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Kinetic arguments for the existence of a single form of intestinal ornithine decarboxylase during the postnatal development of normal and sparse-fur mutant mice and after EGF treatment

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Summary. The K_m for ornithine is remarkably constant during the course of postnatal development in both normal and spf mutant mice even if a large but transient increase in ornithine decarboxylase (ODC) activity is noted. Four hours after EGF injection (4 μg/g b.wt) to 17-day-old normal and spf mice, a marked stimulation of ODC activity is observed but K_m remains unaffected. These data argue against the existence of multiple forms of ODC in the intestinal mucosa of mice.
Key words. Ornithine decarboxylase; small intestine; postnatal development; kinetic properties; EGF treatment; sparse-fur mutant mice.

Ornithine decarboxylase (ODC, EC 4.1.1.17), the first enzyme in the pathway leading to polyamine biosynthesis, catalyses the conversion of ornithine to putrescine. There is increasing amount of experimental evidences indicating that ODC plays a key role during the differentiation and proliferation of a variety of tissues and cells [2–4]. In the mouse [5] and rat [6] small intestine, ODC activity increases during the course of the normal postnatal development as well as following mucosal injury, jejunectomy and during lactation [7]. We have recently demonstrated that the intestinal ODC activity is lower in suckling sparse-fur (spf) mutant mice as compared to normal animals [5]. This strain of mouse exhibits X-linked ornithine transcarbamylase (OTC, EC 2.1.3.3) deficiency and thus represents a useful model to study the effects of an impaired ornithine metabolism on polyamine biosynthesis during the course of postnatal development.

In rat heart [8] and liver [9, 10] as well as in mouse kidney [10, 11], the existence of multiple forms of ODC has been documented. In rat heart, a change in the affinity for ornithine has been observed after hormonal, neuronal and ontogenic stimuli [8] and a heat-sensitive form seems to be preferentially induced after androgen stimulation in mouse kidney [11]. In adult rat ileum [12], ODC activity was found to be stimulated by epidermal growth factor (EGF) and glucagon while duodenal ODC has been shown to increase after EGF injection to 8-day-old suckling mice [13]. One control mechanism proposed for the rapid increase of ODC activity lies in the existence of multiple forms of the enzyme [8–11] with different affinities for L-ornithine [8, 9]. However, this hypothesis has never been tested on intestinal ODC during the course of normal postnatal development nor after hormonal induction. In the present study, we have determined the kinetic parameters of the enzyme in the intestinal mucosa from both normal and spf mice and after EGF treatment. There was no change in the affinity for L-ornithine in either situations, which suggests the presence of a single form of ODC in the intestinal mucosa of mice.

Materials and methods. Sparse-fur hemizygous male mice (spf/Y) were used as experimental animals and normal Swiss ICR male mice as controls. Mutant spf male mice were kindly provided by Dr Ijaz A. Qureshi, form Ste-Justine Hospital where inbreeding was done as previously described [5]. Post-weaning animals were fed ad libitum on mouse Purina chow (Ralston Purina). 17-day-old normal and spf mice were injected s.c. on the dorsal surface with 4 μg of EGF/g b.wt or equivalent volume of water for the control animals, as established previously [14]. The mice were then returned to their mother for the next 4 h. 13-, 17-, 21-, 25-day-old and 8-week-old normal and spf mice were killed by decapitation without being fasted. Controls and EGF-treated animals were sacrificed 4 h after injection. The first 15 cm of the small intestine were removed and rinsed with cold saline. The mucosa was scrapped with a spatula, weighed and used immediately for the determination of ODC activity. Since there was no difference between 17-day-old normal and control animals, they were all combined and included as controls in the table.

The tissues were homogenized in 5 vols of 0.1 M Tris-HCl (pH 7.4) containing 0.1 mM EDTA, 5 mM dithiothreitol and 0.3 mM pyridoxal 5'-phosphate and then centrifuged at 100,000 × g for 60 min. ODC activity was determined in the supernatant by measuring the rate of formation of $^{14}CO_2$ from L-[1-^{14}C] ornithine as previously described [5]. Samples of the supernatant were incubated in the presence of 1 μCi L-[1-^{14}C] ornithine (spec. act. 57 mCi/mmol, Amersham, Oakville, Ontario, Canada) and various concentrations of cold L-ornithine (0.05–3 mM). Blanks were incubated with 10 mM difluoromethyl-ornithine (DFMO) (a gift from Dr P. McCann, Merrell Research Center), a specific inhibitor of ODC. Radioactivity was counted in Aquasol II using a Minaxi Tri-Carb Series 4000, model 4450 scintillation counter

(United Technologies Packard). Results were expressed as picomoles of CO_2 released/h/mg protein ± SEM. K_m and V_{max} were determined according to Eadie[15] and Hofstee[16]. Regression analysis have been performed using an Apple IIe microcomputer and a curve-fitter program (P. K. Warme, Copyright 1980, Interactive Microware Inc.). Proteins were determined according to Lowry et al.[17] using crystalline bovine serum albumin as standard.

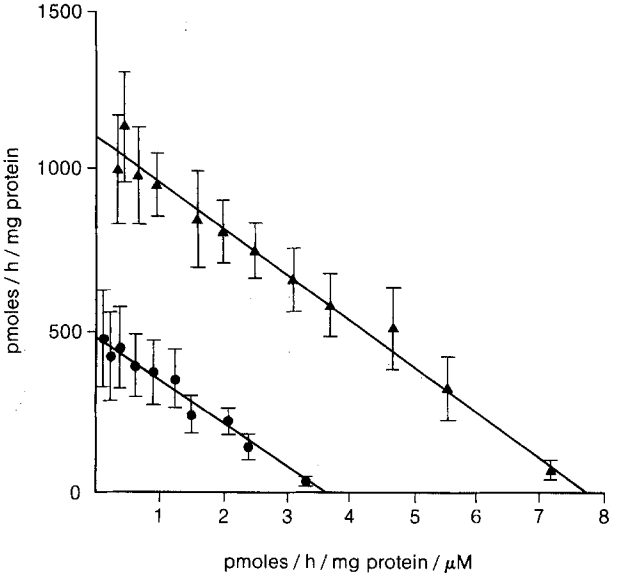
Results and discussion. Many mechanisms have been proposed for the control of ODC activity[2-4, 18]. Among these, the existence of multiple forms of the enzyme has been suggested by several investigators from studies showing enzyme heterogeneity by column chromatography[9, 19, 20] or differences in biological[10, 11] or kinetic[8, 9] properties. In order to verify if the large transitory increase of intestinal enzyme activity observed during the course of the normal development involves the appearance of a new form of ODC, we have determined the kinetic parameters of the enzyme and compared them with the kinetics of ODC in adult mice. As previously demonstrated[5], ODC activity is very low at 13 days of age in both normal and spf mice and increases thereafter. Kinetic studies were then performed on both groups between 17 days and 8 weeks of age. There was no significant difference in the K_m for ornithine from 17 days after birth to the adult period in normal mice, even if the V_{max} of the enzyme showed a large increase after 21 days (table). The same behaviour was also observed in spf mice: the K_m values were identical at all stages of postnatal development and did not differ significantly from the K_m determined in normal mice. Nevertheless, highly significant differences were noted in the V_{max} between 21- and 25-day-old normal and spf mice, which was in complete agreement with the developmental pattern previously reported[5, 6]. There was no sign of heterogeneity in the Eadie-Hofstee plot, over the wide range of substrate concentrations used in these experiments. These data indicate that the lower ODC activity observed in spf mice during the suckling period must be attributed mainly to the presence of less active enzymatic protein in the enterocytes rather than to the presence of a different form of the enzyme.

The ODC activity increases very rapidly in response to a variety of agents such as hormones, drugs and tumor promoters[18]. Only 4 h following injection of 4 µg of EGF to 17-day-old normal mice, ODC activity reached the value normally observed at 21 days, which represents a 13-fold increase over the control value (V_{max}: 94.7 vs 1121.5 pmoles CO_2 released/h/mg protein). However, the K_m was not affected by EGF treatment (143.9 ± 5.8 µM) (fig.). In EGF-treated spf mice, the V_{max} also increased to the 21-day-old value (472.9 ± 14.7 pmoles CO_2 released/h/mg protein in 17-day-old EGF-treated spf mice) but the K_m was not significantly affected (132.2 ± 9.1 µM). The Eadie-Hofstee plots were linear in both EFG-treated normal and spf mice and the slopes were parallel. Based on these kinetic arguments, we can conclude that there is a single form of ODC activity in the intestinal mucosa of normal and mutant mice and that change in affinity for L-ornithine is not responsible for the rapid increase of activity observed only 4 h after EGF administration.

Kinetic properties of ODC in normal and spf mice during postnatal development

	Controls		spf	
	K_m	V_{max}	K_m	V_{max}
17 days	148.1 ± 28.8	94.7 ± 34.3 (6)	N.D.	N.D.
21 days	138.9 ± 21.0	1244.1 ± 103.4 (4)	161.4 ± 8.8	426 ± 83.8 (4)*
25 days	115.7 ± 16.1	1158.4 ± 99.6 (4)	147.9 ± 19.7	556.4 ± 37.3 (3)*
Adults	168.7 ± 27.7	638.5 ± 36.0 (3)	174.7 ± 31.4	778.0 ± 123.4 (4)

K_m (µM ± SEM) and V_{max} (pmoles CO_2 released/h/mg protein ± SEM) were determined by linear regression analysis of the Eadie-Hofstee plot. Number of animals used in each group is indicated between parentheses. N.D., non-detectable value; * significantly different from the control value; $p < 0.0005$.



Eadie-Hofstee plot of ODC activity at varying concentrations of L-ornithine (0.05–3 mM). Normal (▲) and spf (●) mice were killed 4 h after a single injection of EGF (4 µg/g b.wt, s.c.) on the dorsal surface. Each point represents the mean ± SEM of three different determinations, each done in duplicate. Kinetic parameters and coefficients of correlation have been determined by linear regression analysis. Control animals (▲): K_m: 143.9 ± 5.8 µM, V_{max}: 1121.5 ± 19.9 pmoles CO_2 released/h/mg protein, r: 0.984; spf mice (●): K_m: 132.2 ± 9.1 µM, V_{max}: 472.9 ± 14.7 pmoles CO_2 released/h/mg protein, r: 0.982.

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