Drosophila ethanol metabolizing system. Acetaldehyde oxidation in ALDOX-null mutants[1]

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Summary. Aldehyde dehydrogenase (ALDH) activity is demonstrated in two mutant strains of *D. melanogaster* lacking active aldehyde oxidase (ALDOX). The levels of ALDH activity are compared with those found for the wild strain of *D. melanogaster* and its sibling species *D. simulans.* These data provide a rational explanation for the capacity of ALDOX-null flies to detoxify acetaldehyde and use it for energy production.

In the pathway of ethanol (ETOH) metabolism, the acetaldehyde derived from ETOH is much more toxic than ETOH itself. In *Drosophila* species using ETOH as well as acetaldehyde for energy production, rapid oxidation of acetaldehyde by a highly efficient enzymatic system represents a critical step for survival[2]. This important metabolic role has long been attributed to aldehyde oxidase (ALDOX), a flavine-containing enzyme very abundant in *Drosophila*[3,4].

In previous work, we have presented clear-cut biochemical evidence for the existence of aldehyde dehydrogenase (ALDH) in the two sibling species *D. melanogaster* and *D. simulans*[5,6]. This NAD^+-dependent enzyme is known to play a key role in the ETOH metabolic pathway of various animal species including man[7-9]. In *Drosophila* species adapted to environmental ETOH, this enzyme is thought to be essential. In fact, ALDH activity was shown to be directly correlated with alcohol dehydrogenase (ADH) activity and with the degree of tolerance to ETOH and acetaldehyde[10-12].

In a recent paper from Lietaert and coworkers[13], the presence of ALDH was suggested in ALDOX-null mutant flies. But the authors failed to detect any trace of ALDH activity by spectrophotometric assays.

In the present paper, we demonstrate ALDH activity in 2 ALDOX-null mutant strains, Aldox-negative[1] (*Aldox^{n1}*) and maroon-like[1] (*mal^{l}*), and show that the enzyme activities are comparable to that found in the wild strain.

The *Drosophila* flies used in this study were the 2 sibling species *D. melanogaster* from Colmar and *D. simulans* from Villeurbanne (France) kindly made available by Prof. J. David, Laboratoire de Génétique Evolutive, Gif-sur-Yvette, France, and the mutant ALDOX-null strains *Aldox^{n1}* and *mal^{l}* kindly made available by Prof. M.B. Bentley, Department of Biology, University of Calgary, Calgary, Canada. As in our previous experiments[5,6], the flies were grown in a low-density population on an axenic killed yeast medium containing no ETOH[14]. Adult flies, 3-5-day-old, were immobilized by cooling, rapidly frozen by immersion in a bath of liquid nitrogen and homogenized in 10 volumes of 20 mM Tris-HCl buffer at pH 7.0 with a Polytron (Brinkman). The homogenate was centrifuged and treated as described elsewhere[6]. The clear supernatant obtained was stored at −80 °C until used for the enzymatic assays. ALDH activities were measured spectrophotometrically at 25 °C by monitoring the formation of NADH at 340 nm (20-sec intervals) with a Beckman DU 8 UV/VIS computerized spectrophotometer equiped for enzyme kinetics. The standard assay mixture contained 30 mM sodium pyrophosphate pH 10.0; 1.0 mM NAD^+; 0.8 mM pyrazole (to inhibit ADH) and 20 µl of supernatant for a final volume of 1.0 ml. The reaction was started by addition of acetaldehyde 10 µM or 100 µM. To correct for a blank reaction, a control was run simultaneously to each sample with omission of acetaldehyde. The values obtained for the control were automatically substracted from those of the sample.

It is of importance to mention that the assay conditions described above were initially adapted from studies in rodents and man[8,15]. The failure of Lietaert and coworkers[13] to detect ALDH activity in their preparations could be attributed to the use of an enzymatic method specifically devised for yeast ALDH with assay conditions which were far from optimal for *Drosophila* ALDH[16]. In fact, our study on the physico-chemical properties of *Drosophila* ALDH demonstrates that the *Drosophila* enzyme is in many aspects similar to mammalian liver ALDH[6].

In the table, the ALDH activity of 3 strains of *D. melanogaster* (2 mutants ALDOX-null and a wild strain) and the wild strain of *D. simulans* are compared. The fact that ALDOX-null flies possess an ALDH activity which is comparable to that of flies known for their adaptation to environmental ETOH confirms the behavioral data of several authors and provides a positive answer to the so-called 'ALDOX paradox'[13,17,18].

It is of interest to mention that the increase in ALDH activity (%) between the low and the high concentrations of acetaldehyde is much larger in mutant flies than in the wild. This could suggest that ALDH in mutant flies is kinetically different from the enzyme of wild strain. Whether this difference represents an adaptation mechanism to compensate for ALDOX inactivity in the degradation of other aldehyde substrates remains to be investigated.

Comparative study of aldehyde dehydrogenase (ALDH) activities in *D. simulans* and several strains of *D. melanogaster*

Strains/species	ALDH activity (nmoles NADH/min)* Acetaldehyde 10 µM	Acetaldehyde 100 µM	Increased activity** (%)
D.m.: wild	1.38±0.14	1.96±0.12	42
D.m.: Aldox^{n1}	0.65±0.08	1.39±0.10	114
D.m.: mal^{l}	0.77±0.04	1.80±0.21	134
D. simulans	0.79±0.16	1.12±0.13	42

* Each value represents the mean (±SE) of 3 determinations.
** Increased activity between assays done at 10 µM and 100 µM acetaldehyde.

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The intrapulmonary neuroepithelial bodies after vagotomy: demonstration of their sensory neuroreceptor-like innervation[1]

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Summary. In the neonatal rabbit, infranodosal vagotomy destroys most of the intracorpuscular nerve endings of the pulmonary neuroepithelial bodies (NEB), while supranodosal vagotomy leaves these nerve endings intact. We conclude that NEB are mainly innervated by sensory neurons whose cell bodies lie in the nodose ganglion of the vagus nerve. These findings support the hypothesis that although secretory in nature, NEB are neuroreceptor structures.

Apart from single neuroendocrine-like cells[2-6], innervated corpuscles of such cells, termed neuroepithelial bodies (NEB) recently have been identified in the intrapulmonary airway epithelium of man[7], several mammalian species[8-11], and amphibians[12,13]. NEB are composed of high cylindrical non-ciliated epithelial cells that contain serotonin[8,14-16] and peptides[17-19], stored in their intracytoplasmic dense-cored vesicles. Their strategic position at airway bifurcations[7,8], their distinct innervation which includes morphologically afferent (sensory) nerve endings[20,21] and the release by the corpuscular epithelial cells of serotonin upon their exposure to hypoxia[22-24], suggest that they may represent some kind of intrapulmonary neuro(chemo)receptor with local secretory activities and apparently sensitive to the composition of the inhaled air.

A condition to be satisfied on assigning a neuroreceptor function to these NEB is an unequivocal demonstration of the sensory nature of their innervation. In this report, we present neuroanatomical evidence obtained after nerve degeneration experiments and indicating that indeed most of the intracorpuscular nerve endings are sensory.

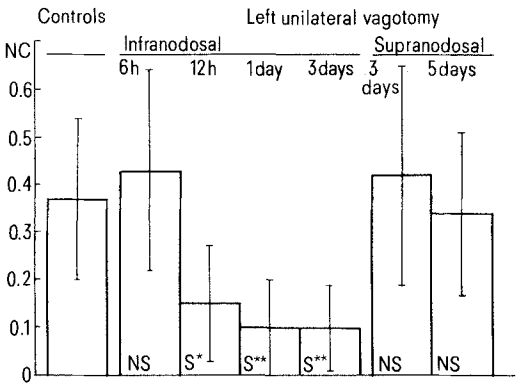
It is classically known that the sensory innervation of most of the thoracic and abdominal viscera, including the lungs[25], originates from pseudounipolar neurons whose cell bodies lie in the nodose ganglion of the vagus nerve[26]. We have thus studied the NEB intracorpuscular nerve endings after section of the vagus nerve either above or below the nodose ganglion, investigating whether the obtained results are compatible with the hypothesis that these nerve endings are sensory (i.e. terminals of processes of neurons whose cell bodies are situated in the nodose ganglion).

Although the anuran lung and its NEB are morphologically very different from the mammalian lung, it may be mentioned that a total vasosympathetic denervation has been carried out on the toad lung[12]. These experiments, however, only demonstrated that the origin of the nerve fibers to the amphibian NEB arises from a source 'extrinsic to the lung'[12].

Material and methods. Left unilateral vagotomy was performed on 17 neonatal rabbits, aged 1-2 weeks. The animals were anesthetized by an i.m. injection of Hypnorm® in doses of 0.05 ml per 100 g b.wt. The left vagus nerve was exposed in the midcervical region.

In 12 rabbits, the nerve was sectioned below the nodose ganglion (infranodosal vagotomy), a short length of it being removed at the same time. The animals were allowed to survive for a time interval varying from 6 h (2 animals) to 12 h (2 animals), 1 day (4 animals) and 3 days (4 animals).

In 5 rabbits, the left vagus nerve was surgically exposed in a cranial direction until the nodose ganglion was reached. The nerve was then sectioned above the nodose ganglion



Comparison of the ratio (NC) of the number of intracorpuscular nerve endings to the number of corpuscular cells in the NEB of control and infranodosally or supranodosally vagotomized left rabbit lungs; various survival times (as indicated on the figure). The SD are in each instance indicated by the brackets. A total number of 134 NEB was studied. Statistical significance: S*, $p < 0.05$; S**, $p < 0.01$; NS, not significant.