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Ontogeny of Stress Effects on Ornithine Decarboxylase Activity in Rats

CYNTHIA M. KUHN, ALBERTO GRIGNOLO AND SAUL M. SCHANBERG

Department of Pharmacology, Duke University Medical Center, Durham, NC 27710

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KUHN, C. M., A. GRIGNOLO AND SAUL M. SCHANBERG. *Ontogeny of stress effects on ornithine decarboxylase activity in rats.* PHARMACOL BIOCHEM BEHAV 18(5) 669–672, 1983.—This study demonstrates that "stress" elicits a specific pattern of organ response in developing rats that is determined by the particular "stress" and by the age of the animal. Maternal deprivation (MD) of preweanling rat pups decreases ornithine decarboxylase (ODC) activity in many tissues, as reported previously, while immobilization (IMM) and cold stress increase ODC activity in liver and heart of neonatal rat. Serum GH is decreased by MD and by IMM, but is not affected by cold stress. Stress-induced ODC elevation increases with age, while MD effects disappear at weaning. IMM and cold increase ODC activity in kidney, liver and heart of adult rats. These effects of IMM are blocked by the ganglionic antagonist chlorisondamine in adult but not in neonatal rats. The results of this study suggest that MD and the "classical" stress paradigms IMM and cold evoke different patterns of organ ODC response in neonatal rats. Furthermore, these findings suggest that the mechanism by which immobilization increases ODC activity changes from a hormonal to a neural mechanism during ontogeny.

Maternal deprivation Stress Ornithine decarboxylase

MATERNAL deprivation (MD) of preweanling rat pups elicits a characteristic pattern of hormonal and biochemical response that includes suppressed organ ornithine decarboxylase (ODC) activity, decreased serum serum growth hormone levels and impaired tissue responsivity to growth hormone [3, 4, 8, 9]. It is not clear if these effects of MD represent a unique response to this environmental manipulation or if other environmental stimuli elicit a similar pattern of response. Several findings support the former hypothesis. The effects of maternal deprivation are reversed only by a very specific form of tactile stimulation which resembles maternal stimulation of the pups, not by any intense stimulus [6]. In addition, at least two different "stresses," immobilization (IMM) and cold, elevate rather than suppress ODC activity in certain organs in adult rats [6]. However, the increase in ODC observed in stressed adults could reflect a difference between the response of adults and that of neonates to these stimuli, rather than a differential effect of specific stressors on ODC activity. Furthermore, at least one maternal deprivation effect, the decrease in growth hormone secretion, is a characteristic stress response in rats which is observed following a number of different environmental manipulations [2].

The goal of the present study was to determine if MD elicits responses that are specific to this manipulation, and to evaluate the effects of other stresses on ODC activity in developing rats. We have compared the biochemical effects of maternal deprivation in neonatal rats to those of IMM and cold stress. In addition, we have further characterized the effects of IMM and cold stress during ontogeny in rats. This study demonstrates that each of these stresses elicits a unique pattern or organ response in developing rats, and so suggests that ODC responds in a specific way both to maternal deprivation and to other "stresses."

METHOD

Animals

Lactating female rats with litters of ten pups and adult male rats were obtained from Zivic Miller Laboratories (Allison Park, PA). All rats were housed in a vivarium with a 12 hr light and dark cycle and an ambient temperature of 22°C. Rats were transferred to the laboratory the night before each experiment.

Stress Paradigms

The maternal deprivation method previously developed in this laboratory was used [3,6]. Pups were removed from the mother, and placed in a plastic cage containing home cage shavings that was inside an "isolette" infant incubator (Air Shields Inc.). The incubator was maintained at 33–34°C and was equipped with a humidifier and handling ports so that experimental manipulations could be done without removing the pups. In this environment, body temperature of MD pups did not differ significantly from that of controls. Controls were removed from the home cage and then returned immediately. In all experiments, pups were randomized and half of each group deprived and half returned to lactating mothers. Previous studies have shown that cross-fostering pups has no effect on tissue ODC activity. Animals were immobilized by placement in wire mesh cages, either in the incubator (for pups), or in the laboratory at room temperature (for adults). Animals were placed in a 4° cold room for cold stress experiments. To prevent an excessive fall in body temperature in younger animals with limited ability to thermoregulate, bedding was placed in the cages to partially insulate animals from the cold. Preliminary experiments demonstrated that extreme hypothermia resulted in a fall in ODC

TABLE 1

EFFECT OF STRESS ON TISSUE ODC ACTIVITY IN NEONATAL RATS

	Tissue ODC Activity (percent control)			
	Heart	Liver	Brain	Serum GH
Control	100 ± 13 (58)	100 ± 24 (49)	100 ± 11 (14)	31 ± 4 (12)
MD	40 ± 9* (57)	14 ± 4* (48)	39 ± 3* (15)	17 ± 2* (12)
MD + IMM	101 ± 14† (35)	98 ± 49† (20)	—	7 ± 2† (7)
MD + Cold	151 ± 32† (35)	140 ± 31† (17)	70 ± 9† (15)	26 ± 3† (7)

Eight to ten day old rat pups were deprived in an incubator, placed in an incubator in wire restraining cages or placed in a 4° cold room for 2 hours. Controls were left with the mother for this time period. At the end of 2 hours, all animals were killed and tissue ODC activity was determined. Results are expressed as percent control ± SEM. GH data are expressed as ng/ml. N is indicated in parentheses.

*Indicates statistically different from control, $p<0.05$ or better.

†Indicates statistically different from deprived animals, $p<0.05$ or better. Control ODC activity=0.487 nmole/g/hr for liver, 1.190 nmole/g/hr for heart and 0.747 nmole/g/hr for brain.

activity, while limited hypothermia induced ODC activity. The amount of bedding was decreased with age, and omitted entirely after day 16. Body temperature of cold-stressed 8–10 day old pups decreased an average of 3.5°C, and body temperature of immobilized pups decreased an average of 1°C. In contrast, body temperature of cold-stressed 35-day-old animals decreased only 1° and body temperature of immobilized 35-day-old animals increased transiently (at 30 minutes) and then remained at normal levels for the duration of the experiment.

ODC Activity

Tissues (brain, heart, liver and kidney) were removed and homogenized 20:1 (w:v) in ice-cold (4°C) Tris buffer (10 mM, pH 7.2) with a Polytron homogenizer and then centrifuged at 27,000×g for 20 minutes. ODC activity in the supernatant was determined by measuring the release of $^{14}CO_2$ from DL-(1-^{14}C) ornithine as described previously [1]. An aliquot (0.8 ml) of the supernatant was added to 0.2 ml of incubation mix to give 0.5 mM dithiothreitol (DTT), 50 μM pyridoxal phosphate, and 0.01 mM (1-^{14}C) ornithine (10^6 CPM). Samples were incubated in vials sealed with serum stoppers for 40 minutes at 37°. The reaction was stopped by addition of 1 ml citric acid (2 M). Evolved $^{14}CO_2$ was trapped in an insert containing hyamine hydroxide and counted by liquid scintillation spectrometry in a toluene-based scintillation fluor containing PPO and POPOP. Serum GH was measured by RIA.

Statistics

Results are expressed as mean±SEM with levels of significance calculated from the raw data by Student's unpaired two-tailed *t* test.

RESULTS

The effects of MD, IMM and cold on tissue ODC activity in neonatal rats (days 8–10) are shown in Table 1. In this and subsequent tables, the ODC data are expressed as percentage of control value to allow easier comparison of stress responses among tissues and ages with widely-varying basal ODC activity. MD decreased ODC activity in all tissues, while IMM elevated ODC activity to levels significantly above that observed in MD pups, and cold increased ODC activity to levels significantly greater than that observed in maternally deprived pups (brain) or non-deprived controls (liver and heart). Serum GH was decreased by MD and IMM but not by cold (Table 1). The effect of pretreatment with the ganglionic antagonist chlorisondamine (CHLOR) on IMM in neonates is shown in Table 2. CHLOR did not block the ODC decline associated with MD or the rise associated with IMM in 8-day-old rats, but it did block IMM effects in 16-day-old rats. The effects of CHLOR on cold responses could not be studied, as treatment of cold-exposed pups with CHLOR caused a severe hypothermia which itself lowered ODC activity.

Stress responses in the heart from day 3 to day 22 are shown in Fig. 1. MD decreased organ ODC activity until day 16, as previously reported [3]. Both cold and IMM elevated ODC activity over the levels observed in MD animals on day

TABLE 2

CHLORISONDAMINE EFFECTS ON STRESS-INDUCED ODC ACTIVITY IN NEONATAL RATS

	Heart ODC Activity	
	Day 10	Day 16
Maternally deprived	100 ± 14 (8)	100 ± 12 (10)
Chlorisondamine + Deprived	106 ± 18 (9)	67 ± 7 (10)
Immobilized	171 ± 27* (7)	400 ± 62* (9)
Chlorisondamine + Immobilized	200 ± 22* (10)	105 ± 12*† (9)

Pups were injected with saline or chlorisondamine (2.5 mg/kg), then deprived in an incubator or immobilized in wire mesh cages for 2 hours. At this time, pups were killed and tissue ODC activity determined. Results are expressed as percent maternally deprived control ± SEM. N is indicated in parentheses.

*Indicates statistically different from control, $p<0.05$ or better.

†Indicates statistically different from immobilized, $p<0.05$. Control ODC activity=0.735 nmole/g/hr for 10 day old pups and 0.680 nmole/g/hr for 16 day old pups.

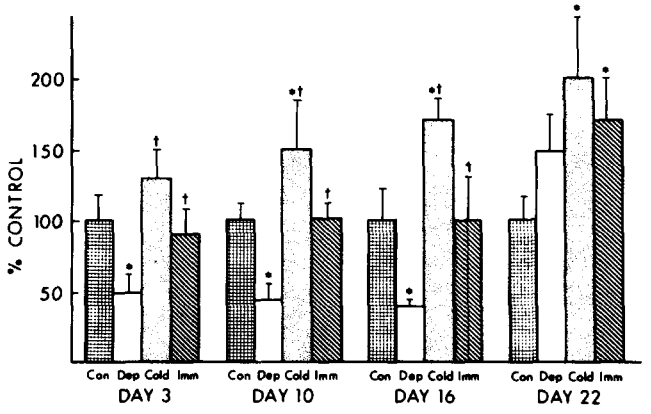

FIG. 1. Effects of maternal deprivation (DEP), cold or immobilization (IMM) on heart ODC activity in developing rats. Rats were left with the mother (control), or stressed for 2 hours, then killed and heart ODC activity was determined. Results are expressed as percent control ±SEM. N=10 or more in each experimental group. *Indicates statistically different from control, $p<0.05$ or better. †Indicates statistically different from deprived, $p<0.05$ or better. Control ODC activity=2.213 nmole/g/hr for day 3, 1.194 nmole/g/hr for day 10, 0.153 nmole/g/hr for day 16 and 0.765 nmole/g/hr for day 22.

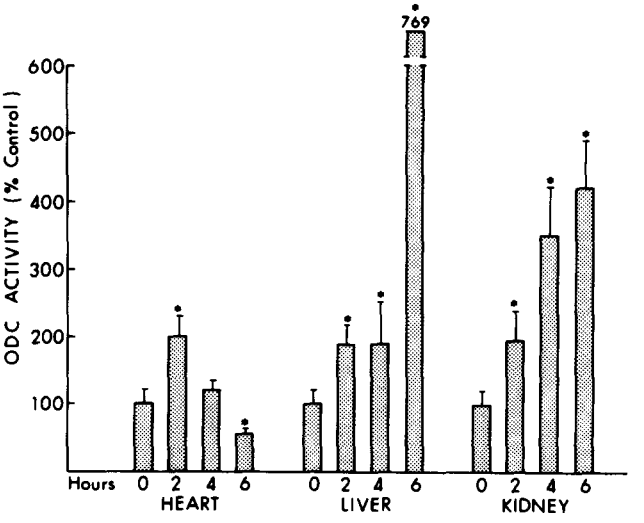

FIG. 2. Effects of immobilization (IMM) on ODC activity in heart, liver and kidney. Thirty-five-day-old rats were immobilized for the indicated time, killed and tissue ODC activity determined. ODC activity is expressed as percent control ±SEM. N=10 or more in each experimental group. *Indicates statistically different from control, $p<0.05$ or better. Control ODC activity=0.510 nmole/g/hr for heart, 0.176 nmole/g/hr for liver and 3.880 nmole/g/hr for kidney.

TABLE 3

EFFECT OF CHLORISONDAMINE ON ODC ACTIVITY DURING IMMOBILIZATION IN 35 DAY OLD RATS

	ODC Activity (Percent control)		
	Heart	Liver	Kidney
Control	100 ± 13 (20)	100 ± 16 (18)	100 ± 17 (16)
Immobilized	297 ± 23* (10)	277 ± 83* (10)	1010 ± 300* (9)
Chlorisondamine	85 ± 14 (20)	78 ± 16 (18)	162 ± 39* (19)
Chlorisondamine + Immobilized	144 ± 21† (10)	117 ± 11† (10)	79 ± 20† (10)

Animals were injected with saline or chlorisondamine, then immobilized for two hours in wire mesh cages and tissue ODC activity determined. Results are expressed as percent control ± SEM. N is indicated in parentheses.

*Indicates $p<0.05$ or better relative to control.

†Indicates $p<0.05$ or better relative to immobilized. Control ODC activity=0.295 nmole/g/hr in heart, 0.238 nmole/g/hr in kidney and 0.399 nmole/g/hr in liver.

3, and the extent of the response gradually increased up to day 22. The developmental profile for liver ODC responses to stress was identical (data not shown).

The effects of IMM in 35-day-old rats are shown in Fig. 2. IMM increased ODC activity in all tissues studied at 2 hours. Liver and kidney ODC activities were increased at 4 and 6 hours. In contrast, IMM evoked a biphasic response in heart: the increase at 2 hours was followed by a decrease at 6 hours. The effect of CHLOR administration on IMM in 35-day-old rats is shown in Table 3. CHLOR blocked the ODC elevation observed after 2 hours of IMM in all tissues. CHLOR itself increased kidney ODC, but the increase was much smaller than that associated with IMM. Cold exposure (2 hours) similarly increased ODC activity in heart, kidney and liver in 35-day-old rats (Table 4).

TABLE 4

EFFECT OF COLD EXPOSURE ON TISSUE ODC ACTIVITY

	ODC Activity (Percent control)	
	Control	Cold
Heart	100 ± 12 (15)	195 ± 29* (15)
Liver	100 ± 18 (13)	231 ± 34* (13)
Kidney	100 ± 14 (11)	282 ± 45* (13)

Twenty five day old rats were placed in a 4° cold box for 2 hours or left in the home cage for the same period of time, killed, and tissue ODC activity determined. Results are expressed as percent control ± SEM. N is indicated in parentheses.

*Indicates $p<0.05$ or better relative to nonstressed controls. Control ODC activity=0.370 nmole/g/hr for heart, 0.30 nmole/g/hr for liver and 1.560 nmole/g/hr for kidney.

DISCUSSION

Disruption of normal mother-infant interactions is a profound "stress" for most mammals that impairs behavioral and somatic development, alters sympathetic nervous system function and inhibits growth hormone secretion [5, 7, 11, 12]. The results of this study indicate that at least one of the responses to maternal deprivation, the decline in ODC activity, reflects a specific response to this particular environmental manipulation that differs from responses to other "stresses." We have shown that maternal deprivation and the "classical" stress paradigms immobilization and cold evoke distinct biochemical responses in developing rat pups. While ODC activity decreases in all organs during maternal deprivation, as previously shown in this laboratory [3,4], ODC activity in liver and heart increases after IMM or cold.

IMM and cold stresses were necessarily superimposed upon maternal deprivation, raising the possibility that these manipulations simply reversed the effects of maternal deprivation. This is particularly likely with IMM, as tactile stimulation provided by the wire mesh cage could reverse the effects of MD [6]. However, our results indicate that the increase in ODC activity observed during these stresses represents a distinct response rather than simply a reversal of maternal deprivation effects. First, the changes in GH secretion associated with IMM are the reverse of those expected if this manipulation simply reversed MD. IMM caused a decline in GH even greater than that caused by MD, while tactile stimuli increase GH [6]. The inability of IMM to reverse MD effects on GH is consistent with our studies showing that maternal deprivation effects are reversed only by a specific form of tactile stimulation. Second, although the effects of maternal deprivation disappear at weaning, IMM and cold responses continue to increase with age. Finally, the results show that different mechanisms trigger responses to IMM and MD in 16-day-old pups. The ganglionic antagonist chlorisondamine blocked IMM effects but not those of maternal deprivation.

Although two of the stress procedures used in this study did affect body temperature, changes in body temperature cannot explain stress effects on ODC activity for several reasons. First, we have shown previously that the ODC response to MD is not affected by a range of environmental temperatures within normal physiologic limits for 8–10-day-old pups [3,4]. Furthermore, the changes in body temperature associated with cold and IMM were not consistent with the changes in ODC. First, the degree of ODC induction did not correlate well with the degree of temperature change in cold stressed animals, as body temperature decreased much more in 10-day-old pups than in 35-day-old rats, but ODC increased less in the younger animals. In addition, IMM decreased body temperature in 10-day-old pups, but actually increased body temperature in 35-day-old rats, while ODC activity increased at both ages.

This study demonstrates that behavioral manipulations of developing rats have pronounced effects on at least one important marker of cellular growth and differentiation, ornithine decarboxylase activity, and suggest that further investigation of behavioral modulation of ODC activity might reveal the mechanisms by which such manipulations affect growth and behavioral development. The variation of direction, duration, magnitude and physiologic mediator of the ODC response to stress with the particular stressor, the age of the animal, and the organ which is studied, further demonstrates that careful evaluation of the pattern of physiologic response to stress is a more powerful tool than is evaluation of a single parameter.

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