

Reciprocal functions of the enzymes NBAD-synthase and NBAD-hydrolase in epidermal and neural tissue of flies

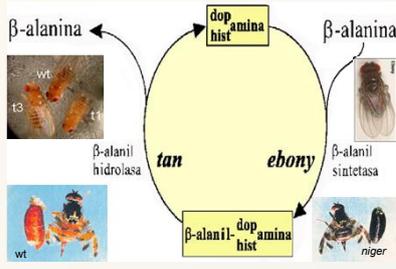
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Introduction

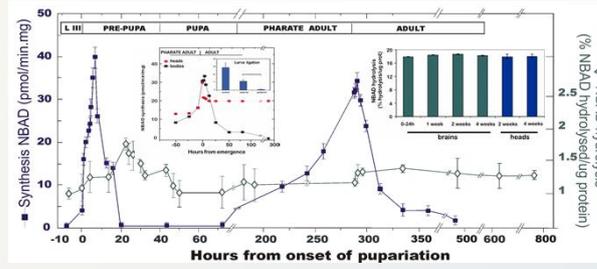
The acylation of neurotransmitters is important for the homeostasis of neural system but also gives rise to important metabolites in insects. The best known are N- β -alanyldopamine (NBAD), the main sclerotization precursor of insect brown cuticles, and carcinine (N- β -alanylhistamine), an essential compound of the visual system. Key enzymes of this metabolism are NBAD-synthase (NBAD-S) and NBAD-hydrolase (NBAD-H) which synthesizes and hydrolyzes, respectively, β -alanyl derivatives of different neurotransmitters. In our laboratory we have characterized these enzymes in epidermal and nervous tissue during the whole life cycle. They function together in a system regulating the levels of NBAD and dopamine during cuticle sclerotization, innate immune response and in nervous tissue and the levels of histamine and carcinine in the optic lobes. NBAD-synthase is coded by the genes *niger* in *Ceratitis capitata* and *ebony* in *Drosophila melanogaster*, whereas NBAD-hydrolase is coded by the gene *tan* in *D. melanogaster* (it is unknown in *C. capitata*).

NBAD-S and NBAD-H metabolism



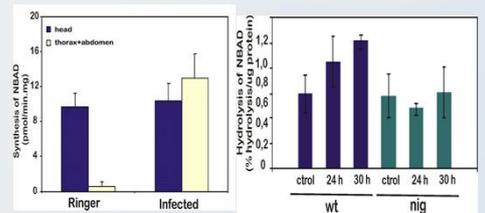
Metabolism of synthesis and hydrolysis of beta-alanyl derivatives. In italics are the genes names, *ebony* and *tan* (*D. melanogaster*). t1 and t3 are two different *tan* mutants.

NBAD-S and NBAD-H activity during the life cycle of *C. capitata* (epidermal and neural tissue)



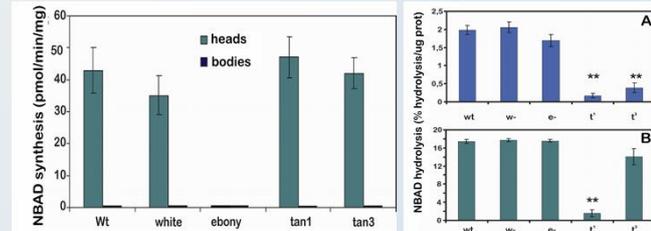
Epidermal activity of NBAD-hydrolase (\diamond) and NBAD-synthase (\blacksquare) during the life cycle of *C. capitata*. Inset: left, NBAD-synthase activity in heads and bodies of pharate adults and imagoes. The bar figures show the ligand of 3th instar larvae to demonstrate the response of epidermal NBAD-S to 20-OH ecdysone. Right inset: NBAD-hydrolase activity in nervous system (brains and heads).

Response of NBAD-S and NBAD-H against bacterial infection (immune challenge) in *C. capitata* adults



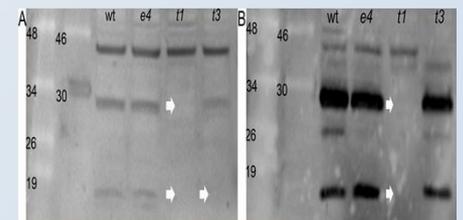
NBAD-Hydrolase in immune response of 1 week *C. capitata* adults wt and *niger*. Insects were injected with sterile Ringer solution or *E. coli* and analyzed for NBAD-synthase (24 h after injection) and NBAD-hydrolase induction (24 h and 30 h after injection). Control: mock injection. In *niger* mutant, unable to synthesize NBAD, there were no differences in NBAD-hydrolase activity between control and infected individuals.

NBAD-S (left panel) and NBAD-H (right panel) in heads and bodies of *D. melanogaster* adults



NBAD-synthase (left) and NBAD-hydrolase (right; A: bodies; B: heads) activity in body and head extracts of 1 week adults of *D. melanogaster* wt and mutants: wt; Canton S; w-; *white1116*; e-; *e4*; t1; *tan1*; t3; *tan3*. Heads represent mainly neural activity; bodies represent mainly epidermal activity.

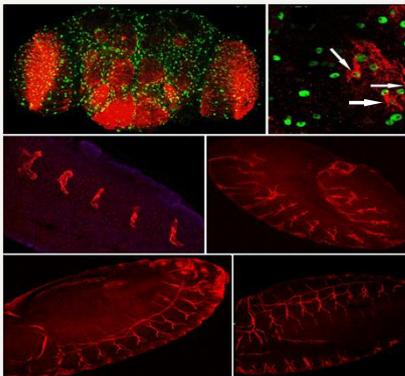
Immunodetection (western blots) of NBAD-hydrolase in adults of *D. melanogaster*



Western Blot analysis of body (A) and head (B) extracts of wt, e4, t1 and t3 strains using Tan antibody. White arrows point at the missing bands in *tan* mutants. Numbers indicate MW of markers (M).

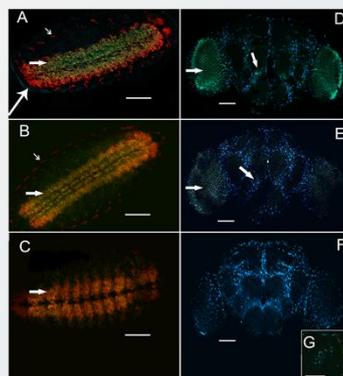
In situ expression of NBAD-S y NBAD-H in adults brain ganglia and embryos of *D. melanogaster*

NBAD-synthase



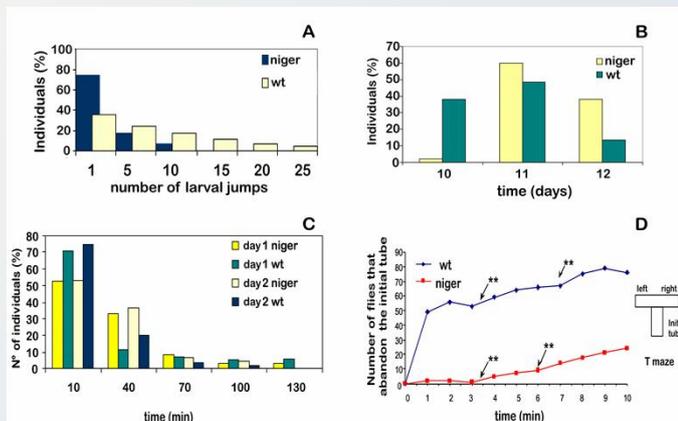
Confocal microscopy images of Ebony expression in *D. melanogaster*. (A) Expression of Ebony (red) and Repo (green) in the whole brain. (B) Detailed image showing Repo staining in the nucleus and Ebony staining in the cytoplasm of some glia cells (arrows). (C-E) Expression of Ebony in embryos: Ebony presence in trachea of embryonic stages 11 to 15.

NBAD-hydrolase



Confocal microscopy images of *D. melanogaster* Tan expression. Big arrows indicate Tan expression. Small arrows in A and B indicate ELAV expression in peripheral nervous system. Bars are 50 μ m. A: Expression in wt embryo of Tan (green), Elav (neuronal marker; red; overlapping is yellow) and Ebony (light blue). B-C: Expression of Tan (green), Elav (red) in t3 (B) and t1 (C) embryo. D-F: Expression of Tan (green) and Repo (blue) in the whole brain of wt (D), t3 (E) and t1 (F). G: Detailed image showing the faint Tan (green) and Repo (blue) expression in the medulla of the optic lobe of t1 flies.

The mutant *niger* shows several physiological impairments



A: Number of jumps made by wt or *niger* larvae after the abandonment of the food plate. B: Developmental time: days from oviposition until larval jump in order to initiate metamorphosis. Mutant *niger* larvae jumped one day later than wt larvae. C: response to light stimulus; time from light turned on until larvae jumped to abandon the food. D: Exploratory behaviour: number of flies that left the initial tube and walked through the left or right side on a T maze (** flies that walked back to the initial tube).

Conclusions

- During the development NBAD-synthase is expressed in epidermis in two narrow windows, when the insect needs to sclerotize the cuticle. This enzyme is induced by 20-OH ecdysone at the moment of cuticle sclerotization.
- NBAD-H, otherwise, in epidermis showed a constitutive activity during the life cycle.
- Both enzymes showed constitutive activity in neural tissue during the whole life cycle.
- In *C. capitata*, the epidermal NBAD-synthase was activated after the microbial challenge, whereas NBAD-hydrolase responded to the synthesis of NBAD, but not to the microbial infection.
- NBAD-synthase is expressed in different brain regions, in the glia. In embryos is expressed in the developing tracheae (epidermal expression).
- NBAD-hydrolase is expressed in neurons of the optic lobes in adults and in central nervous system in embryos.
- The mutant *niger* showed some physiological impairments. It showed less response to the light stimulus, a delayed in the life cycle and a lesser exploratory capacity.

