Activity of orotate metabolizing enzyme complex and various urea-cycle enzymes in mutant mice with ornithine transcarbamylase deficiency[1]

I. A. Qureshi, J. Letarte and R. Ouellet

Laboratoire d'Endocrinologie, Métabolisme et Nutrition, Centre de Recherche Pédiatrique, Hôpital Sainte-Justine, 3175 Chemin Ste-Catherine, Montréal (Qué., Canada H3T 1C5), 2 February 1981

Summary. The overall activity of the enzyme complex consisting of orotate phosphoribosyl transferase and orotidine monophosphate decarboxylase, and of various enzymes of the urea cycle, has been studied in sparse-fur (spf) mutant mice with ornithine transcarbamylase deficiency. The enzyme complex has a lower overall activity, which could be caused by disturbed pyrimidine metabolism due to hyperammonemia. Other enzymes of the urea cycle do not show any significant change.

Sparse-fur (spf) mutation arose spontaneously in the progeny of an irradiated male mouse at Oakridge, and has been maintained on various genetic backgrounds[2]. The mode of transmission of the spf gene is x-linked, and the biochemical phenotype is characterized by an abnormal activity of ornithine transcarbamylase (OTC)[3]. Evidence has been presented in respect of altered kinetics of the enzyme and excessive orotic aciduria in affected hemizygote males and heterozygous females[4]. This has similarities to the OTC deficiency hyperammonemia in children[5]. Since the orotate metabolism in these animals is disturbed, we planned to study its effect on the activity of the orotate metabolizing enzyme complex, comprising orotate phosphoribosyl transferase (PRTase) and orotidine monophosphate (OMP) decarboxylase, as well as other enzymes of the urea-cycle.

Materials and methods. The animals used in the experiment were the progeny of breeding stock supplied by Dr L. B. Russell of Oakridge National Laboratories, USA[3]. 6 males each were classified into affected hemizygotes and normals, according to their morphological phenotype, as well as orotate excretion[4]. The animals were sacrificed by guillotine, their livers removed and stored immediately at - 70 °C until enzyme analyses.

A radiochemical method[6] was employed which measures the rate of conversion of orotic acid to uridylic acid, a process requiring the activity of both orotate PRTase and OMP decarboxylase. {7-^{14}C} orotic acid was used as substrate and $^{14}CO_2$ released by the 2nd enzymatic step was collected and measured. 0.1 ml of a 1:20 diluted liver homogenate was incubated with 0.9 ml of 0.062 M sodium phosphate buffer, pH 7.5, containing 0.25 µmoles 5-phosphorylribose-1-pyrophosphate, 3 µmoles $MgCl_2$ and 0.5 µCi {7-^{14}C} orotic acid (0.25 µmoles). Reaction was terminated with 0.2 ml 1N H_2SO_4, after 1 h of incubation at 37 °C. $^{14}CO_2$ content was collected in hyamine hydroxide and radioactivity counted in 15 ml POPOP/PPO in a liquid scintillation counter (Packard, Model 338).

The activity of OTC was measured by the colorimetric method of Ceriotti[7] as adapted to liver tissue[8]. Carbamyl phosphate synthetase (CPS-1) and argininosuccinate synthetase (ASS) were determined by the method of Nuzum and Snodgrass[9]. Argininosuccinate lyase (ASL) was measured by a modification of the method of Takahara and Natelson[10]. Arginase (ARG) was determined by the technique of Shih et al.[11].

Results of enzyme activity were expressed as µmole of product formed (urea-cycle enzymes) or µmole of $^{14}CO_2$ produced (orotate metabolizing enzyme complex) per g liver · h and also per mg protein · h. Liver protein was measured by the method of Lowry et al.[12].

Results and discussion. Data presented in the table indicate that the activity of OTC follows the same pattern as described earlier[4], with affected hemizygotes having only 14% of the activity of the normal group. CPS-1, ASL and ARG activities are increased in affected hemizygotes, but these are not significantly different. On the other hand, the activity of orotate metabolizing enzyme complex is significantly lower in the affected hemizygotes as compared to normals.

The excessive synthesis and excretion of orotate in spf mice is caused by a structural defect in the OTC molecule. This results in the accumulation of carbamyl phosphate (CP) in the mitochondria which cannot be condensed to citrulline. Excess CP shifts to the cytoplasm, to stimulate the CPS-II in the pyrimidine pathway, which causes accumulation of orotate at the rate-limiting enzyme step of orotate PRTase[13]. In the present study, the decrease in the overall activity of orotate PRTase and OMP decarboxylase which

Activity of orotate-metabolizing enzyme complex and various urea cycle enzymes in spf mice

Enzyme	No. of animals	Normal mice	spf mice	p-values[a]
Orotate enzymes[b]	5	7.19 ± 0.27[c] (1785 ± 58) (1785 ± 58)	4.73 ± 0.53 (837 ± 120) (837 ± 120)	< 0.01
CPS-I	5	3.66 ± 0.66[d] (590 ± 76)	5.9 ± 0.76 (942 ± 120)	> 0.05 N.S.
OTC	6	103.8 ± 12.4 (27075 ± 4539)	14.3 ± 0.60 (2628 ± 173)	< 0.001
ASS	5	0.29 ± 0.07 (46.5 ± 9.4)	0.27 ± 0.03 (42.6 ± 3.4)	> 0.05 N.S.
ASL	6	0.82 ± 0.06 (209 ± 12)	1.11 ± 0.13 (217 ± 38)	> 0.05 N.S.
ARG	6	37.9 ± 2.9 (9645 ± 714)	43.1 ± 2.5 (8065 ± 812)	> 0.05 N.S.

[a] p-values calculated by Student's t-test, on the basis of enzyme activity/mg protein. [b] Overall activity of orotate PRTase and OMP decarboxylase. [c] Mean ± SEM nmole$^{14}CO_2$/mg protein h. (Values in parentheses are per g liver). [d] Mean ± SEM µmole product/mg protein · h. (Values in parentheses are per g liver). N.S. Not significant.

exist as a bifunctional complex in mammals (as reviewed by Levine et al.[14]) could be caused by a repression of orotate PRTase. Hoogenraad and Lee[15] have shown that uridine can inhibit de novo synthesis of pyrimidine in cultured hepatoma cells by an effect on the level of activity of orotate PRTase. Uridine, uracil and certain other pyrimidine metabolites are in excess in OTC deficiency hyperammonemia and orotic aciduria[5]. The excess of these pyrimidine metabolites could, therefore, be the cause of a repression of synthesis of the orotate metabolizing enzyme complex in spf mice.

Slightly higher activities of CPS, ASL and ARG in spf mice, which are statistically non-significant, would not have any importance, as individual enzyme deficiencies in hereditary urea cycle disorders are not known to cause any remarkable changes in other enzymes of the cycle[16].

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Diolefin analog of a sex pheromone component of *Heliothis zea* active in disrupting mating communication

D.A. Carlson and J.R. McLaughlin

Insects Affecting Man and Animals Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 14565, Gainesville (Florida 32604, USA), and Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 14565, Gainesville (Florida 32604, USA), 21 April 1981

Summary. A hydrocarbon (*Z*)-1,12-heptadecadiene, was synthesized from the major sex pheromone of the corn earworm, *Heliothis zea* (Boddie). In field tests, significant disruption of the sexual behavior of *H. zea* males was obtained, comparable to the pheromone (*Z*)-11-hexadecenal. This novel diolefin should be inexpensive, exhibit improved stability, and may lead to improved methods of control.

Disruption of mating communication in moths via air permeation has been accomplished with pheromones and also with non-pheromonal chemicals having structural properties similar to the pheromone components of the target species. The corn earworm, *Heliothis zea* (Boddie), and the tobacco budworm, *H. virescens* (F.) utilize (*Z*)-11-hexadecenal (HDA) as a major component of the females' sex pheromone. A structurally related analog, (*Z*)-9-tetradecen-1-ol formate, was an effective disruptant of mating communication in air permeation trials against these species[2,3]. An olefin, (*Z*)-5-hexadecene, was found to disrupt mating communication in *Chilo suppressalis* (Walker) in field tests, and also inhibited the response of males to pheromone-releasing females in a trap[4].

Aldehyde pheromones are expensive, tend to polymerize when stored in bulk, and present longevity problems in the field due to air oxidation and photosensitivity. Alternative materials with useful behavioral effects, greater stability and reduced cost are potentially useful in insect control. We report the 1st field tests of (*Z*)-1,12-heptadecadiene, a non-oxygenated, doubly-unsaturated hydrocarbon analog of a natural pheromone component that disrupts mating communication of *H. zea*. This analog was synthesized from (*Z*)-11-hexadecenal (Chemical Sample Co., Columbus, Ohio, USA) via a Wittig reaction using methyltriphenylphosphonium bromide (Ventron, Danvers, Massachusetts, USA) and *n*-butyl lithium (PCR, Gainesville, Florida, USA) (Carlson, unpublished data). The diolefin was eluted from a silica gel column with hexane and analyzed by GC on a 1.8 × 2 mm glass column packed with 3% OV-1 held at 110 °C. The major peak contained 99.8% of the material obtained, and eluted at the equivalent of 16.7 carbons, compared to paraffin standards, while the formate eluted at 17.0 and the starting HDA eluted at 17.7 carbon equivalents (table). The increased volatility of DO suggests that equiv-

(*Z*)-11-hexadecenal and some analogs: GC retention indices, retention times

Structure	Notation	RI[a]	Tr/min[b]
(*Z*)-1,12-heptadecadiene	DO	16.70	6.74
(*Z*)-5-heptadecene	Olefin	16.85	7.25
(*Z*)-9-tetradecen-1-ol formate	Formate	17.00	7.74
(*Z*)-11-hexadecenal	HDA	17.75	11.37

[a] Retention indices in carbon number equivalents. [b] Retention times by gas chromatography.