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THE EFFECT OF HYPERTHERMIA ON ORNITHINE DECARBOXYLASE ACTIVITY IN DIFFERENT RAT TISSUES

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Abstract—Hyperthermia produced a decrease of ornithine decarboxylase activity in different tissues of adult rats. The fall in ornithine decarboxylase was dependent on time of exposure and temperature. The decay of ornithine decarboxylase activity in liver, brain, kidney, heart, spleen and testes was rather similar. The $t_{1/2}$ for liver ornithine decarboxylase determined by the hyperthermic treatment (40° ambient temperature) was 20 min. Ornithine decarboxylase activity was recovered in all tissues exposed to the hyperthermic shock after a period of 4 hours, although the degree of recovery was dependent on the type of tissue. The effect that hyperthermia produces on ornithine decarboxylase activity in rats could be related to an inhibition in the synthesis of active enzyme rather than to a specific degradation or inactivation of ornithine decarboxylase molecule.

Ornithine decarboxylase (EC 4.1.1.17, ODC) is the rate-limiting enzyme in the synthesis of polyamines by animal cells [1–5] and seems to be a key regulatory enzyme in the growth process [6]. Its activity is subject to a variety of influences, being the mammalian enzyme induced in most tissues in response to several growth-promoting stimuli, and exhibiting wide fluctuations in activity depending on the type of tissue and animal age [7–9]. The enzyme presents a remarkable rapid rate of turnover with $t_{\frac{1}{2}}$ of about 15 min [7], and therefore its activity can vary rapidly in response to changes in the rate of its synthesis or degradation; hence the enzyme amount decreases significantly *in vivo* by inhibitors of protein synthesis [7, 10–12].

Hyperthermia is a physiological situation produced in thermoregulatory animals in response to several agents [13], that causes changes in some biochemical processes both in cell cultures and in whole animals [14]. A decrease in the incorporation of labelled amino acids into proteins as well as disaggregation of polyribosomes have been observed in a variety of animals [15–17]. A fall in the rate of synthesis of proteins should affect mainly those proteins with high rate of synthesis, such as ODC. Although hyperthermia greatly affects the activity of ODC in cell cultures [18–20], the studies on the effect of whole body hyperthermia on ODC activity in animals are scarce and incomplete. In young rats hyperthermia produces a decrease of ODC activity in brain but not in testes [15]. In mice, raising the core body temperature causes the decrease in kidney ODC activity [21]. Moreover, some adverse effects of hyperthermia in the rat depend on the duration and extent of temperature elevation [22]. The aim of the present work is to determine the effect of different grades of hyperthermia on ODC activity from several rat tissues, both during the hyperthermic period itself and in the recovery period after the heat shock.

MATERIALS AND METHODS

Chemicals. DL-[1-^{14}C]-Ornithine (54 mCi/mmol) was obtained from Amersham. Cycloheximide and Actinomycin D were purchased from Sigma. All other chemicals and reagents were of the highest purity commercially available.

Animals. Male rats of the Sprague–Dawley strain, weighing 150–200 g were used for all the experiments. In order to induce hepatic ornithine decarboxylase, all animals were given an intraperitoneal injection of thioacetamide (150 mg/kg of body weight) 20 hr before killing. Acute hyperthermia was induced by placing the rats in an incubator chamber with controlled temperature and humidity. Rectal temperature was monitored with a thermometer (Ellab TF3) using RM6 thermocouple probes. Control rats were maintained at room temperature (22–24°). Rats were killed by decapitation, and liver and the other organs removed and frozen in liquid nitrogen. After homogenization by Polytron in 25 mM sodium phosphate buffer pH 7.2 containing 0.25 M sucrose, 2 mM dithiothreitol, 0.1 mM EDTA and 0.1 mM pyridoxal phosphate, the extracts were centrifuged at 105,000 *g* for 1 hr and the supernatants used to measure ODC activity.

Ornithine decarboxylase assay. ODC was determined by measuring the rate of $^{14}CO_2$ evolved from L-[1-^{14}C]-Ornithine. The assay mixture contained 25 mM sodium phosphate buffer pH 7.2, 2 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM pyridoxal phosphate, 0.3 mM L-ornithine, 0.25 μCi of ^{14}C-ornithine and 100 μl of enzymatic extract in a total volume of 0.25 ml. The reaction was carried out at 37° in tubes sealed with rubber stoppers suspending paper filter discs containing hyamine hydroxide 1 M in methanol. After 1 hr the reaction was stopped by

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Table 1. Effect of hyperthermia on ODC activity in liver of thioacetamide-treated rats

Temperature of exposure (°C)	Time of exposure (min)	ODC activity*	Increase in body temperature (°C)†
22	25	203.5 ± 55.4 (6)	—
38		176.3 ± 52.3 (6)	2.36 ± 0.40
40		217.4 ± 40.9 (6)	2.43 ± 0.46
42		181.8 ± 50.3 (7)	4.55 ± 0.25
22	45	209.9 ± 59.0 (6)	—
38		126.6 ± 23.5 (7)‡	3.53 ± 1.20
40		69.6 ± 19.0 (6)§	4.90 ± 0.26
42		68.0 ± 29.4 (6)§	6.80 ± 0.60

* Values are means ± SD. Number in parentheses refers to the number of animals. Activity is expressed as nmoles CO_2/hr·g tissue.

† Increase at the end of hyperthermic treatment. The mean rectal temperature of control animals maintained at room temperature (22°) was 36.8 ± 0.56°.

‡ $0.01 < P < 0.001$. § $P < 0.001$

the addition of 0.5 ml of 3 M citric acid. After 45 min, the paper discs were removed and counted on toluene-based scintillation mixture. Activity was expressed as nmoles of CO_2 released per hour.

Amino acid analysis. Amino acids were determined from deproteinized samples by an amino acid autoanalyzer (Rank–Hilger Chromaspeck) equipped with a fluorometric detector.

RESULTS

Effect of hyperthermia on hepatic ODC in adult rats

Hyperthermia produced the decrease of ODC activity in adult rats treated with thioacetamide. The fall in ODC activity was dependent both on temperature and duration of exposure. After 25 min of exposure to the elevated temperatures selected (38, 40 and 42°) no significant diminutions in the activity of ODC were observed. However, exposures for 45 min produced significant decreases in ODC activity in the three experimental temperatures used (Table 1). While in the rats exposed to 40° or 42° the ODC decrease was greater than when exposure was 38°, no significant variations were observed between 40° and 42°. The fact that exposure to high ambient temperature for 25 min did not produce any significant fall in ODC activity must be related to the slow increase in body temperature at the beginning of the hyperthermic treatment, as tested by continuous recording of rat rectal temperatures (the time needed to reach rectal temperatures above 39° oscillated about 20 min depending on the value of exposed temperature—results not shown).

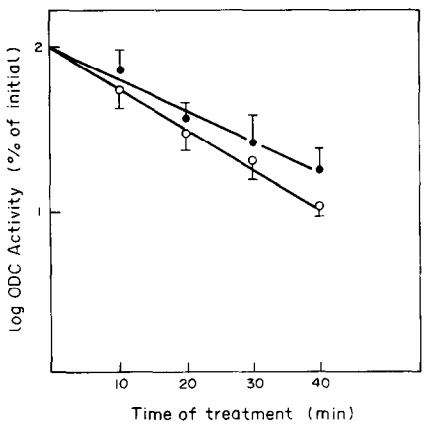

Fig. 1. Loss of ODC activity from rat liver after cycloheximide or hyperthermic treatment: (○) cycloheximide (10 mg/kg); (●) hyperthermia (40°). Time was taken from the moment rats reached 39° body temperature. Each value is the mean ± SD of individual determinations from 4 rats.

Changes in hepatic ODC activity during hyperthermia and recovery

To study the decay of ODC activity in liver, rats were exposed at 40° for different periods of time started from the moment the rats reached 39° of rectal temperature. Figure 1 shows the time-dependent variation of ODC activity in the hyperthermic rats and in rats treated with cycloheximide (10 mg/kg). The decay was rather similar, being $t_{\frac{1}{2}} = 15$ min when protein synthesis was almost totally inhibited by cycloheximide and 20 min the apparent $t_{\frac{1}{2}}$ determined under hyperthermic conditions. Figure 2 shows the thermal sensitivity of hepatic ODC under *in vitro* conditions. The semi-inactivation period was 150 min at 42° and 41 min at 45°, and these values are much longer than those observed at lower tem-

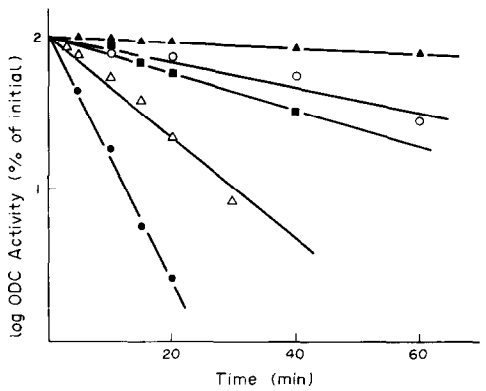

Fig. 2. *In vitro* thermoinactivation of ODC activity from rat liver: (▲) 42°; (○) 45°; (■) 48°; (△) 49.5°; and (●) 52°.

Table 2. Levels of some free essential amino acids in the soluble fraction of liver extracts from rats exposed for 45 min at different hyperthermic conditions

Amino acid*	Control (22°)	Temperature of exposure 38°	40°	42°
Tyrosine	2.28 ± 0.51	2.91 ± 0.51	2.90 ± 0.56	5.12 ± 2.92
Leucine	4.05 ± 0.28	5.64 ± 0.44	6.42 ± 0.83	6.82 ± 2.21
Valine	3.66 ± 0.63	3.97 ± 0.48	6.87 ± 0.79	7.11 ± 2.89
Isoleucine	1.55 ± 0.28	2.76 ± 0.99	2.39 ± 0.21	2.48 ± 1.28
Methionine	2.08 ± 0.29	3.28 ± 1.75	3.01 ± 0.33	3.41 ± 1.33
Lysine	8.54 ± 0.68	8.16 ± 2.65	13.28 ± 4.05	12.50 ± 6.41

* Values are expressed as nmoles/mg protein (mean ± SD).

peratures under *in vivo* conditions. These results show that the fall of hepatic ODC activity produced by hyperthermia in rats is not directly related to a non-metabolic thermolability of the enzyme. Changes in the rates of synthesis or degradation of ODC could be responsible for the effect produced by hyperthermia on ODC levels.

The determination of free amino acid levels in the soluble fraction of liver extracts from rats exposed to hyperthermic conditions showed a significant increase in the concentration of some essential amino acids (Table 2). These values were very similar to those found after 30 min of cycloheximide treatment (data not shown). This rise in the free amino acids levels can be produced either by a decrease in the biosynthesis of proteins or by a higher proteolytic activity. In Table 3 the effect of cycloheximide or hyperthermic treatments alone are compared with the combined treatment of cycloheximide plus hyperthermia. No significant differences were observed. This result indirectly indicates that the decrease in ODC activity produced by hyperthermia could be related to an inhibition of its synthesis rather than to an accelerated degradation of ODC.

Figure 3 shows the changes in hepatic ODC during the period of hyperthermia and recovery. The dramatic decrease in ODC activity produced by the thermal shock is followed by a progressive increase through the recovery period at 22° ambient temperature. After 4 hours of recovery the levels of hepatic ODC were similar to controls. Treatment with cycloheximide in the beginning of the recovery period prevented the recuperation of liver ODC but actinomycin D did not, suggesting that the increase of ODC levels in the recovery period is dependent on protein synthesis and independent on transcription.

Effect of hyperthermia on ODC activity in other rat tissues

Table 4 shows the effect of hyperthermia (40° for 45 min) on ODC activity in other rat tissues such as brain, kidney, spleen, heart and testes. A significant decrease in ODC activity was observed in the tissues, all of them having a similar pattern. However, the recovery under normothermic conditions varied from one tissue to another. While in liver and spleen the activity was fully recovered, brain showed a two-fold increase, and heart, kidney and testes did recover only partially, less than 50% the activity of controls, at least during the period of 4 hours.

DISCUSSION

Both hyperthermia and enzyme-activated suicide inhibitors of ODC, such as α-difluoromethylornithine, have been shown to adversely affect rapid growing in normal or neoplastic tissues [23–28] and there are some experimental trials using them in cancer therapy [25, 27, 29–31]. Besides, both treatments can produce undesirable effects in normal cells at least in some periods of growth, mainly during embryonic and fetal development [23, 28, 32].

Table 3. Effect of combined treatment of cycloheximide and hyperthermia on hepatic ODC levels

Treatment	Time (min)	Activity remaining (%)
None	—	100
Hyperthermia (40°C)*	10	82.6
	20	43.1
	30	27.0
Cycloheximide (10 mg/kg)	10	54.0
	20	36.0
	30	25.0
Hyperthermia plus cycloheximide†	10	49.5
	20	38.2
	30	27.6

* The time of hyperthermia was taken from the moment that rats reached 39° rectal temperature.

† Cycloheximide was injected i.p. when rats had reached 39° rectal temperature.

All values represent the mean of three determinations.

Table 4. Effect of hyperthermia and recovery on ODC in different tissues

Tissue	Initial activity*	% Activity remaining after hyperthermia†	% Activity after recovery‡
Liver	2.68	48	123
Brain	0.15	20	184
Heart	0.09	27	36
Kidney	0.26	34	40
Spleen	0.21	28	82
Testes	0.79	43	45

* Expressed as nmoles CO_2/hr · mg protein.
† Exposed at 40° for 45 min.
‡ After 4 hr recovery at room temperature; the number of animals was 8 and all values are the mean of three determinations of the extract of pooled organs.

Although the pharmacological effect of α-DFMO is related to the depletion of polyamines [27], the cytocidal effect of hyperthermia can be the result of a multifactorial action, though some relationship appears to exist between hyperthermia and polyamine levels [20, 33–35]. In this context, inactivation of ODC by heat has been reported both in cell cultures [18–20] and in whole animals [15, 21]. Despite inhibition of protein synthesis having been postulated as the cause of ODC fall [15, 19] it has been recently shown that the heat-shock produced the fall of ODC in kidney while protein synthesis remained unchanged in this tissue [21]. Moreover, it has been reported that heating at 42° for 15 min induced ODC activity in several rat tissues and, accordingly, that ODC may be a heat-shock protein [36]. These apparently contradictory results must be analyzed in the light of the complexity of the biochemical effect of hyperthermia, especially on the process of protein synthesis. In this regard, exposure at temperature above 42° inhibited total protein synthesis, while temperatures below 41° did not affect the translational process [37]. Also, the effect of hyperthermia on protein synthesis in intact animals seems to be dependent on factors such as the kind of tissue, age, and type of animal [15, 38]. Moreover, the evaluation of protein synthesis by measuring the incorporation of labelled amino acids into protein is subjected to some ambiguities, and it is also known that heat-shock induces a mechanism of translational control which promotes the translation of the messenger RNA's of the heat-shock proteins but reduces the translation of other messengers [39].

Our results show that the decrease in ODC activity produced by hyperthermia depends on the elevation of body temperature and duration of the exposure. A time of exposure of 25 min or shorter is not sufficient to produce a significant decrease in ODC activity. However, 45 min of exposure at 38° produced 40% inactivation of ODC and at 40° or 42° this value rose to 70%. These results can be explained if one assumes that a critical body temperature is needed to produce changes in the turnover of ODC. There is a first period during the exposure in which the body temperature is under a critical value and no effects on ODC activity can be detected. When this limit is exceeded, the ODC level will considerably decrease with time. According to Table 1, one can assume that this limit in body temperature lies between 39–40°.

As mentioned above, one of the main reasons for the fall of ODC by hyperthermia could be related to the effect that heat produces on protein synthesis. However, other processes might be responsible for the loss of ODC activity. Our *in vitro* experiments on heat inactivation of ODC from liver extract show the possibility that an extremely high thermal lability of the ODC molecule as the cause of the inactivation *in vivo* can be ruled out. The elevation of free amino acid levels in the liver of hyperthermic rats shown in Table 3 can, in principle, be related either to an inhibition of protein synthesis or to an increased proteolytic activity (or both). The role that proteolysis may play in the changes in ODC activity in the hyperthermic state must not be underestimated, since stimulation of muscle protein degradation by hyperthermia or fever has been described [40]. If an increased proteolytic degradation of ODC was produced by hyperthermia, the combined treatment with cycloheximide and hyperthermia must give an accelerated fall in ODC when compared with treatments with cycloheximide or hyperthermia alone, but our results show that this is not the case.

The effect that hyperthermia provokes on liver ODC activity is transient, since the activity is recovered after the hyperthermic shock. Figure 3 shows that the recovery of activity is proportional to the recovery time, reaching the control value of ODC activity after a period of 4 hr. The facts that treatment with cycloheximide during the recovery abolishes the rise of ODC activity and that ODC recovery in the presence of Actinomycin D is complete indirectly suggest that the increase of ODC activity during recovery is dependent on protein synthesis and independent of transcription.

Although in young rats ODC activity is affected by hyperthermia in brain but not in testes [15] and in rabbits hyperthermia produces dissaggregation of polysomes in brain but not in kidney [41, 42], our results show that hyperthermia in adult rats decreases ODC levels in all the tissues studied, including liver, brain, heart, kidney, spleen and testes. The heat sensitivity is rather similar in all of them and the fall in ODC seems to be independent of the level of activity. However, the recovery of ODC after hyperthermic shock considerably differs from one tissue

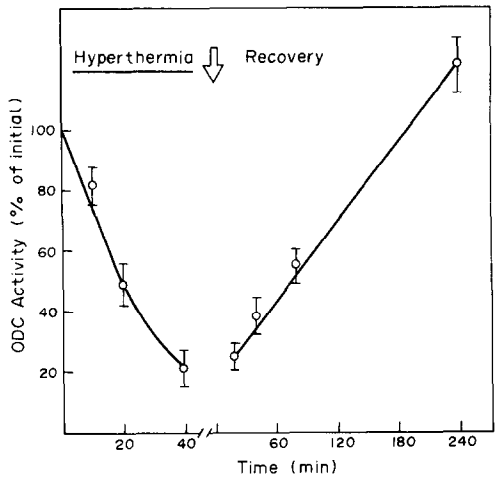

Fig. 3. Decay and recovery of ODC activity during and after hyperthermic treatment. Rats exposed to hyperthermic conditions, 40° ambient temperature. After the hyperthermic shock (45 min), the rats were returned to an ambient temperature of 22°; rectal temperature decreased under 39° after 15 min and from that moment rats were killed at different periods of time (recovery time).

to another. This interesting feature is difficult to analyze mainly because of the great complexity of mechanisms regulating ODC activity [3, 5, 43].

In conclusion, our results show that hyperthermia decreases considerably the ODC activity in all rat tissues which have been tested, this fall being dependent on both length and temperature of exposure. Moreover, the recovery of ODC activity in the period of a few hours after the end of the hyperthermic shock also depends on the type of tissue, since the pattern of recovery for every tissue is different. This indicates that the polyamine levels could be altered mainly in those tissues where the recovery of ODC activity is slow or incomplete. Although the teratogenic effects of hyperthermia are related to a complex action of temperature on different structures, our results suggest that hyperthermia could produce more severe effects on the growth or differentiation of tissues of high ODC activities, independently from other effects that such treatment can produce, at a general level, in structures that have nothing to do with ODC and polyamines. This selectivity in the action of heat on ODC in different tissues can be of interest in relation to the application of hyperthermia in cancer therapy. In this context, preliminary studies carried out in our laboratory on ODC from Harding-Passey mouse melanoma, have shown that the recovery of ODC is a slow process in this tumour [44] and that the effectiveness of the hyperthermia therapy could be partially related to that.

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