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Characterization of third chromosome dominant α-methyl dopa resistant mutants (*Tcr*) and their interactions with *l(2)amd* α-methyl dopa hypersensitive alleles in *Drosophila melanogaster*

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Summary

In *Drosophila melanogaster* two alleles at the *Third chromosome resistance* locus (*Tcr*; 3-39·6) were isolated in a screen of EMS mutagenized third chromosomes for dominant resistance to dietary α-methyl dopa, α-MD, a structural analogue of DOPA. Both alleles of *Tcr* are recessive lethals exhibiting partial complementation. Almost half (48·3%) of the Tcr^{40}/Tcr^{45} heterozygotes die as embryos but some survive past adult eclosion. Both the embryonic lethal phenotype and the adult phenotype suggest that *Tcr* is involved in cuticle synthesis. *Tcr* mutants suppress the lethality of partially complementing alleles at the α-MD hypersensitive locus, *l(2)amd*. The viability of Tcr^{40}/Tcr^{45}, however, is not increased by the presence of a *l(2)amd* allele. The possibility that the *Tcr* and *l(2)amd* mutations reveal a catecholamine metabolic pathway involved in cuticle structure is discussed.

1. Introduction

The metabolism of biogenic amines in insects is an intriguing example of evolutionary conservatism. Not only do these compounds act as neurotransmitters and modulators of adenylate cyclase activity, but they are also essential for the formation and stabilization (sclerotization) of the insect cuticle.

Studies of these compounds and the enzymes involved in their synthesis and degradation, especially in a genetically well characterized organism such as *Drosophila melanogaster*, should contribute not only to our understanding of this complex developmental process but also to our knowledge of catecholamine metabolism.

Numerous enzymes have been implicated in the complex events involved in formation and sclerotization of the insect cuticle (for reviews see Brunet, 1980; Wright, 1987). The best characterized genetically and biochemically is dopa decarboxylase (DDC, EC 4.1.26, 3-4-dihydroxy-L-phenylalanine-carboxylase; for reviews see Wright, 1977, 1987). DDC converts DOPA (3,4-dihydroxy-L-phenylalanine) to dopamine, a precursor of *N*-acetyldopamine and *N*-β-alanyldopamine, two compounds which appear to play a major role in sclerotization (Karlson & Sekeris, 1976; Hopkins *et al.* 1982).

α-methyl dopa (α-MD, α-methyl dihydroxyphenylalanine) is a structural analog of DOPA. When fed to developing larvae of both *D. melanogaster* (Sparrow & Wright, 1974) and *Lucilia cuprina* (Turnbull & Howells, 1980), α-MD is toxic, causing death at the next molt. Both the time of death and the abnormal outer epicuticle in *L. cuprina* fed the inhibitor (Turnbull *et al.* 1980) suggest that lethality is the result of abnormal sclerotization.

Initially it was assumed that α-MD induced mortality was due to inhibition of DDC. Surprisingly, however, screens to recover *D. melanogaster* mutants hypersensitive to α-MD toxicity produced no mutations affecting the *Dopa decarboxylase* (*Ddc*; 2-53·9) locus. Instead they revealed another locus, *amd*, which maps directly adjacent to *Ddc* at 53·9 on the second chromosome (Sparrow & Wright, 1974; Wright *et al.* 1976). Mutants of the *amd* locus, *l(2)amd* alleles, exhibit dominant lethality on low concentrations of α-MD and recessive lethality on media without the inhibitor. Lethality of the homozygotes occurs late in

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embryogenesis and the cuticles of those larvae which hatch have no structural integrity. Some *l(2)amd* heterozygotes are partially complementary, producing morphologically normal adults, but at a lower frequency than expected.

Analysis of the two loci in *D. melanogaster* indicates that the toxic effect of α-MD is due to its interaction with the product of the *amd* locus. The *l(2)amd* mutations have absolutely no effect on the activity or amount of DDC (Sparrow & Wright, 1974; Clark *et al.* 1978). Furthermore, flies with 150% DDC activity not only fail to exhibit increased resistance to the inhibitor but actually have an increased sensitivity to α-MD (Marsh & Wright, 1986). Recent studies (Black *et al.* 1987) have shown that *l(2)amd* mutants lack a compound identified as a catecholamine by low resolution mass spectroscopy and other criteria.

To explore further the *in vivo* effect of α-MD, we have isolated two third chromosome mutations resistant to the inhibitor, *Third chromosome resistant* (*Tcr* alleles), and investigated their interactions with the *amd* locus. The results reported below suggest that *Tcr* and *l(2)amd* mutations may define a previously unrecognized pathway involved in cuticle structure and catecholamine metabolism.

2. Materials and methods

Except where otherwise noted, all gene and chromosome designations are those of Lindsley & Grell (1968).

Media to which α-MD was added was similar to that described by Sparrow & Wright (1974).

(i) *Isolation of third chromosome mutations resistant to α-MD*

bw; *TM3*, *Ser*/*Sb* virgins were mass mated to lethal free *bw*; *st* males which had been mutagenized with ethyl methanesulphonate according to the method of Lewis & Bacher (1968). *bw*; *TM3*, *Ser* males carrying mutagenized *bw*; *st* chromosomes were mated individually to *bw*/*bw pr*; *TM3*, *Sb Ser*/*st* virgins, and the progeny raised on media containing a lethal concentration (0·8 mM) of D,L-α-MD. In 674 of the 1715 crosses, F_2 males were used to resolve for possible mosaics (Jenkins, 1967). All putative mutants were re-tested on the same concentration of the inhibitor.

(ii) *Mapping of two α-MD resistant mutations*

Resistance to lethal concentrations of α-MD of the two strains tested segregated with the mutagenized third chromosomes. A preliminary mapping of Tcr^{45} with a multiply marked third chromosome placed the resistant locus between *hairy* (*h*; 3-26·5) and *thread* (*th*; 3-43·2). A more precise mapping for the resistance of Tcr^{40} was performed using the multiply marked third chromosome *ru h th st cu sr* e^s *ca*. Progeny of the mapping cross were raised on media containing 0·7 mM-D,L-α-MD. As a control, wild-type flies (Oregon-R and Canton-S) were raised on the same media.

(iii) *Effective lethal phase and mutant phenotypes*

In order to determine the effective lethal phase and observe any lethal phenotypes of *Tcr*, reciprocal matings of Tcr^{45} *st*/+ with Tcr^{40} *st*/+ were made. Eggs were collected and placed on square pieces of blotting paper (50 eggs/square). One square was placed in each vial. After 48 h the unhatched eggs were removed and transferred to another piece of blotting paper in a new vial. This procedure allowed more time for the embryos to hatch and prevented other larvae from moving the eggs off the paper. After another 24 h the chorion of the eggs was removed using double stick tape, the eggs mounted and scored for fertilization. The vials were all placed at 25 °C.

The number of pupae was counted and this number subtracted from the number of hatched eggs to determine how many died in the larval stages. Adults were scored for *st* eyes. Dead pupae were dissected from their pupal cases and examined for phenotype.

(iv) *Interaction between the* Tcr *and* amd *loci*

Interaction between *Tcr* and *amd* was determined by examining the effects of a mutant allele of one locus on partially complementing alleles at the other locus. Males of the following genotypes: $l(2)amd^{H1}$ *cn bw*/*SM5*; *TM3*, *Sb Ser*/Tcr^{40} *st* or $l(2)amd^{H1}$ *cn bw*/*SM5*; *Gl*/Tcr^{45} *st* were mated to virgin females that were either *SM5* or *CyO* and heterozygous for another *l(2)amd* allele at 3–5 pairs per vial. Partially complementing *l(2)amd* heterozygotes could be identified by either the absence of *Cy* (from *SM5* or *CyO*) or by homozygous *cn bw* eyes. The presence of a *Tcr* null allele was indicated by the absence of dominant markers *Gl* or *Sb Ser*. The effects of *l(2)amd* alleles on Tcr^{40}/Tcr^{45} complementation were tested with $l(2)amd^{H26}$ *Bl*/+; Tcr^{40} *st*/+ crossed to *bw*/+; Tcr^{45} *st*/+. Reciprocal crosses were used to look for any maternal effects. Eggs from this cross were picked (50 eggs/vial), allowed to develop at 25 °C and scored as for the effective lethal phase egg pick. This protocol was followed to insure uncrowded conditions since the only Tcr^{40}/Tcr^{45} adults recovered have come from uncrowded cultures. The presence of $l(2)amd^{H26}$ was ascertained by the presence of *Bl* since they are closely linked (0·9 map units apart) and Tcr^{40}/Tcr^{45} heterozygotes were scored by their rough eyes; a characteristic of the *Tcr* mutant phenotype.

To determine if any *Tcr* allele induced increase in complementation was dependent upon at least partially functional *amd* gene product, $l(2)amd^{H1}$ *cn bw*/*Sp* bw^D; Tcr^{40} *st*/*TM3*, *Ser* males were crossed to virgins heterozygous for a deficiency, *Df(2L)TW130*, of *amd*.

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Table 1. *Segregation of resistance to* L-α-*MD with the third chromosomes of* Tcr^{40} *and* Tcr^{45} [a]

α-MD (mM)	No. of eggs	*TM3, Ser/Tcr⁴⁰ st* × *TM3, Sb Ser/Tcr⁺ st** [b] Adult genotypes: *TM3, Sb Ser/Tcr⁴⁰ st*	*Tcr⁴⁰ st/Tcr⁺ st**	*TM3, Ser/Tcr⁺ st*
0	249	63	53	62
0·1	276	78	61	2
0·2	286	56	37	0
0·3	287	41	12	0
0·4	121	8	0	0

α-MD (mM)	No. of eggs	*TM3, Sb Ser/Tcr⁴⁵ st* × *TM3, Ser/Tcr⁺ st* [c] Adult genotypes: *TM3, Ser/Tcr⁴⁵ st*	*Tcr⁴⁵ st/Tcr⁺ st*	*TM3, Sb Ser/Tcr⁺ st*
0	271	50	63	63
0·1	257	71	58	26
0·2	241	60	61	7
0·3	265	50	43	0
0·4	178	11	12	0

[a] Data from reciprocal crosses were pooled since no maternal effect was found.
[b] *TM3, Sb Ser/Tcr⁺ st** was a control stock carrying a mutagenized *Tcr⁺ st** chromosome isolated from the same screen and carried through the same crosses as Tcr^{45}.
[c] *TM3, Ser/Tcr⁺ st* was a control stock carrying a non-mutagenized *Tcr⁺ st* and carried through the same crosses as Tcr^{40}.

3. Results

(i) *Recovery of mutations*

Eighty putative, α-MD resistant mutations were found among 1715 ethyl methanesulphonate mutagenized chromosomes. Interestingly, all were recovered from those crosses in which the extra generation to resolve for mosaicism was not included and, as expected, most failed to maintain the resistant phenotype in subsequent tests. Two, Tcr^{40} and Tcr^{45}, were chosen for further investigation as they consistently demonstrated high viability on 0·8 mM-D,L-α-MD, a concentration well above the LD_{100} for wild type.

Table 2. Tcr^{40} st/Tcr^{45} st *effective lethal phase*

Cross[a]: *Tcr⁴⁰ st/+* × *Tcr⁴⁵ st/+*	Dead or eclosed: Embryos (480)	Pupae (366)	Adults (352)
Wild-type	—	2	343
Expected (%) (Expected = 75% of 480 = 360)	—	0·6%	95·2%
Tcr⁴⁰/Tcr⁴⁵	58[b]	11	9
Expected (%) (Expected = 25% of 480 = 120)	48·3%	9·2%	7·5%
Unknown	0	1	0

[a] Data from reciprocal crosses were pooled since no maternal effect was observed.
[b] *Tcr⁻* embryos have a pale cephalopharyngeal apparatus.

Table 1 shows the resistance of the two strains to α-MD and the segregation of this phenotype with the mutagenized third chromosomes. The L form of the inhibitor, which is approximately twice as toxic as the D,L compound (Sparrow & Wright, 1974), was used in this experiment. The LD_{50} for both mutations was found to be above 0·3 mM. The LD_{50} for wild type is less than 0·1 mM.

The mutagenized third chromosomes of both strains were found to behave as recessive lethals on media with or without the inhibitor. Crosses between Tcr^{40} and Tcr^{45} stocks showed that the heteroallelic combination (Tcr^{40}/Tcr^{45}) are poor partial complementors (less than 20% complementation, see Tables 2 and 4) indicating that they are alleles and that recessive lethality is an additional phenotype.

(ii) *Genetic localization*

Preliminary mapping of Tcr^{45} with a multiply marked third chromosome placed the locus between *h* and *th*. A finer mapping was performed for Tcr^{40} by crossing Tcr^{40}/*ru h th st cu sr* e^s *ca* virgins with *ru h th st cu sr* e^s *ca* males on 0·7 mM-DL-α-MD. Control bottles: Oregon R; 10 females and 7 males, and Canton S; 7 pairs of parents, failed to produce progeny at this inhibitor concentration. The mapping cross was set in a total of 12 bottles; one with seven pairs of parents, the remainder with 10 females and 5–10 males, and the progeny scored for *h* and *th*. A total of 1790

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Table 3. *Effects of* Tcr⁻ *alleles on viability of partially complementing* l(2)amd *alleles*

Basic cross: Virgins *l(2)amd⁻/Cy* balancer × *l(2)amd^{H1} cn bw/Cy* balancer; *Tcr⁻ st*/marker males	Expected (%)	
	Control without *Tcr⁻*	Experimental with *Tcr⁻*
l(2)amd^{H82}/l(2)amd^{H1}; Tcr^{40}/+	1·2	111·0
l(2)amd^{H82}/l(2)amd^{H1}; Tcr^{45}/+	2·4	74·1
l(2)amd^{H89}/l(2)amd^{H1}; Tcr^{40}/+	84·0	124·0
l(2)amd^{H89}/l(2)amd^{H1}; Tcr^{45}/+	64·7	69·6
l(2)amd^{H149}/l(2)amd^{H1}; Tcr^{40}/+	22·3	110·9
l(2)amd^{H1}/Df(2L)TW130; Tcr^{40}/+	0	0

progeny were obtained: 1392 h^+ th^+, 310 *h* th^+, 82 h^+ *th* and 6 *h th*. The position of the *Tcr* locus was calculated to be approximately 39·6 by determining the percentage of crossovers between *h* and *Tcr* and multiplying by the standard map distance between *h* (3-29·5) and *th* (3-43·2).

(iii) *Effective lethal phase and mutant phenotype*

The results of the lethal phase experiment indicated that slightly less than half (48·3%) of the *Tcr^{40} st/Tcr^{45} st* individuals die during embryogenesis (Table 2). These heterozygotes lived for 48 or more hours after egg deposition but failed to escape from the egg. They appeared to be mature first instar larvae except that their cephalopharyngeal apparatus was usually underpigmented and they were very slow to darken after death compared to other embryonic lethals.

Those *Tcr^{40} st/Tcr^{45} st* heterozygotes which completed pupariation survived to become pharate adults with 45% of these eclosing from their pupal cases. The adults which eclosed were 7·5% of the expected and usually found dead or dying in the vial food. Adults which were not in the food were usually unable to walk on their tarsi and walked instead on their metatarsi or tibia with uncoordinated leg movements.

Both the pharate adults which failed to eclose and the eclosed adults had a pleiotrophic phenotype. In addition to abnormal walking behaviour, these phenotypes were observed: dark, rough often reduced eyes with black spots; missing bristles, especially from the head; one to all of the ocelli missing; notching of the wings; reduced scutellum; *Minute*-like bristles mixed with wild-type bristles; unexpanded ptilinum; reduced sex combs and a greyish abdomen. The expressivity of these traits was variable and only the rough eyes had full penetrance. Individuals unable to eclose were sometimes successful in splitting open their operculum but unable to crawl out of the pupal case. Others could not open their operculum either because they were too far away from it or because they lacked an adequate ptilinum. A few seemed to completely lack a ptilinum. The pupal cases all had normal coloration and appeared to be appropriately hardened. One female survived to produce two progeny. All but two of the 20 adults and dead pupae were *st* and these two had a dark eye phenotype. *Tcr⁻* flies have dark eyes except when they are also homozygous *st*, in which case they have *st* eyes, indicating that *st* is epistatic to *Tcr* for eye colour.

(iv) *Interaction between* Tcr^{40} *and the* amd *locus*

All partially complementing *l(2)amd* heterozygotes tested showed an increase in viability when either *Tcr^{40}* or *Tcr^{45}* was present (Table 3). The failure to produce any progeny hemizygous for a *l(2)amd* allele (*l(2)amd/Df(2L)TW130; Tcr/+*) when either *Tcr* allele was present indicates that at least a partially functional *amd* protein is required before a *Tcr* allele can suppress the lethality caused by *l(2)amd.*

No increase in complementation between the two *Tcr* alleles was seen when a *l(2)amd* allele was also present. *l(2)amd^{H1}* failed to increase the number of *Tcr^{40}/Tcr^{45}* progeny produced (data not shown) when the crosses were done with 3–5 pairs per vial. Also,

Table 4. *Effects of* l(2)amd^{H26} *on the viability of* Tcr^{40}/Tcr^{45}

		Adult phenotypes[b]		
Fertile eggs	Dead[a] *Tcr⁻* embryos	wild type and *l(2)amd^{H26} Bl*	*Tcr⁻*	*l(2)amd^{H26} Bl; Tcr⁻*
Cross: Virgins *l(2)amd^{H26} Bl/+; Tcr^{40} st/+* × *bw/+; Tcr^{45} st/+* Males				
294	47 (63·9%)[c]	135	4 (10·9%)	1 (2·7%)
Cross: Virgins *bw/+; Tcr^{45} st/+* × *l(2)amd^{H26} Bl/+; Tcr^{40} st/+* Males				
315	15 (19%)	215	7 (17·8%)	7 (17·8%)

[a] *Tcr⁻* embryos have a pale cephalopharyngeal apparatus.
[b] Adults in this table include those individuals that died as pharate adults and those which eclosed from the pupal case.
[c] % of expected.

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l(2)amd^{H26} did not increase complementation between *Tcr*^{40} and *Tcr*^{45} in uncrowded (50 eggs/vial) conditions (Table 4). *l(2)amd* alleles have a maternal effect on *Tcr*^{40}/*Tcr*^{45} embryos, causing an increase in embryonic mortality. The results in Table 4 show that a maternally derived *l(2)amd*^{H26} chromosome increased embryonic mortality approximately three fold (63·9% versus 19% embryonic mortality) and in another cross (data not shown) using *Sco* as a dominant marker, embryonic lethality was also increased approximately three-fold (81·2% versus 29%) due to a maternally derived *l(2)amd*^{H21} chromosome.

4. Discussion

Two third-chromosome mutations, *Tcr*^{40} and *Tcr*^{45}, conferring dominant resistance to dietary concentrations of the enzyme inhibitor α-MD lethal to control stocks were isolated and characterized. The mutations were found to be recessive lethal alleles, defining the *Tcr* locus which was mapped between *h* and *th* at approximately 39·6 on the third chromosome. The effective lethal phase and lethal phenotypes at two stages have been reported as well as the interaction between *Tcr* and a locus which affects sensitivity to α-MD, *l(2)amd*. *l(2)amd* alleles cause a dominant hypersensitivity to α-MD whereas mutations at *Tcr* confer dominant resistance.

A critical period for *Tcr* expression is during embryogenesis. This was determined by the lethal phase experiment from a mating of *Tcr*^{40}/+ with *Tcr*^{45}/+. It was assumed that all progeny with at least one wild type *Tcr* allele survived to the adult stage (75% of the progeny), while those which died were heterozygous for the two *Tcr* alleles. 95·8% of the expected wild-type progeny were recovered as either identifiable dead pupae (0·6%) or eclosed adults (95·2%), indicating that almost all of the dead progeny could be accounted for by the expected number of *Tcr*^{40}/*Tcr*^{45} heterozygotes. The greatest lethality of *Tcr*^{40}/*Tcr*^{45} individuals occurred during embryogenesis when 48·3% of the expected number of *Tcr*^{40}/*Tcr*^{45} heterozygotes died. Most of the dead embryos had an underpigmented cephalopharyngeal apparatus (mouthparts) and were slow to darken after death when compared to other embryonic lethals. An examination of the eggs 48 h after laying showed that the unhatched larvae were active and looked like wild-type mature first instar larvae except for the pale mouthparts. One can infer that the failure of the larvae to hatch is a result of incompletely sclerotized mouth hooks as indicated by the lack of pigmentation.

Lethality during the pupal stage occurs because the *Tcr*^{40}/*Tcr*^{45} heterozygotes cannot eclose. In some instances they were unable to split open their operculum, either because they were too far from it, or because their ptilinum did not expand enough to force it open. Other individuals could open the operculum but were unable to escape from the pupal case. Whether this latter failure is due to a lack of coordinated movement or to a cuticle which is not hard enough to permit them to crawl out could not be determined. When removed from the pupal case, these individuals were capable of movement but it was very spastic. This abnormality may be the result of uncoordinated muscular movements or inability of their legs to support their body.

The adult *Tcr*^{40}/*Tcr*^{45} heterozygotes that did eclose were usually found stuck in the food, dead or dying, but one female lived to produce two offspring indicating that the females are at least partially fertile. The adults as well as the pupae have a pleiotropic phenotype. Although most of the phenotypes might be due to cell death (missing ocelli, missing bristles, notching of the wings, dark spots in the eyes, reduced eyes, reduced scutellum, reduced ptilinum and reduced sex combs), not all can be easily explained that way (dark eyes and the soft cuticle).

The soft adult cuticle and underpigmented embryos whose mouthparts are probably not hard enough to rip through the egg membrane, together with the fact that these *Tcr* mutants were isolated by their dominant resistance to a compound (α-MD) known to interfere with cuticle synthesis (Turnbull *et al.* 1980), strongly suggests that the *Tcr* gene is required for normal cuticle production. The two other loci known to be affected by dietary α-MD, *Ddc* and *l(2)amd*, are apparently also involved in cuticle synthesis (Wright *et al.* 1981; Wright, 1987).

Alleles at both the *Ddc* and *l(2)amd* loci exhibit intracistronic complementation, and the *Ddc* locus has been identified as the structural locus for DDC (Wright *et al.* 1976). In the case of the *amd* locus, the enzyme produced by the wild type allele is necessary for the synthesis of an, as yet, unidentified catecholamine (Black *et al.* 1987).

The interactions between *Tcr* and *l(2)amd* were examined by looking at the effects of a mutant allele at one of the loci on the viability of partially complementing alleles at the other locus. Either allele of *Tcr* caused an increase in the viability of partially complementing *l(2)amd* alleles. At least some functional *amd* gene product is necessary for this interaction to occur since, even in the presence of *Tcr* mutants, *l(2)amd* hemizygotes remain completely lethal. On the other hand, not only did two different alleles of *l(2)amd* fail to increase the viability of *Tcr*^{40}/*Tcr*^{45} heterozygotes, but if the chromosome bearing the *l(2)amd* allele was maternally derived, the viability of *Tcr*^{40}/*Tcr*^{45} embryos was decreased. It is clear from these results that the products of the two loci are not competing for the same substrate because a reduction in either enzyme would increase the amount of substrate available to the other and that should increase the viability of partially complementing alleles at the second locus.

D. melanogaster DDC appears not to be significantly inhibited by α-MD *in vitro* (Black &

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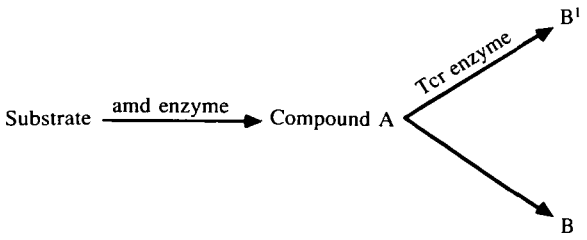

Fig. 1. Proposed branch in the catecholamine metabolic pathway based on the interaction between mutants in the *amd* locus and the *Tcr* locus. See text for details.

Smarrelli, 1986). Furthermore, genetic results indicate that in *D. melanogaster* inhibition of DDC is not the major cause of α-MD induced lethality (Wright *et al.* 1981). If lethality were caused by a failure to produce enough dopamine, anything which reduced the amount of DDC present should decrease resistance to α-MD. Just the opposite occurs. Individuals with a Ddc^- allele are more resistant to α-MD, while individuals with an extra dose of the *Ddc* gene are more sensitive (Marsh & Wright, 1986). Thus the actual *in vivo* effect of α-MD appears to result from inhibition of another enzyme which also uses DOPA as its substrate. Increased DDC removes more DOPA from the pool, thus reducing the substrate available to this enzyme and enhancing the sensitivity of the organism to the inhibitor.

The data obtained so far strongly suggest that toxicity of α-MD is due to inhibition of the protein produced by the *amd* locus. This locus is directly adjacent to *Ddc* and exhibits an extensive sequence homology with *Ddc* (Eveleth *et al.* 1986; Marsh *et al.* 1986). Comparison of the deduced primary and secondary structures of the products of these loci (Eveleth & Marsh, 1986) suggest that they resulted from gene duplication. Thus it is quite likely that both share DOPA as a substrate, and possibly their subsequent divergence has rendered the *amd* product more sensitive to α-MD. The genetic evidence supports this view. Thus far, mutations to hypersensitivity have been recovered only at *amd*. Furthermore, three wild type doses of the *amd* locus increase resistance to α-MD (Marsh & Wright, 1986).

The observed interaction between the *amd* and *Tcr* loci has provided evidence for another metabolic pathway in *Drosophila* which appears to be involved in the formation of the cuticle. The data available thus far can be explained by the simple scheme presented in Fig. 1. The substrate, probably DOPA, is converted by the amd enzyme to a catecholamine, compound A, which is then converted to B^1 by an enzyme produced by the *Tcr* locus, and to B by the enzymatic product of an as yet unidentified gene. The recessive lethality of mutations at the *Tcr* locus indicates that some B^1 is necessary for viability, but B is the more vital of the two compounds. A reduction in the activity of the amd enzyme, either by α-MD inhibition or by incomplete complementation among *l(2)amd* alleles results in complete or partial lethality by reduction in B. In either case, heterozygosity for the *Tcr* mutation would increase the amount of A available resulting in an elevation of B compatible with increased viability. The amd protein is placed first in the pathway because at least some activity of this enzyme is necessary for these interactions to occur: the *Tcr* mutations do not confer absolute resistance to the inhibitor (Table 1) and a genotype which provides little or no amd protein shows no response to *Tcr* (Table 3). While other, more complicated models can be presented, such as *Tcr* coding for a protein required to produce an enzyme co-factor or a transport protein, this simple scheme can account for all of the observed data. The fact that the *l(2)amd* adult escaper phenotype is different from the *Tcr* escaper phenotype would be due to the increased need of compound B over compound B^1. Whenever enough A is present to permit adequate B to be produced, more than enough B^1 will be made. The *l(2)amd* escapers probably have just enough B but ample B^1 from the Tcr enzyme so that no *Tcr* phenotype is produced. A similar argument can be used to explain the difference in the embryonic phenotype.

We have recovered mutants at a new locus which is involved in cuticle synthesis. A scheme has been presented in which all of the observations on α-MD toxicity and mutant interactions can be accounted for by placing the catecholamine produced by the amd enzyme before a branch point in a metabolic pathway with the *Tcr* gene product being an enzyme in one of the branches. The substrate of the amd enzyme, in this model, appears to be the catecholamine DOPA. The evidence supports *amd* and *Tcr* being in a previously uncharacterized branch of a catecholamine metabolic pathway involved in cuticle synthesis, and if our scheme is correct there is at least one other enzyme in the pathway.

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