females and in DAN mice near unity (Martin *et al.* 1984; Middleton *et al.* 1987). The DAN phenotype is due to segregation of alleles at a testosterone-responsive gene *Hdc-a* in the gene complex, [*Hdc*], but

the genetic control of the DBA/2 phenotype has not been analysed previously.

It has already been demonstrated that the C57BL/10 and DBA/2 inbred strains have allelic differences in three genes within the histidine decarboxylase gene complex (Martin & Bulfield, 1984*b*). The sex difference levels in DBA/2 mice is unlikely to be due to an allelic difference of the structural gene, *Hdc-s* (Martin *et al.* 1984), or the concentration gene, *Hdc-c* (Martin & Bulfield, 1984*a*) as these would affect both sexes equally. The third allelic difference at the *Hdc-e* gene (Martin & Bulfield, 1984*b*, DBA/2 is repressed by oestradiol and C57BL/10 is induced by oestradiol) also cannot satisfactorily explain the sex differences in the two strains. The repression of HDC in DBA/2 mice by oestrogens is only expressed at pharmacological levels of oestradiol (R. Webb, personal communication). At physiological levels of oestradiol, DBA/2 seems to behave normally, in that, ovariectomy (i.e. removal of the major oestradiol source) does not result in an increase in HDC levels but lowers them as expected (Martin *et al.* 1984*b*). Furthermore, the other oestradiol repressible strain, C57BL/6, has the same sex difference in HDC levels as C57BL/10 (Martin & Bulfield, 1986). This suggests that the DBA/2 phenotype is due either to low levels of circulating testosterone or that [*Hdc*] in DBA/2 is less sensitive to testosterone.

Serum testosterone levels in DBA/2 males are higher than those in C57BL/10 males (Table 1), therefore the high DBA/2 male levels of HDC cannot be caused by reduced circulating levels of testosterone. The DBA/2 HDC phenotype must therefore be due to a lower sensitivity of this strain to testosterone. This sensitivity cannot be a general property of all testosterone-responsive genes in DBA/2 mice as the testosterone-inducible enzyme ODC has higher levels in DBA/2 animals (Middleton *et al.* 1987) paralleling their high plasma levels of testosterone (Table 1). These results suggest that the testosterone insensitivity of DBA/2 mice is specific to HDC, and this is confirmed by the lower response of HDC levels in DBA/2 males than C57BL/10 males to repression by testosterone implants (Table 2; although it is impossible to quantify the repression in C57BL/10 animals as HDC levels in both treated and untreated

animals are so low). The data do, however, indicate that the histidine decarboxylase gene complex in DBA/2 males is less sensitive to testosterone repression than that in C57BL/10 males.

The sensitivity to testosterone segregates as a single gene amongst the BXD recombinant inbred strains between DBA/2 and C57BL/6; the strain distribution pattern in BXDs is consistent with the difference being at or near the gene complex for HDC on chromosome 2 (Table 3). Further genetic evidence that the gene for the relative testosterone insensitivity of DBA/2 mice is near [*Hdc*], comes from the backcross animals from the DBA/2-DAN cross (Fig. 1) and the association of DBA/2-like phenotype and the *agouti* locus on chromosome 2. This cross also supports the conclusion that the DBA/2 phenotype must be independent of the structural gene *Hdc-s* and the concentration gene *Hdc-c*, as both DBA/2 and DAN carry the same alleles at both these genes (Middleton *et al.* 1987).

The DBA/2 inbred strain has a single allelic difference in a testosterone sensitivity gene which is segregating in crosses with C57Bl/10 and DAN animals and between the BXD RI strains. The simplest explanation is that DBA/2 has a third allele at the testosterone sensitivity locus, *Hdc-a*, which we call *Hdc-a^d*. This explanation would account for the HDC sex ratio phenotype, and the relatively high HDC levels on testosterone implantation observed in DBA/2 mice. It is also likely that the C3H/He inbred strain also carries the *Hdc-a^d* allele as it has the same HDC sex ratio and testosterone repression phenotypes as DBA/2.

It is now possible to assign alleles to all four genes of [*Hdc*] for the four known haplotypes (Table 4). Once the molecular arrangement of the structural gene is known, our knowledge of the genetic elements at this complex and their interactions will become clearer.

References

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

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

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

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

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

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.

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.

Middleton, R. J., Martin, S. A. M. & Bulfield, G. (1987). A new regulatory gene, *Hdc-a*, determines the responsiveness of mouse kidney histidine decarboxylase to testosterine. *Genetical Research* **49**, 61–67.

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

Rosengren, E. (1962). Formation of histamine in the pregnant mouse. *Experientia* **18** (1), 176–177.