Mutations at the *stm A* locus of *Drosophila melanogaster* confer resistance to the sodium channel neuropoison veratridine

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Adult males of the temperature sensitive paralytic mutant *stambh* A^1 (2–56.8) of *Drosophila melanogaster* are resistant to the lethal effects of the sodium channel neuropoison veratridine. Four alleles were isolated on the basis of their inability to complement the paralytic phenotype of *stm* A^1. Flies of all these alleles also showed resistance to veratridine, demonstrating that the two phenotypes are genetically inseparable. Two other paralytic mutants of *Drosophila* known to be resistant to veratridine are also cross-resistant to DDT and pyrethroids. This report demonstrates the increasing prospects of using paralytic behaviour mutants of *D. melanogaster* to understand the genetic basis of neurotoxin and insecticide resistance mechanisms.

DROSOPHILA MELANOGASTER is an ideal insect to study the genetic basis of neurotoxin and insecticide resistance mechanisms because of the availability of a large number of neurological and behavioural mutants and extensive genetic manipulation techniques. Despite these, it remains under-utilized to answer questions related to insecticide resistance.

There are eighteen genetic loci in *D. melanogaster* which mutate to a behavioural phenotype of temperature-sensitive paralysis[1–5]. Conditional paralysis is believed to be due to defective neurotransmission involving voltage-gated sodium channels[6,7]. The primary target of the neurotoxin veratridine and the insecticides DDT and pyrethroids is the neuronal *trans*-membrane sodium channel[8,9]. Temperature-sensitive paralytics could therefore serve as valuable material to study the genetic basis of neurotoxin and insecticide resistance mechanisms.

I report here the resistance of adult flies of the reversible temperature sensitive paralytic mutant *stambh* A^1 (2–56.8) to the lethal effects of the sodium channel neuropoison veratridine.

The original *stambh* A^1 (*stm* A^1) stock was identified (on a Canton-S wild type background) and was mapped and described by Shyngle and Sharma[5]. Briefly, homozygous flies showed 100% paralysis within 4 minutes at 38° C and recovered to normalcy within 5–6 minutes when brought back to 23° C. Variously diluted solutions of veratridine (Sigma) were made in 2% sucrose and applied to 1.5 cm Whatman No. 1 filter paper discs in standard fly vials. Control flies were fed on 2% sucrose. All feeding was done at 23 ± 2° C. Only male flies aged 2–3 days were used for the assay. Dead flies were scored after 2, 4, 6 and 24 h of commencement of feeding. Probit analysis of lethality data was performed according to Finney[10].

It was observed that adult males of *stm* A^1 were markedly resistant to the lethal effects of veratridine at 24 h when compared to Canton-S wild type males which were susceptible (Figure 1). There was 100% kill of CS males after 24 h exposure to 150 μg veratridine, whereas *stm* A^1 flies only showed 10% kill. Probit analysis of a large set of data showed that *stm* A^1 was 1.5 times more resistant to veratridine than CS at the LC_{50} level (Figure 2). The dose response curves of the two strains were non-identical and non-parallel, the difference in response being more pronounced at higher

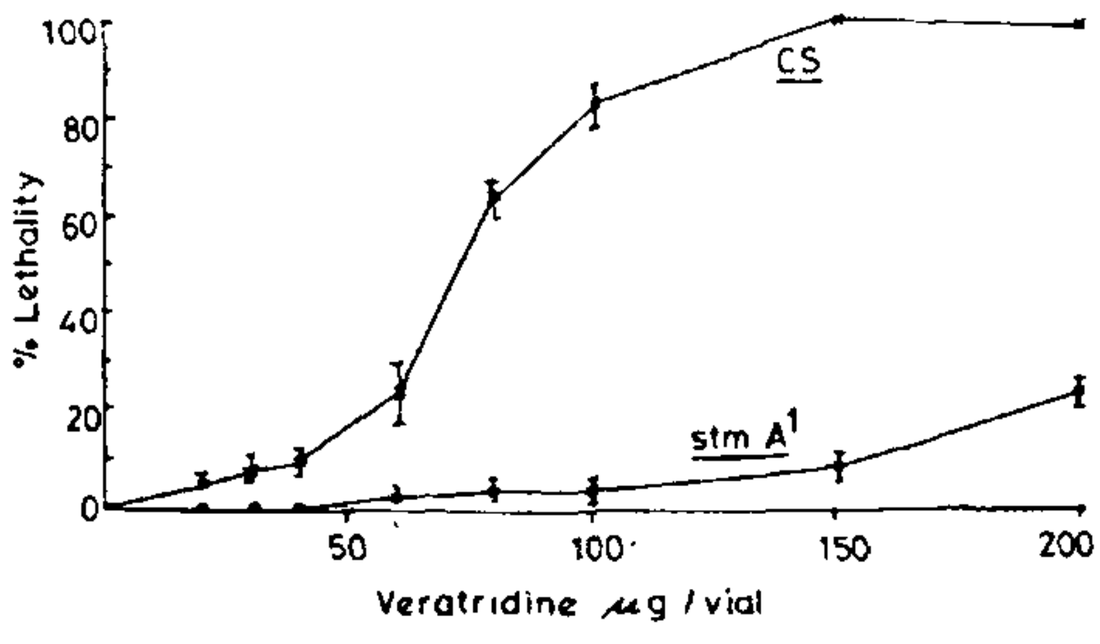

Figure 1. Dose response of veratridine dependent lethality of *Canton S* and *stm* A^1 males at 23° C. Number of flies at each point = 125.

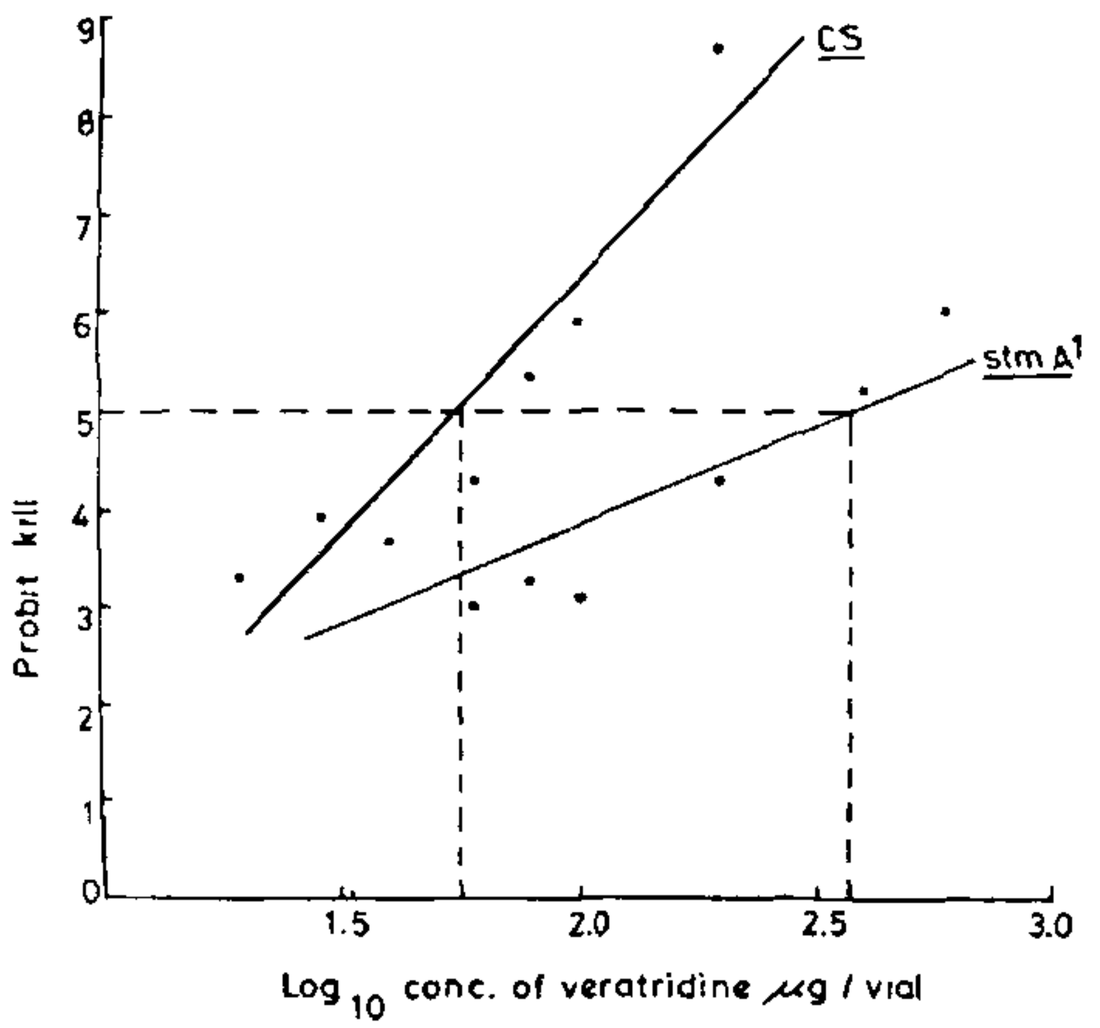

Figure 2. Probit kill curves of veratridine-dependent lethality of *Canton S* and *stm* A^1 males at 23° C, $n = 1$, 125 for each strain. *CS*: Regression coefficient = 5.305211; LC_{50} = 1 80, 95% confidence limits = 0 91–2.03. *stm* A^1. Regression coefficient = 1.898851; LC_{50} = 2.63, 95% confidence limits = 2.33–3.02.

levels than at lower ones. There was a marked difference between the two strains in the time course of lethality (Figure 3) with CS males showing a 60% increase in kill between 2 and 4 h during which *stm* A^1 males were totally unaffected by the toxin.

Four alleles of *stm* A^1 were isolated using EMS and P element mutagenesis. EMS mutations were induced on a lethal-free wild type CS second chromosome. EMS-fed males were mass-mated to *b stm* A^1 virgins. (Description of all markers and balancers in this report can be found in Lindsley and Zimm[11-14].) $3.9-10^4$ F_1 males (genotype *b stm* A^1/b^+ *stm* $A^?$) were screened for paralysis at 38°C, of which 3 paralysed and later recovered at 23°C. The b^+ *stm* A^x chromosomes were recovered by mating the selected males to *In (2LR) al dp b Pm/In (2LR) Cy al dp cn* virgins and intermating phenotypically b^+ *Pm* males and virgin female progeny. These were confirmed as alleles after retesting their ability to non-complement *stm* A^1 and were given the numbers *stm* A^2, *stm* A^7 and *stm* A^{12}. Only *stm* A^2 was homozygous viable and has been used in this study. P element induced alleles of *stm* A^1 were isolated essentially according to the crossing scheme of Robertson *et al.*[15]. Three confirmed alleles (now numbered) *stm* A^{p1}, *stm* A^{p2} and *stm* A^{p4} were isolated. All these were homozygous viable.

Homozygous adult males of the four new alleles of *stm* A^1 showed 100% paralysis at 38°C and likewise, 100% recovery from paralysis at 23°C, but with different patterns. The time taken for 50% paralysis and for 100% recovery of homozygous males of all alleles is shown in Table 1. *stm* A^2 and *stm* A^{p1} were faster paralysing than *stm* A^1. Males of all *trans*-allelic heterozygous combinations also paralysed at 38°C (data not shown) from which it was possible to decipher the dominance/recessive relationship among them as follows:

stm A^2 > *stm* A^{p1} > *stm* A^1 > *stm* A^{p2} > *stm* A^{p4}

Adult males of all the four alleles showed high levels of resistance to veratridine after 24 h compared to the wild type control (Table 2). Probit tranformation of the lethality values showed that at the LC_{50} level each allele was significantly different from thc control. No attempt to relate the severity of the paralytic phenotype with LC_{50} levels has been made although it appears that faster paralysing alleles *stm* A^{p1} and *stm* A^2 are more resistant than slower paralysing ones.

Here I demonstrate that the *stm* A^1 mutant of *D. melanogaster* which was isolated for its paralytic behaviour at 38°C is resistant to the lethal effects of veratridine at 23°C (a temperature wherein it is behaviourally normal). To demonstrate that veratridine resistance and paralysis are phenotypic manifestations of the same mutational lesion, four alleles of *stm* A^1 based on their inability to complement its temperature-sensitive paralytic behaviour were isolated. All these alleles also showed resistance to veratridine. If the two effects in *stm* A^1 were due to mutations in two independent genes then mutant alleles selected for paralysis should not be simultaneously expected to show veratridine resistance. It is therefore concluded that the mutation-causing paralyis also confers veratridine resistance, and that the two effects are genetically inseparable.

The primary target of the alkaloid veratridine as well as insecticides DDT and several pyrethroids is the voltage-sensitive sodium channel of nerve cells[8,9]. Temperature-sensitive paralytic mutants of *Drosophila* are believed to be defective in neurotransmission involving voltage-sensitive sodium channels[6,7]. Resistance to veratridine and cross-resistance to DDT and natural and synthetic pyrethroids are already reported in two of

Table 1. Paralysis (at 38° C) and recovery time (at 23° C) of various *stm A* alleles. $n = 25$ for each genotype

Genotype	Time taken (min; sec) for 50% flies to paralyse	Time taken (min; sec) for 100% recovery after 100% paralysis
stm A^1	02;42	05;30
stm A^2	01;42	03;00
stm A^{p1}	01;50	03;30
stm A^{p2}	02;30	09;30
stm A^{p4}	03;10	10;30

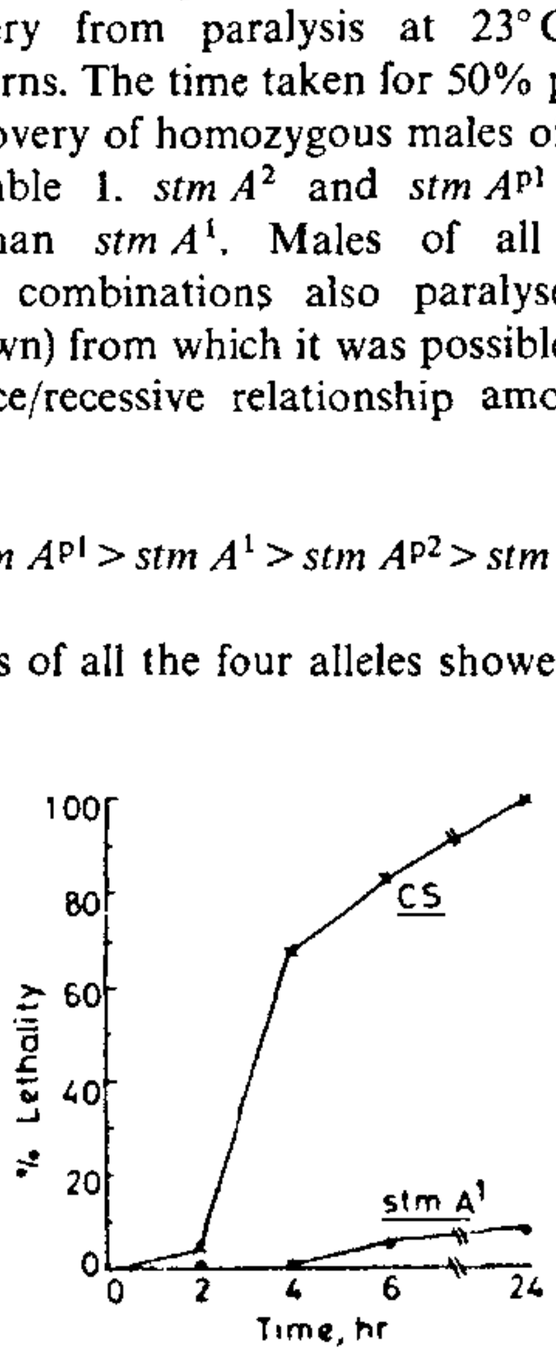

Figure 3. Time course of veratridine-dependent lethality of *Canton-S* and *stm* A^1 males at 23° C. Dose = 150 μg/vial.

Table 2. LC_{50} values of veratridine on adult males of *Canton S* and various *stm A* alleles. $n = 1$, 125 for each strain. Doses of veratridine (μg/vial) = 0, 20, 40, 50, 100, 150, 200, 400 and 600

Genotype	LC_{50} (μg/vial)	95% confidence limits
+/+ (CS)	1.75	0.91 2.02
stm A^2	3.42	3.12 3.74
stm A^{p1}	3.41	3.14 3.93
stm A^{p2}	2.44	2.21 3.25
stm A^{p4}	2.22	2.02 2.78

the 18 genetically characterized temperature-sensitive paralytic mutants, viz. *no action potential*^{ts} and *paralysed*^{ts} (refs 16–18). This report on veratridine resistance of yet another ts paralytic mutant shows the need to screen other members of its class for their resistance to neurotoxins and insecticides.

Resistance to veratridine, DDT and pyrethroids is correlated with reduced neuronal sensitivity in *nap*^{ts} (ref. 17) which, in turn, is due to a reduction in the number of sodium channels[19]. There have been no neurophysiological studies on *stm A* to demonstrate either of these possibilities. There is evidence that delayed onset of lethality to toxins and pesticides, similar to that reported in *stm A*^{1} (Figure 3), is consistent with resistance mechanisms involving reduced target sensitivity[17,20,21]. This does not rule out the possibility of other mechanisms like efficient detoxification or reduced penetration of the toxin. However, because the behavioural paralytic phenotype and veratridine resistance of *stm A*^{1} are mutationally inseparable it is reasonable to expect a mechanism of resistance involving neuronal functions rather than one involving metabolic differences. This question needs more investigation.

In conclusion, this preliminary report on the resistance of *stm A* to veratridine, suggests that paralytic mutants of *D. melanogaster* should be increasingly used to study mechanisms of neurotoxin and insecticide resistance. The availability of P element alleles of *stm A*, in addition, open up the future possibility of its molecular cloning.

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Germ cell cytotoxicity in tumour-bearing mouse subjected to single therapeutic dose of *cis*-diamminedichloroplatinum (II)

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Cytotoxic potential of cisplatin on testicular germinal cells of sarcoma-bearing mice has been assessed at single therapeutic dose at certain chosen post-treatment time points. It was observed that the drug affected testicular germinal cells of both tumour and nontumour mice to a significant extent and cytotoxicity was manifested in the form of structural chromosome aberrations and precocious desynapsis of sex bivalent. It was further observed that both differentiating spermatogonia and primary spermatocytes were equally sensitive to the clastogenic action of cisplatin. The possible significance of the findings has been discussed.

DAMAGE to testicular germinal cells is a potential side effect of cancer chemotherapy. This is of particular concern to cancer patients of reproductive age who are subjected to prolonged course of chemotherapy. Information on the nature and extent of germinal cell damage in response to drug exposure will be of value for better understanding of drug action and better monitoring of chemotherapy. This can be achieved by employing tumour-bearing mouse as experimental model because of the similarity, in many respects, in the spermatogenesis of man and mouse[1,2]. It is known that any damage to spermatogonial stem cells would result in long term infertility and genetic risk[3,4]. Meiotic chromosome analysis at diak-meta. I constitutes an important parameter of evaluating germ cell damage at different phases of spermatogenesis depending on the time interval chosen between the time of drug exposure and the cell sampling.

Cisplatin, the commercially available preparation of *cis*-diamminedichloroplatinum (II), is an effective antineoplastic drug widely used at present to combat various forms of human malignancies[5]. The drug is at the same time a mutagen, a teratogen and a suspected carcinogen[6]. Naturally the potential hazards of its wide application cannot be ignored. Here we