

Alteration of biogenic amines in *Drosophila virilis* under stress.

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We present here the results of studies of the content of noradrenaline (NA), octopamine (GA), and their precursors tyrosine (Tyr), tyramine (TA) and DL-B-(3,4-dihydroxyphenyl) alanine (DOPA) in individuals of line 101 and 147 of *D. virilis* under normal and stressful conditions, using a simple method based on high-performance liquid chromatography with electrochemical detection that obviates the necessity of previous sample purification. In individuals of the resistant line (101) contents of OA, Tyr, NA, and DOPA were lower than in the flies of the sensitive one (147) under normal conditions. Heat stress caused an increase

in the content of GA, Tyr, NA, and DOPA in the resistant line whereas those titers in the sensitive line did not change. The lines did not differ in the level of TA under normal conditions and the amine content did not change under stress.

Dynamics of the contents of Tyr, OA, NA, and DOPA in females of line 101 are shown in Figure 1. It is well seen that the contents of Tyr, OA, NA, and DOPA sharply increase already after 15 min at 38°C and start to decrease after 1 h for Tyr and NA, and after 2 h of stressor action for OA and DOPA. DOPA content increases up to 1 h of stressing and after longer stress (2 h) the content of NA rises again.

It has been shown earlier that the activities of the enzymes of biogenic amines synthesis sharply decrease in individuals of the line 101 of *D. virilis* already after 30 min of stressing (Rauschenbach *et al.*, 1995; Sukhanova *et al.*, 1997). It has been also demonstrated that OA is synthesized in the insect's nervous tissue and released from the *corpus cardiacum* and from certain nerve terminals in the flight

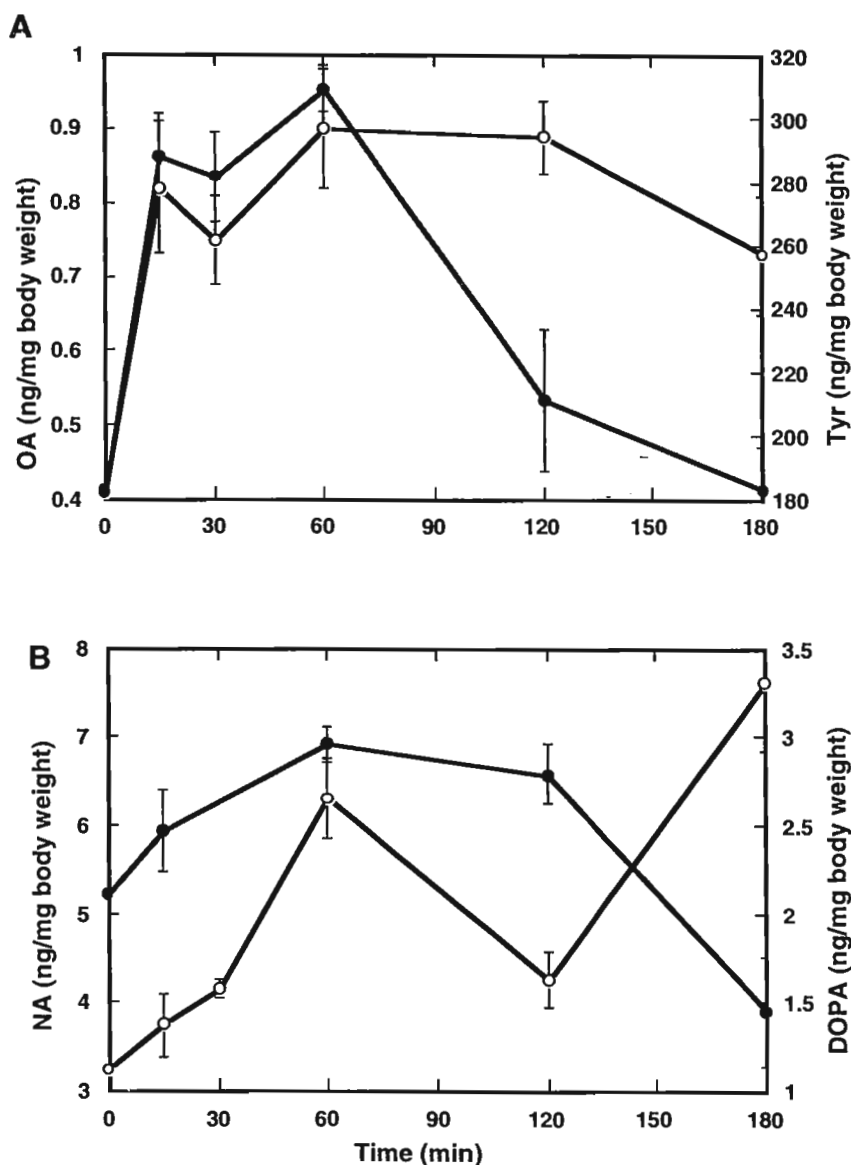


Figure 1. Time course of changes in A) OA (o) and Tyr (•), and B) NA (o) and DOPA (•) content in the *D. virilis* 101 line.

muscles (Orchard, 1982) into hemolymph (Davenport and Evans, 1984; Hirashima and Eto, 1993). Bearing in mind these data and present results (the increase of Tyr, OA, NA, and DOPA contents after 15 min of stressor action) the most likely explanation of the observed changes in the levels of biogenic amines and their precursors in *D. virilis* under stress is as follows. A sharp increase in the contents of DA and OA results from their release from depot. The increase of product content may decrease the activity of the synthesizing enzymes, tyrosine hydroxylase (TH) and tyrosine decarboxylase (TDC), via a feedback mechanism, and we have demonstrated precisely this (Rauschenbach *et al.*, 1995; Sukhanova *et al.*, 1997). A sharp decrease in TH and TDC activities may, in turn, lead to the increase of Tyr pool, and this, again, we have precisely shown (Figure 1A). An increase in Tyr content would decrease the activity of alkaline phosphatase (Aph), the Tyr-controlling enzyme. Indeed, it has been demonstrated that the activity of Aph decreases under stress (Sukhanova *et al.*, 1996).

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Regulation of the expression of tissue-specific esterase in *Drosophila*.

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The esterase genes family was described in *Drosophila virilis* and *D. melanogaster* (Korochkin, 1980). The deduced amino acid sequences of tissue specific esterase S *D. virilis* and esterase 6 *D. melanogaster* reveals 50% homology. The exon-intron structure of both genes is similar (Sergeev *et al.*, 1995). The *est6* gene of *D. melanogaster* has only one promoter while gene *estS* of *D. virilis* has two.

Drosophila melanogaster was transformed with the esteraseS gene from *D. virilis*. This gene is strongly activated in ejaculatory bulbs in mature males of *D. virilis*. The closely related gene from *D. melanogaster* is activated in ejaculatory ducts. A genomic copy of this gene including 400 bp of 5' regulatory region was integrated into the genome of *Drosophila melanogaster* (Figure 1). The tissue- and stage-specific expression of genomic copy of the esterase S gene integrated into the *D. melanogaster* genome is the same as in *D. virilis*. The products of the transferred genes were detected in ejaculatory bulbs of transgenic flies (Korochkin *et al.*, 1995). The results suggest that this specificity is evidently determined by the regulatory region of the esterase S gene and controlled by cis mechanism and at the transcriptional level: *estS* gene of *D. virilis* is transcriptionally active only in ejaculatory bulbs of transgenic flies (Figure 2). "Transgenic product" could be transferred from male ejaculatory bulbs of transgenic flies into female genitals upon copulation, with subsequent degradation there. The using of lacZ reporter gene shows that tissue and stage specificity depends upon a relatively small 5' regulatory region of the esterase S gene. A 396 bp fragment of the regulatory region of the *estS* gene upstream to the major transcription initiation site can switch on the *estS* gene in many places of the developing animal, but DNA upstream from this fragment inhibits this gene activity in all organs excluding the ejaculatory bulb. Removal of different parts in 5' and 3' regions of the *estS* gene shows that deletions in 5' regulatory region of the *estS* gene up to 115 bp to the major transcription initiation site can reduce the transcription *in vitro* four-fold. The DNA fragments with a 5' region containing 830, 750, 450, 390 bp to the major transcription initiation site were also used in such experiments. The efficiency of *in vitro* transcription of these deletion constructions was practically the same. Computer analysis of the region from -390 to -60 to the major transcription initiation site shows that this region contains many sequences similar to