endogenous opioid activity attenuates restraint stress-induced potentiation of morphine catalepsy, as also does an increase in central DA activity. Since restraint stress-morphine interaction appears to involve several neurotransmitter systems it is cogent to explain it in terms of a central neuromodulator system which could induce the observed changes. PGs have been postulated to function as modulators of central synaptic transmission, and have been proposed as the first mediator of stress[9]. Restraint stress has been reported to enhance rat brain PG levels[10]. PGs of the E series have been shown to enhance rat brain serotonin[11] and cholinergic[12] activity, and to inhibit the release of DA from the rat striatum[13]. Recent studies indicate that PGD_2, the major rat brain PG, also enhances central serotonergic activity in this species[14]. Restraint stress-induced increase in rat brain serotonin has been shown to be antagonized following pretreatment with PG synthesis inhibitors[15]. While literature on the inter-relationship between central opioid and PG systems is sparse, it is known that naloxone inhibits the increase in rat brain PGs induced by met-enkephalin[16]. In a recent study[17], PGE_1-induced catalepsy in rats was shown to be attenuated following treatments disigned to reduce central serotonin and Ach, and to specifically enhance DA levels. Naloxone also antagonized PGE_1 catalepsy.

Experimental catalepsy is known to result from an interplay between central neurotransmitter systems. Thus, a decrease in central DA activity and an increase in brain serotonergic and cholinergic activities has been envisaged as the primary mechanism of experimental catalepsy[2,18]. It appears likely that PGs, released during restraint stress, potentiate morphine catalepsy by enhancing rat brain serotonin[11,14] and Ach[12], and reducing DA[13] activity. This is indicated by the attenuating effect of the PG synthesis inhibitors on stress-morphine interaction. Treatments which reduce central serotonin or Ach and enhance DA levels would then be expected to partially antagonize restraint stress-induced potentiation. Experimental stress is known to enhance rat brain endorphin level[19]and the inhibition induced by naloxone on stress-morphine interaction is in keeping with this observation.

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Ethanol metabolizing system in *Drosophila melanogaster:* subcellular distribution of some main enzymes

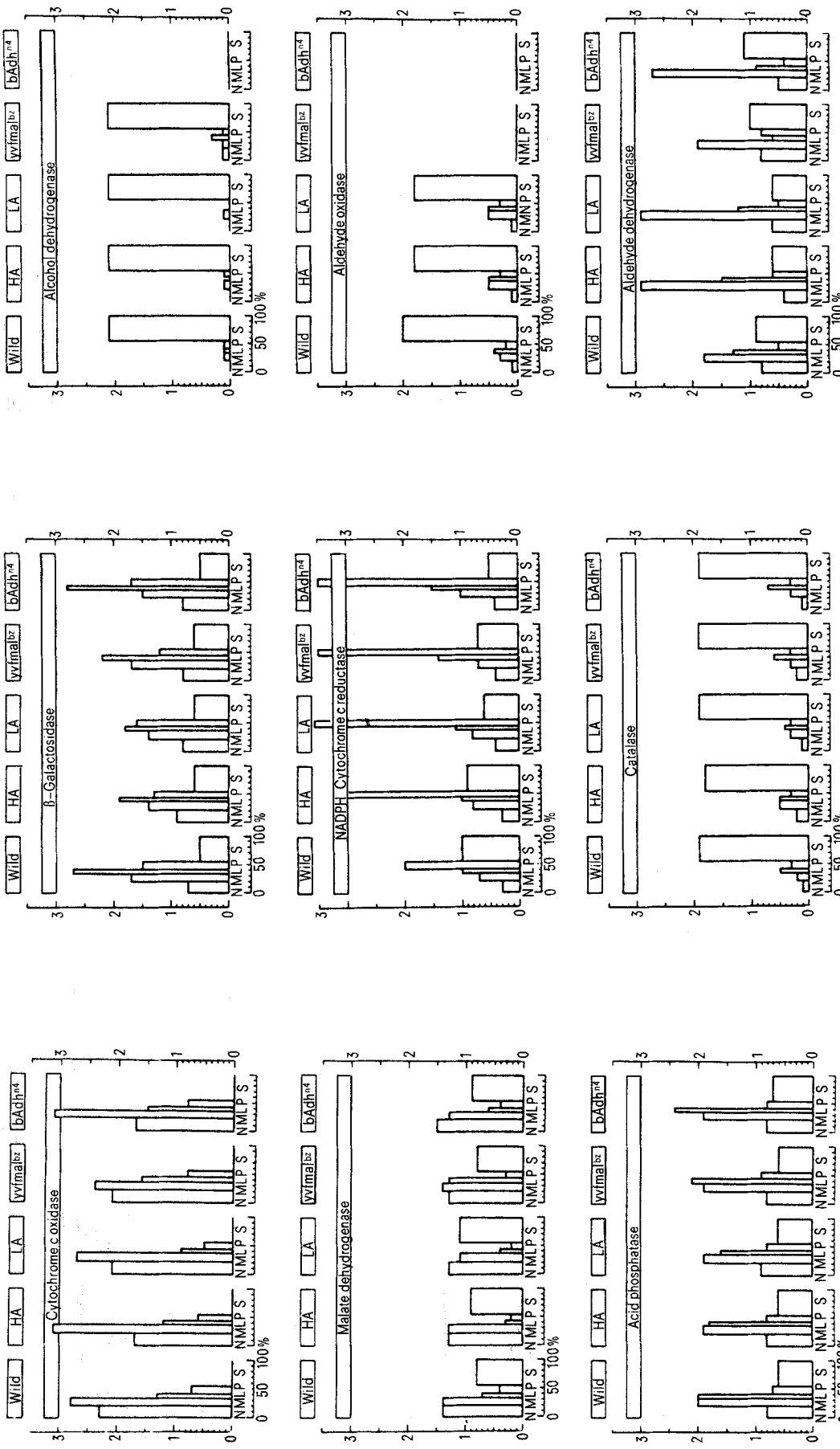
B. Chanteux[1], M. Libion-Mannaert[1], C. Dernoncourt-Sterpin[1], S. Wattiaux-De Coninck[2] and A. Elens[1]

Facultés Universitaires N.D. de la Paix, 61, rue de Bruxelles, B-5000 Namur (Belgium), 21 November 1984

Summary. The subcellular distribution of some enzymes which play a part in ethanol metabolism have been determined by differential centrifugation of homogenates of adult *D. melanogaster* flies of various genotypes. Aldehyde dehydrogenase, recently discovered in *D. melanogaster*, is present in the five genotypes studied. It has been found however to be, in vitro at least, most active in a strain lacking both alcohol dehydrogenase and aldehyde oxidase.
Key words. D. melanogaster; aldehyde dehydrogenase; catalase; alcohol dehydrogenase; aldehyde oxidase; ethanol metabolism.

Flies of five *D. melanogaster* genotypes were submitted by us to homogenization and subcellular fractionation according to a method originally described for rat liver[3], and slightly modified[4]. Five fractions were isolated. A nuclear fraction (N) was first separated from a total cytoplasmic extract (E). Then from the cytoplamic extract, four fractions were isolated: a heavy mitochondrial fraction (M), a light mitochondrial fraction (L), a microsomal fraction (P), and a final supernatant (S). Our main interest was in alcohol dehydrogenase (ADH), catalase, aldehyde oxidase (AO), and aldehyde dehydrogenase (ALDH). The enzymatic activities were determined according to Sofer and Ursprung for ADH[5], to Baudhuin et al. for Catalase[6], to Courtright for AO[7], and to Crow et al.[8] and Eckfeldt et al.[9] for ALDH. We used cytochrome *c* oxidase[10] and malate dehydrogenase[11] as marker enzymes for the mitochondria, while acid phosphatase[3] and beta-galactosidase[12] were used for lysosomes, and NADPH cytochrome *c* reductase[13] was the marker enzyme for endoplasmic reticulum. In the case of peroxisomes, catalase was the reference enzyme[5].

Besides the *bAdh*^{n4}, used in our previously described experiments[4], which lacks both ADH and AO, four other *D. melanogaster* genotypes were examined. The strain *y v f mal*^{bz} (Johns Hopkins University, Dept of Biology), lacks AO but has a normal ADH activity. Both HA and LA lines are the result of a long term selection for the 'male sexual activity', combined with brother-sister mating, which has given, after 330 generations, a 'highly active' line HA and a 'lowly active' line LA[14]. As in our previous experiments[15-17], our laboratory *wild* $e^{\pm}$ strain was used



Distribution pattern of enzymes. Ordinate: relative specific activity of fractions (percentage of total recovered activity/percentage of total recovered proteins). Abscissa: relative protein content of fractions (cumulatively from left to right). N: nuclear fraction, M: heavy mitochondrial fraction, L: light mitochondrial fraction, P: microsomal fraction, S: supernatant.

Specific activities of the enzymes (units of enzyme activity/mg of protein). For acid phosphatase, beta-galactosidase, and aldehyde dehydrogenase, the values have been multiplied by 10^2

Enzymes	Specific activities Strains wild e^+ Mean	σ	HA Mean	σ	LA Mean	σ	yvf mal^{bz} Mean	σ	$bAdh^{n4}$ Mean	σ
Cytochrome c oxidase	0.1636	0.0160	0.1395	0.0368	0.1695	0.0535	0.1454	0.0254	0.1496	0.0391
Malate dehydrogenase	2.1352	0.5756	1.6434	0.3473	1.4683	0.3460	2.0636	0.4652	2.4134	0.3949
Acid phosphatase	2.7093	0.4037	2.4348	0.8748	2.9246	1.7960	3.3772	0.8349	3.0263	0.7651
β galactosidase	0.7077	0.1623	0.9545	0.0940	0.8137	0.1656	0.6525	0.0864	0.9235	0.1410
NADPH cytochrome c reductase	0.1790	0.0344	0.1314	0.0099	0.1219	0.0173	0.1563	0.0324	0.1206	0.0384
Catalase	0.0529	0.0130	0.0474	0.0241	0.0579	0.0089	0.0674	0.0087	0.0638	0.0240
Alcohol dehydrogenase	0.3032	0.0280	0.1350	0.0345	0.1539	0.0322	0.3262	0.1118	–	–
Aldehyde oxidase	5.5915	1.0535	2.5416	1.0281	2.4723	0.5790	–	–	–	–
Aldehyde dehydrogenase	2.8450	2.2997	3.8454	1.6485	4.3905	0.6647	2.1533	0.0115	5.1252	1.1137

as control: this strain has a high activity of ADH as well as of AO. The HA and LA lines are endowed with normally high ADH and AO activities. All these genotypes differ in tolerance to ethanol and in oviposition preferences for ethanol supplemented mediums, in relation to their ADH activity levels[15-17]. As in our previous experiments, adult flies (from 5 to 10 days of age) were used[4,15-17].

Our main observations can be summarized as follows:

The specific activities of the reference enzymes, for the five *D. melanogaster* genotypes, are very similar. The ADH activities of the HA and LA lines were lower than expected from previous studies[17]. To obtain enough flies for the centrifugation experiments, it was necessary to relax the inbreeding selection and to culture the lines 'in mass' for about three to five generations. The change in ADH activity seems to be a consequence of inbreeding relaxation: similar changes have been observed under similar conditions for other physiological and morphological characteristics[18,19]. The AO activity is also lower than in the *'wild'* strain. As already known, the *y v f* mal^{bz} strain lacks AO but not ADH; the $bAdh^{n4}$ strain lacks both ADH and AO. ALDH is present in all five genotypes (table).

The distribution patterns of the reference enzymes and of the main ethanol metabolizing enzymes in the five fractions are shown in the figure, for the five genotypes.

A high proportion of cytochrome *c* oxidase is present in M and N fractions. Malate dehydrogenase is found in M and N fractions and also in the soluble fraction. Two malate dehydrogenases (MDH) are known in *Drosophila;* while one is found in the supernatant fraction after centrifugation (s-MDH), the other, m-MDH, is a mitochondrial enzyme[20]. The presence of large amounts of the two mitochondrial enzymes in the N fraction denotes a high sedimentation coefficient and the presence of large mitochondria. About 40% of the lysosomal enzymes were recovered in M and L fractions. However, the relative specific activities were about the same in L and M fractions for acid phosphatase. Beta galactosidase was purified the most in the L fraction. A high proportion of the two enzymes is present in the soluble fraction. Catalase, which is considered as a peroxisomal reference enzyme and also plays a part in the oxidation of ethanol into acetaldehyde, was recovered mainly in the S fraction and did not seem to be associated with a particulate fraction. By electron microscopy (not shown), peroxisomes were not detected in the isolated fractions. NADPH cytochrome *c* reductase was purified the most in the microsomal fraction P.

The distribution of the enzymes playing a role in ethanol and acetaldehyde metabolism was compared with the distribution of the reference enzymes. In *D. melanogaster* flies endowed with ADH activity, the enzyme was obtained mainly in the soluble fractions. In *wild* e^+ strain and HA and LA types, most of the AO was unsedimentable, but a small proportion of the enzyme was associated with heavy and light mitochondrial fractions, especially in HA and LA flies. Here special attention must be given to ALDH, recently discovered in *D. melanogaster* flies[4,21].

In mammals, ALDH is principally associated with mitochondria but is present in the cytosol as well[8,9,22]. In *D. melanogaster,* the distribution pattern of ALDH, as compared with those of cytochrome *c* oxidase and malate dehydrogenase, supports the assumption that this enzyme could be associated with mitochondria. However, a high proportion of ALDH is also found in the S fraction of the five *D. melanogaster* genotypes. Perhaps two different ALDH isoenzymes exist in *D. melanogaster* as in mammals: an essentially mitochondrial ALDH and another soluble one[23]. Indeed, it has recently been suggested that the ALDH of 'AO-null' strains is kinetically different from the ALDH of *wild* strains[21]. These two enzymes may differ as well in subcellular localization (e.g. as the two malate dehydrogenases differ[20]).

AO, a very abundant enzyme in *D. melanogaster,* which can also be present in rodents[24], was formerly credited with an almost exclusive role in acetaldehyde detoxification in *Drosophila.* Today, we know that the role of AO has been overestimated[25]. The presence of ALDH has been demonstrated first in *D. melanogaster* strains lacking ADH[21], and later in the $bAdh^4$ strain lacking both ADH and AO[4]. In the present experiments, ALDH is found in all the five genotypes studied. It seems possible that this enzyme could be present in other *D. melanogaster* strains as well. This is particularly interesting, if one remembers that ALDH is the main enzyme of acetaldehyde detoxification in mammals[26]. In mammalian liver, ethanol oxidation proceeds essentially via cytosolic ADH, but the role of peroxisomal catalase has also been demonstrated[27-30]. The role of the so-called 'microsomal ethanol oxidizing system (MEOS)' is also important. The acetaldehyde resulting from ethanol oxidation is transformed into acetate mainly by the mitochondrial ALDH, although some action of the cytosolic ALDH is not excluded[26]. All these enzymes, with the exception of MEOS, are also present in *D. melanogaster.*

However, these similarities do not demonstrate that ADH, catalase, ALDH, or AO play the same role in *Drosophila* as in mammals. From in vitro observations on homogenates of flies, one may not conclude that the same enzymes have the same activities in vivo. Enzymes of a metabolic chain must be present in the same cell, and sometimes in the same compartment. It is to be noted that in this study the homogenates originated from different organs. As a preliminary to the study of ethanol metabolism pathways, the tissular localization of the various enzymes will have to be ascertained.

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2 Département de Chimie Physiologique FNDP, Namur.

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Elastase digested urokinase (ED-UK)

H. Sumi, T. Kosugi*, N. Toki** and H. Mihara

*2nd Department of Physiology, Miyazaki Medical College, Miyazaki 889-16 (Japan), *2nd Department of Physiology, School of Medicine, University of the Ryukyus, Okinawa 903-01 (Japan), and ** Biochemistry Section, Michael Reese Research Foundation, Chicago (Illinois 60616, USA), 7 November 1983*

Summary. Elastase digested urokinase (ED-UK) was prepared from human high mol. wt urokinase (HMW-UK). It resembled low mol. wt urokinase (LMW-UK) in its mol. wt, specific activity, and active sites. The steady-state kinetic parameters of each enzyme for the activation of human Glu-plasminogen also resembled each other, as did their amidase parameters (with pyro-Glu-Gly-Arg-pNA).
Key words. Urokinase; elastase; enzymatic degradation.

Urokinase (EC 3.4.99.26), a serine protease produced in the kidney and found in the urine, is a potent activator of plasminogen and has been employed as a thrombolytic agent[1,2]. Two major molecular forms occur in current therapeutic preparations: a high mol. wt form (HMW-UK), and a low mol. wt form (LMW-UK)[2,3]. Both enzymes consist of two chains[4-8], designated as the B chain and A or A_1 chains[9,10], respectively, which are connected by a single interchain disulfide bond[7,8]. Since transformation of HMW-UK to LMW-UK readily occurs in solutions without protease inhibitors[4,11], or by enzyme treatments[12-14], LMW-UK is thought to represent an enzymatic degradation product of native HMW-UK. However, the detailed mechanism of degradation and how the related enzymes really act are not yet well understood. In this study, we succeeded in preparing and characterizing a new fibrinolytic enzyme, ED-UK, derived from HMW-UK.

Materials and methods. The following substances were used: hog pancreas elastase (type II) from Sigma Chemical Co., USA; pyro-Glu-Gly-Arg-pNA (S-2444) and H-D-Val-Leu-Lys-pNA (S-2251) from Kabi Group, Inc., USA; and HMW-UK and LMW-UK, purified by affinity chromatography and gel filtration as reported previously[7,15]. UK activity was determined by assessing its ability to hydrolyze pyro-Glu-Gly-Arg-pNA[16]. The enzyme activity was expressed in international units (IU) with Japanese Standard Urokinase (MW 003) as a standard. Active site titration with p-nitrophenyl-p'-guanidinobenzoate (NPGB) was carried out as described by Chase and Shaw[17]. Steady-state kinetic parameters of activation of Glu-plasminogen were determined in a coupled assay in which the plasmin formed was monitored by hydrolysis of H-D-Val-Leu-Lys-pNA at pH 7.4 and 37 °C, as described previously by Wohl et al.[18]. The preparation of [N^{α}-(ε-aminocaproyl)-DL-homoarginine hexylester]-Sepharose and other procedures of affinity chromatography were as described previously[15]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with 10% gels by the method of Weber and Osborn[19]. Isoelectric focussing was performed according to the method of Vesterberg and Svensson[20] using ampholytes of pH 3.5–10.5. Determinations of amino acid composition were performed by the technique of Gros and Labouesse[21]. Immunological methods with rabbit anti HMW-UK and anti human kidney HMW-UK sera were carried out as described previously[22].

Results and discussion. 5.50 mg of highly purified HMW-UK (spec. act. = 106,700 IU/mg protein, 92% active) was dissolved in 0.5 ml of 0.05 M phosphate buffer (pH 7.49) containing 0.15 M NaCl and 1.5 µg of hog pancreas elastase, and incubated for 30 min at 25 °C. Under these conditions, the HMW-UK molecule was digested and produced a new enzyme, ED-UK, with approximately 83% of the activity of the intact enzyme still being retained. By SDS-PAGE under nonreducing conditions, the material showed two main protein bands with molecular weights of approximately 31,000 and 19,000. The former had enzyme activity and was named ED-UK, whereas the latter had no activity. When the reactant was reduced with 0.1 M β-mercaptoethanol, the ED-UK band migrated to a position showing that it had a slightly lower mol. wt of about 30,000, and dis-