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Tactile and Nutritional Aspects of Maternal Care: Specific Regulators of Neuroendocrine Function and Cellular Development (41779)

SAUL M. SCHANBERG, GARY EVONIUK, AND CYNTHIA M. KUHN

Department of Pharmacology, Duke University Medical Center, Durham, North Carolina

Development in mammals is influenced profoundly by many environmental stimuli, and those provided by the mother are the most critical for survival and growth. Disruption of the mother–infant relationship elicits marked behavioral and physiological responses in the offspring ranging from transient changes in body temperature, heart rate, and locomotor activity following short periods of separation, to marked retardation of growth and behavioral development following more long-term separation (1–4).

A growing body of evidence demonstrates that extremely specific sensory cues from the mother regulate different physiological and behavioral responses in young animals. For example, nipple attachment in rats is promoted by specific organic substances on the ventral surface of the mother, and thermal input from the mother modulates locomotor activity in weanling-age rat pups, while compounds secreted by the mother's GI tract "orient" pups to the nest (5–8).

Studies in this laboratory recently have shown that mother–pup interactions also have marked effects on biochemical processes in the developing pup. These biochemical processes, like behavior, respond to extremely specific environmental cues. The findings of our studies, which are described in the following review, have shown that mother–pup interactions seem to be important regulators of physiologic as well as behavioral function. We have found that sensory stimuli associated with the mother elicit coordinated physiological and biochemical responses which vary with the nature of the stimulus. While some environmental stimuli are important regulators of growth and development, others subserve quite different functions, like maintaining tissue sensitivity to specific hormones.

The first group of studies we wish to discuss are those demonstrating that active tactile stimulation of preweanling pups by the mother provides specific sensory cues that maintain normal growth and development. The data that will be discussed demonstrate that restriction of active tactile interaction between mother and pup produces at least three different abnormalities in biochemical processes involved in growth and development. The second group of studies that will be presented demonstrate that specific nutritional stimuli regulate metabolic functions involved in maintaining energy balance. The latter studies show that specific nutrients control tissue sensitivity to several endogenous hormones which regulate energy metabolism.

Tactile Stimuli and Growth. Ornithine decarboxylase (ODC), the first enzyme in the synthesis of the polyamines putrescine, spermine, and spermidine, is one important regulator of growth and differentiation that is affected by mother–pup interactions. The end products of this enzyme are intimately involved in regulation of protein and nucleic acid synthesis (9, 10), and activity of this enzyme is thought to be a sensitive index of environmental effects on biochemical and physiological processes in the developing animal.

ODC activity in both neonatal and adult rats responds markedly to various "stresses" and the pattern of tissue response is determined by the nature of the environmental stimulus, or "stress." Separation of preweanling rat pups from the mother (maternal deprivation, or MD) is one "stress" that profoundly affects tissue polyamine systems in developing animals. MD causes an immediate and marked decrease in tissue ODC activity and in tissue putrescine concentration. These changes occur in all tissues that we have studied including brain, liver, heart, kidney, lung, and spleen, and in all brain regions (Figs. 1, 2). ODC activity normalizes as soon as pups are returned to the mother (Fig. 3). This marked effect of maternal deprivation is ob-

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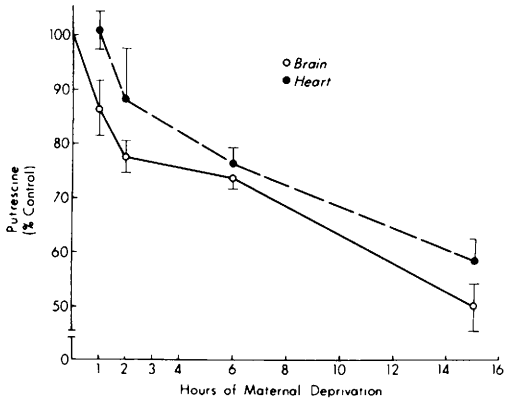

FIG. 1. Effect of maternal deprivation on brain and heart ODC activity in 10-day-old rat pups. All values are expressed as means ± SEM. $N = 5$ in each group. All differences significant, $P < 0.05$ or better.

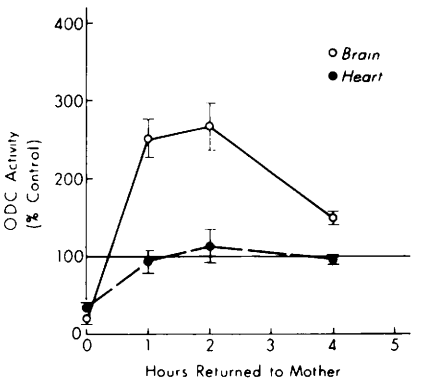

FIG. 3. Comparison in 10-day-old rat brain and heart of the recovery of ODC activity after a 2-hr deprivation and return to the mother. All values expressed as means ± SEM. $N = 5$ in each group. Brain and heart values are significantly below control ($P < 0.05$) at 2 hr. Brain values are significantly above control values at each point after return ($P < 0.05$).

served in preweanling pups, from postnatal Days 1 to 20, when it disappears abruptly (Fig. 4).

The decline in ODC associated with maternal deprivation does not result from a change in body temperature, exposure to an unfamiliar environment, or other nonmaternal stimuli (11). Similarly, we have shown that interruption of feeding does not mediate the fall in ODC, as placing preweanling rat pups with a mother rat whose nipples have been ligated does not cause a similar uniform decrease of ODC activity in all tissues (12). The latter demonstration is extremely important, as feeding represents one of the major components of the mother–pup interaction during the first 2 postnatal weeks, with pups feeding an average of every 10 min (13). Furthermore, auditory, visual, and olfactory stimuli, all of which play a role in maintaining mother–pup contact at various times during the development of preweanling rats, do not influence ODC activity (Kuhn and Schanberg, unpublished observations), and so are not involved

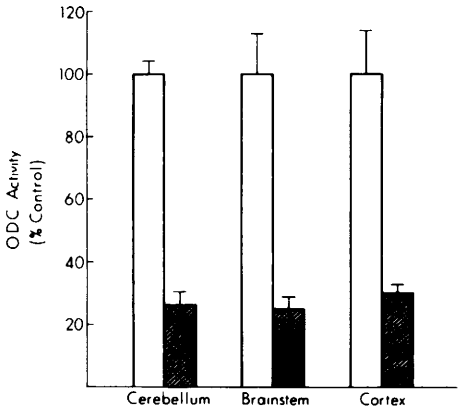

FIG. 2. Effect of maternal deprivation on ODC activity in different regions of 10-day-old rat brain. All values are expressed as means ± SEM. All differences are significant, $P < 0.05$ or better.

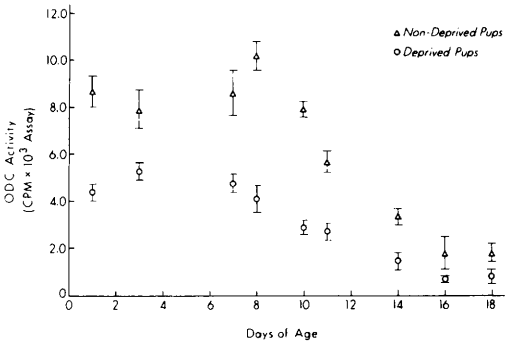

FIG. 4. Effect of a 2-hr maternal deprivation on preweanling rat brain ODC activity in pups of different ages. All values are expressed as means ± SEM. $N = 5$ in each group. All differences significant, $P < 0.05$ or better.

in the ODC response to maternal deprivation. None of these sensory stimuli are functional at birth, but mature at various times during the first 2 to 3 postnatal weeks. Therefore, the finding that these stimuli do not affect ODC is consistent with our observation that maternal deprivation effects are observed somewhat uniformly from the day of birth until weaning.

Interruption of active tactile interaction between mother and pup seems to be the trigger for the decline in ODC activity during maternal deprivation. We have shown that placing pups with a mother rat that has been anesthetized with urethane to prevent active interaction but not lactation (13) changes tissue ODC activity in the same way as maternal deprivation (Table I). This finding is rather striking, as the decrease in ODC activity occurs in the presence of the many sensory cues that are passively transferred (olfactory, auditory, etc.) as well as in the presence of interactions among littermates. Furthermore, when pups are stimulated with a pattern of tactile stimulation roughly approximating that of maternal grooming, ODC activity is increased to normal levels in all tissues although other forms of sensory stimulation of equal intensity were ineffective (Fig. 5). This finding is of particular interest because tactile stimulation has proved to be an important stimulus for growth and development in a number of species, including humans (14).

TABLE I. BRAIN ODC ACTIVITY OF 10-DAY-OLD RAT PUPS

Experimental condition of pups	*N*	ODC activity (% control)
Group 1		
With nonanesthetized mother	18	100 ± 13
With anesthetized mother	19	44 ± 4*
Group 2		
Injected with saline	11	100 ± 16
Injected with urethane	10	119 ± 15

Note. Group 1 rat pups were placed with anesthetized (urethane) or nonanesthetized mothers for 2 hr. Group 2 pups were injected intraperitoneally with urethane (1.1 g/kg of body weight) or 0.9% saline and returned to their lactating mothers. All pups were killed 2 hr after placement with the mother, and the ODC activity was determined. Brain ODC activity is expressed as a percentage of that of littermate controls.

* Significant difference from controls.

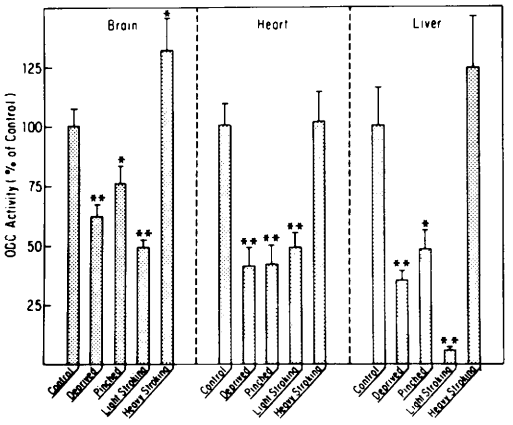

FIG. 5. Effect of different stimuli on tissue ODC activity in maternally deprived rat pups. Pups were deprived for 2 hr and either left untouched or stroked heavily, stroked lightly, or pinched every 5 min during the deprivation. Results are expressed as percentage of control ± SEM. $N = 8$ in each group.

* = $P < 0.05$ different from control.

** = $P < 0.001$ different from control.

The physiologic "signal" which triggers the decline in ODC activity following interruption of maternal–pup interaction is still unknown. The uniform decline in tissue ODC activity throughout the body suggests that some general endocrine or metabolic response to the withdrawal of maternal stimulation mediates this fall. This hypothesis is strengthened by our finding that ODC decreases during MD even when innervation of peripheral tissues is not yet functional, or when innervation is blocked pharmacologically with propranolol or atropine (12) (unpublished observations).

ODC activity is such an accurate and sensitive index of cell growth and development that its decline during maternal deprivation may represent a potential biochemical correlate of the marked suppression of growth and development that results from maternal–infant separation. This finding further suggests that disruption of ODC activity might be a specific biochemical mechanism through which environmental stimuli affect growth and development rather than simply a biochemical "marker" for environmental effects.

Stress affects secretion of many hormones

and neurotransmitters in developing rats. The change in ODC activity during maternal deprivation suggested that secretion of one or more of the many hormonal regulators of ODC was affected by this manipulation. In additional studies, we have shown that this is the case, and in fact that maternal deprivation elicits an unusual if not unique neuroendocrine response. This response represents the second mechanism controlling growth and development that is disrupted by maternal deprivation.

When preweanling rat pups are separated from the mother, there is a selective decrease in growth hormone secretion from the anterior pituitary (Table II), while serum levels of other stress-responsive hormones including prolactin, TSH, and corticosterone do not change if nutrition and other nonmaternal aspects of the environment are controlled. This selective decrease in growth hormone is somewhat unusual as stress usually elicits a complex pattern of endocrine response which in the rat usually involves decreased growth hormone and TSH and increased corticosterone and prolactin secretion. The inhibition of growth hormone secretion during MD could affect development significantly over a prolonged period of deprivation, as circulating growth hormone is responsible for generation of substances called somatomedins which are among the major regulators of skeletal and possibly organ growth.

TABLE II. CONCENTRATION OF GROWTH HORMONE IN SERUM OF MATERNALLY DEPRIVED RAT PUPS

Experimental conditions	*N*	Growth hormone (% control)
Nondeprived	30	100 ± 3
Deprived		
1 hr	10	53 ± 8*
2 hr	16	60 ± 6*
6 hr	5	59 ± 12*
Deprived and returned		
15 min	5	155 ± 32
1 hr	8	99 ± 7
2 hr	13	94 ± 7
4 hr	5	100 ± 4

Note. Results are expressed as percentages of nondeprived controls ± SEM. Growth hormone concentrations in controls were 29 ± 1 ng/ml.

* Statistically different from control ($P < 0.05$, Student's *t* test).

Growth hormone is a well-known regulator of ODC activity in brain as well as in peripheral tissues (15). Therefore, the finding that growth hormone secretion decreased during maternal deprivation provided a possible explanation for the fall in tissue ODC activity. To investigate this possibility, we injected pups with ovine growth hormone at the beginning of the separation procedure to prevent the decrease in ODC activity. Rather than restoring ODC activity to normal levels as predicted, we found that tissue ODC became completely and selectively unresponsive to growth hormone during maternal deprivation: growth hormone was completely unable to induce ODC activity in liver or brain of deprived rats, although it caused a significant induction in normal rats (Table III). Responsivity was restored when pups were returned to the mother. Although tissues of maternally deprived rat pups do not respond to growth hormone, a number of other hormones including cyclic AMP, insulin, and the glucocorticoid hormone dexamethasone still induce ODC activity normally (Table IV). This selective loss of tissue response to growth hormone represents the third major defect in growth-regulating processes that is disrupted by maternal deprivation.

These three defects (decrease in tissue ODC activity, fall in growth hormone secretion, and loss of tissue sensitivity to exogenous growth hormone) appear to be regulated by the same sensory stimulus: active tactile interaction between mother and pups. Tactile stimulation of maternally deprived rat pups returns all three parameters to normal (Table V, unpublished observations). In addition, placing the pups with a urethane-anesthetized mother to eliminate maternal tactile stimulation of the pups decreases both serum growth hormone and liver ODC responses to exogenous growth hormone.

A specific component of the neonate–mother interaction, active tactile stimulation of the pups, regulates many physiologic responses which are related to growth and development. Tissue ODC activity, growth hormone secretion and tissue responsivity to the

TABLE III. LIVER ODC RESPONSE TO GH FOLLOWING RETURN TO MOTHER

	ODC activity (% control)	N
Control	100 ± 25	10
Control + growth hormone	597 ± 132*	10
Deprived	39 ± 5*	10
Deprived + growth hormone	25 ± 3	10
Returned to mother	203 ± 54	10
Returned to mother + growth hormone	982 ± 186*	10

Note. Pups were maternally deprived for 2 hr and killed or returned to the mother. Two hours after return pups were injected sc with vehicle or GH (100 μg). Liver ODC activity was determined 4 hr after hormone administration. Results are expressed as percentage of control ± SEM. Control ODC activity was 40 nCi/30 min/g.

* $P \leq 0.001$ relative to control.

action of growth hormone are disturbed by the interruption of this particular aspect of mother–pup interaction. This finding suggests that interruption of the mother–infant interaction, e.g., the absence of the mother from the nest for prolonged periods of time, would trigger a shift of cell function from a pattern of growth and development to a more conservative strategy of maintenance of basic metabolic processes for survival. These studies show that specific sensory stimuli can elicit a coordinated response involving integration of many physiologic processes, and further, that the specific biochemical mechanisms which mediate this pattern of response can be identified.

TABLE V. EFFECT OF TACTILE STIMULATION ON MATERNALLY DEPRIVED PUPS

	Activity (% control)
Brain ODC	
Control	100 ± 7
Deprived	64 ± 6*
Deprived + heavy stroking	120 ± 13**
Heart ODC	
Control	100 ± 10
Deprived	47 ± 9*
Deprived + heavy stroking	102 ± 14**
Liver ODC	
Control	100 ± 15
Deprived	19 ± 3*
Deprived + heavy stroking	48 ± 10**
Serum GH	Serum level (% control)
Control	100 ± 23
Deprived	38 ± 8*
Deprived + heavy stroking	105 ± 27**

Note. Pups were maternally deprived for 2 hr, then deprived for 2 more hr and either left untouched or stroked heavily 10–20 times every 5 min. Control pups were left untouched with the mother for 4 hr. Results expressed as percentage control ± SEM. Control ODC activity = 0.235, 0.076, and 0.308 nmol/Orn/g tissue-hr, respectively, for brains, hearts, and livers. Control serum GH level = 68 ng/ml. $N \geq 9$ for all groups.

* $P < 0.02$ or better compared to controls.

** $P < 0.05$ or better compared to deprived pups.

These studies of the biochemical basis of growth retardation of maternally deprived rats have added significance because a clinical cor-

TABLE IV. EFFECT OF HORMONES ON LIVER ODC ACTIVITY IN MATERNALLY DEPRIVED RAT PUPS

Drug	Dose (μg)	ODC activity (% control) Control	N	Deprived	N
Vehicle		100 ± 22	(60)	11 ± 3	(60)†
oGrowth hormone	100	437 ± 136*	(20)	11 ± 2	(20)*,†
oPlacental lactogen	100	315 ± 54*	(10)	10 ± 1	(10)*,†
Dexamethasone	200	366 ± 62*	(10)	532 ± 51	(10)*
Dibutyryl cAMP	800	518 ± 172*	(10)	539 ± 183	(10)*
PGE-1	50	510 ± 66*	(10)	620 ± 213	(10)*
Insulin	10	1306 ± 422*	(15)	2040 ± 594	(15)*

Note. Pups were maternally deprived for 2 hr, injected sc with vehicle or hormone, and killed 4 hr later. Results expressed as percentage of control ± SEM. Control ODC activity was 37 nCi/30 min/g tissue.

* $P < 0.05$ or better relative to vehicle-treated control.

† $P < 0.001$ relative to paired control.

relate of this condition has been described. "Psychosocial dwarfism" is a retardation of growth and behavioral development in children which shows a startling similarity to the animal model we have just described, as it is characterized by a selective disruption of growth hormone secretion, a suppression of growth in the presence of normal nutrition, and a selective loss of tissue responsivity to growth hormone (16, 17). This condition is thought to result from a withdrawal of "loving care" by the mother or other caretaker, and all the defects disappear when the child is placed in a "normal" home environment (18). Although it clearly is not possible to model human maternal behavior in the rat, the similarities in the physiologic responses of humans and rats to a similar disruption of maternal–infant interactions suggest a common phenomenon. We feel that the animal model developed in this laboratory can provide exciting new information about the biochemical mechanisms mediating this clinical disorder.

Nutritional Stimuli and Metabolic Regulation in Developing Rats. More recent studies from this laboratory have shown that food, another critical aspect of the mother–pup interaction, regulates an entirely separate constellation of physiologic responses in the pup. Food ingestion even in the normal adult animal provokes a complex series of physiologic reactions involving changes in gut motility, cardiovascular function, secretion of specific hormones, and many metabolic functions. Many of these changes also occur in the feeding neonate. Our studies have shown that food ingestion both provides necessary nutrients and regulates tissue metabolic functions that utilize these nutrients.

During our studies of liver ODC induction by growth hormone, we found that short-term separation of preweanling rat pups from the mother caused the disappearance of liver ODC induction by both growth hormone and a different group of hormones. After only 2 hr of food deprivation, liver ODC induction by α and β adrenergic agonists and by the pancreatic hormone glucagon was dramatically reduced or eliminated, although responses to a number of hormones including dexamethasone, insulin, and cyclic AMP remained normal (Table VI).

TABLE VI. EFFECT OF HORMONES ON LIVER ODC ACTIVITY IN MATERNALLY DEPRIVED PUPS

	ODC activity	
	Control (% control)	Deprived (% control)
Vehicle	100 ± 25 (60)	24 ± 7[a] (60)
Phenylephrine	375 ± 71[a] (18)	38 ± 6[a,b] (20)
Isoproterenol	533 ± 176[a] (10)	26 ± 12[a,b] (10)
Glucagon	962 ± 436[a] (12)	46 ± 4[a,b] (14)
Vasopressin	259 ± 50[a] (10)	18 ± 7[a,b] (10)
Dexamethasone	1538 ± 198[a] (10)	1584 ± 454[a] (10)
PGE-1	691 ± 132[a] (10)	1190 ± 91[a] (10)
Dibutyryl cyclic AMP	518 ± 172[a] (5)	539 ± 283[a] (5)

Note. Pups were deprived for 2 hr, then injected with vehicle, phenylephrine (5 mg/kg), isoproterenol (0.1 mg/kg), glucagon (5 mg/kg), vasopressin (50 μg/kg), dexamethasone (5 mg/kg), PGE-1 (2.5 mg/kg), or dibutyryl cyclic AMP (25 mg/kg). Control pups were left with the mother throughout the experiment and were injected at the same time as deprived animals. Results are expressed as percentage of control ± SEM. Numbers in parentheses, number of rats. Control ODC activity = 0.649 ± 0.160 nmole/g/hr.

[a] $P < 0.05$ or better relative to vehicle-injected control.

[b] $P < 0.05$ or better relative to drug-injected control.

This effect proved to be quite distinct from the effects of maternal deprivation on GH responsivity. First, it was mimicked by placing pups with a mother rat whose nipples had been ligated (Table VII). As pups placed with a nipple-ligated mother receive normal maternal care but not food, these findings show that food, rather than tactile stimulation, is the important environmental regulator of ODC induction by these agonists. Additional support for nutritional mediation of this response was provided by the restoration of adrenergic agonist action when pups were fed by intragastric cannula. By feeding the pups cow milk at 75-min intervals, it was possible to completely restore the ability of isoproterenol (Figs. 6, 7) as well as phenylephrine and glucagon to induce liver ODC activity (unpublished observations). In contrast to the other biochemical defects associated with maternal deprivation, ODC induction was affected only in the liver, not in other peripheral tissues like the heart (Fig. 8). Finally, the developmental time course of this response differed from that observed for basal ODC activity during touch deprivation. Although the defect caused by nutritional deprivation is

TABLE VII. EFFECT OF HORMONES ON LIVER ODC IN FOOD-DEPRIVED RAT PUPS

	ODC activity	
	Control (% control)	Deprived (% control)
Vehicle	100 ± 22 (41)	42 ± 6[a] (44)
Phenylephrine	324 ± 3[a] (20)	67 ± 10[a,b] (20)
Isoproterenol	1194 ± 513[a] (10)	74 ± 39[a,b] (10)
Glucagon	397 ± 75[a] (10)	41 ± 7[a,b] (10)
Dexamethasone	782 ± 145[a] (5)	883 ± 151[a] (5)
Growth hormone	405 ± 42[a] (10)	461 ± 39[a] (10)

Note. Pups were placed with nipple-ligated mothers for 2 hr then were injected with vehicle, phenylephrine (5 mg/kg), isoproterenol (0.1 ml/kg), glucagon (5 mg/kg), dexamethasone (5 mg/kg), or growth hormone (5 mg/kg). All animals were killed 4 hr after injection. Control pups were left with the mother throughout the experiment and injected at the same time as food-deprived pups. Numbers in parentheses, number of rats. Results are expressed as mean ± SEM. Control ODC activity = 0.187 ± 0.040 nmole/g/hr.

[a] $P < 0.05$ or better relative to vehicle-injected control.

[b] $P < 0.05$ or better relative to drug-injected control.

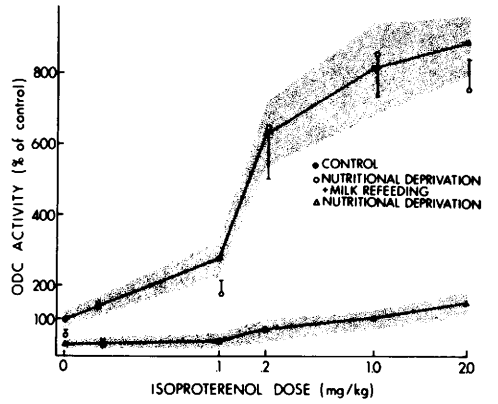

FIG. 7. Effect of increasing doses of isoproterenol on hepatic ODC activity in control, nutritionally deprived (ND), and ND refed pups. Rat pups were placed with control or nipple-cauterized mothers for 2 hr, then injected with varying doses of isoproterenol or vehicle and killed 4 hr later for assay of ODC. Pups receiving milk were intubated and fed 0.2 ml at intervals of 75 min throughout the experiment. SEM for control and ND pups is indicated by the shaded areas. Two-way ANOVA indicates significant ($P < 0.01$) effect of nutrition and isoproterenol.

most pronounced up to the time of weaning, a similar loss of liver responsivity to adrenergic agonists occurs in postweanling animals when the time of food deprivation was lengthened considerably (Table VIII).

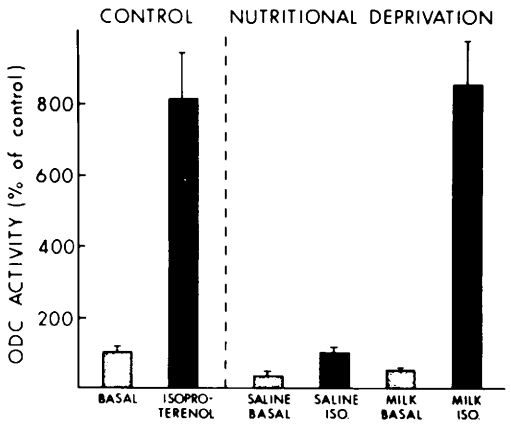

FIG. 6. Effect of nutritional deprivation and milk refeeding on hepatic ODC activity after ODC induction. Rat pups were placed with control or nipple-cauterized mothers for 3 hr, then injected with isoproterenol, 1 mg/kg (solid bars) or vehicle (shaded bars) and killed 4 hr later for assay of liver ODC. Pups receiving oral solutions were intubated and fed at intervals of 75 min throughout the experiment. ODC activity is expressed as percentage of control ± SEM.

The first question raised by our experiments was the identity of the specific nutrient that maintained liver ODC responsivity to this group of hormones. This nutrient, like tactile stimulation, must serve as the initial stimulus to elicit a chain of physiologic responses. To identify the food substance involved in this response, food-deprived neonatal rats were fed a number of nutrients by intragastric cannula. Major chemical classes (protein, fat, carbohydrate) were investigated first by feeding animals skim milk (containing protein and carbohydrate but almost no fat), a casein hydrolysate which provided a fairly pure source of protein, or the carbohydrate lactose. The results of these studies (Table IX) showed clearly that only carbohydrates effectively restored liver ODC induction by isoproterenol in food-deprived rat pups. Further investigation of carbohydrate regulation of ODC responsivity showed that glucose restored the action of all three hormones affected by nutritional stimuli (phenylephrine, isoproterenol, and glucagon) (Table X). This action appears to reflect a true

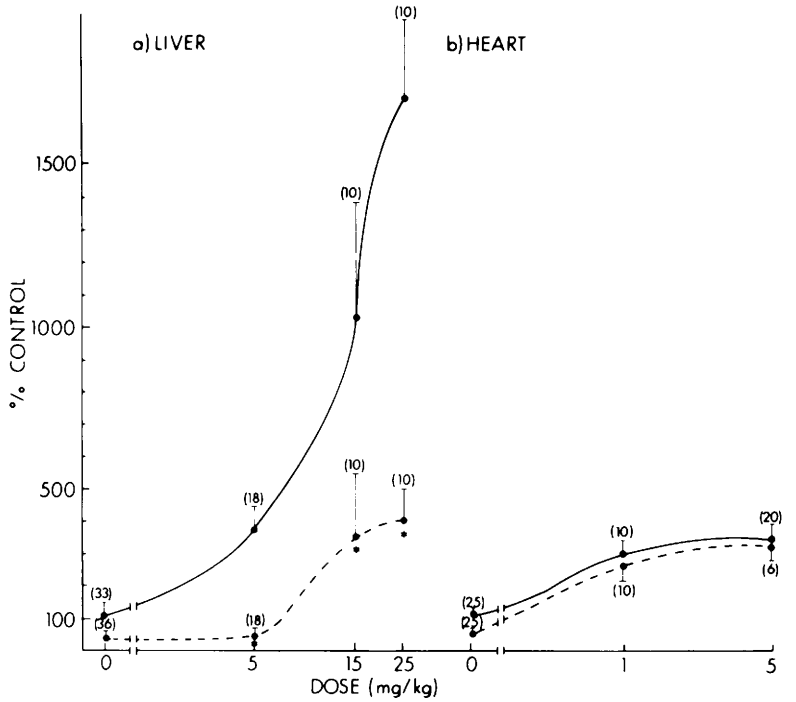

FIG. 8. Effect of increasing doses of phenylephrine on tissue ODC activity in control (solid line) and deprived (dotted line) pups. (a) Liver ODC, (b) heart ODC. Pups were deprived for 2 hours, then injected with saline or phenylephrine and killed 4 hr after injection. Control pups were left with the mother throughout the experiment. Results are expressed as percentage of control ± SEM. * Indicates statistically different from control, $P < 0.05$ or better.

nutritional effect of glucose rather than some nonspecific, non-nutritive effect, as the natural (D) but not the inactive (L) isomer of glucose

TABLE VIII. FOOD-DEPRIVATION EFFECTS ON 26-DAY-OLD RATS

	Liver ODC activity (% control)
Saline	100 ± 21 (15)
Phenylephrine	323 ± 58* (15)
Food-deprived + saline	52 ± 7* (15)
Food-deprived + phenylephrine	144 ± 28** (16)

Note. Experimentals were deprived of food for 48 hr, during which time water and 0.9% NaCl were freely available. They were then injected with saline, phenylephrine (5 mg/kg), or isoproterenol (1 mg/kg). Controls were injected at the same time as food-deprived rats and all rats were killed 4 hr after injection of drugs. Results are expressed as mean ± SEM.

* $P < 0.05$ or better relative to saline-injected control.

** $P < 0.05$ or better relative to drug-injected control.

TABLE IX. EFFECT OF ORAL NUTRIENTS ON ODC INDUCTION BY ISOPROTERENOL

	ODC activity	
	Control	Isoproterenol
Control with mother	100 ± 11	913 ± 135*
Nutritionally deprived + saline	51 ± 7	99 ± 19
Nutritionally deprived + skim milk	61 ± 21	1160 ± 163*
Nutritionally deprived + casein	50 ± 10	242 ± 43
Nutritionally deprived + lactose	81 ± 15	1177 ± 255*

Note. Rat pups were placed with control or nipple-cauterized mothers for 2 hr, then injected with isoproterenol or vehicle and sacrificed 4 hr later for assay of ODC and blood glucose. Pups receiving oral solutions were intubated and fed at intervals of 75 min throughout the experiment. ODC activity is expressed as percentage of control. $N \geq 9$ for all groups.

* $P < 0.05$ of controls.

TABLE X. EFFECT OF NUTRITIONAL DEPRIVATION AND GLUCOSE REFEEDING ON ADRENERGIC AND HORMONAL INDUCTION OF HEPATIC ODC

	ODC activity		
		Nipple-cauterized mother	
Injection	Normal mother	Saline feeding	Glucose feeding
Saline	100 ± 7	49 ± 4*	123 ± 21
Isoproterenol (1 mg/kg sc)	1070 ± 138†	99 ± 19*	1177 ± 255†
Phenylephrine (5 mg/kg sc)	424 ± 77†	69 ± 10*	687 ± 157†
Glucagon (100 μg)	823 ± 152†	108 ± 12*	1042 ± 129†
Insulin (10 mg)	1504 ± 208†	1021 ± 155†	1254 ± 261†

Note. Rat pups were placed with control or nipple-cauterized mothers for 2 hr, then injected with drug or vehicle and sacrificed 4 hr later for assay of ODC. Pups receiving oral solutions were intubated and fed at intervals of 75 min throughout the experiment. ODC activity is expressed as percentage of control (control activity = 0.8419 nm CO_2/g-hr.) $N \geq 9$ for all groups.

† $P < 0.002$ compared to appropriate vehicle-injected control (same column).

* $P < 0.001$ compared to appropriate vehicle- or drug-injected control with normal mother (same row).

restores the liver ODC response to isoproterenol (Table XI).

Several different carbohydrates were investigated next to determine whether the maintenance of ODC responsivity to adrenergic agonists and glucagon was dependent on the glucose molecule itself or its metabolic products. Glucose and galactose (the natural carbohydrates in milk), sucrose, fructose, and a combination of pyruvate and lactate were tested. Sucrose was tested as a control for the effect of carbohydrates in the gut, as this disaccharide is not metabolized or absorbed in preweanling rats. Fructose, pyruvate, and lactate are metabolic derivatives of glucose that are taken up into the liver and utilized at various levels in the glycolytic pathway.

Only the natural disaccharide lactose, and its immediate breakdown products glucose and galactose, restored liver ODC induction by isoproterenol (Table XI). Even though the metabolic derivatives of glucose returned the somewhat depressed blood glucose levels to normal, none of these compounds restored liver ODC responsivity. Sucrose affected neither blood glucose nor liver ODC induction by isoproterenol.

These findings suggest that the glucose molecule itself, or some physiologic response triggered by glucose, maintains liver ODC responsivity to adrenergic agonists and glucagon. This could happen by one of at least two different mechanisms. First, it is possible that short-term food deprivation decreases levels of some metabolite of glucose that is necessary

TABLE XI. EFFECT OF CARBOHYDRATE ADMINISTRATION ON HEPATIC ODC INDUCTION BY ISOPROTERENOL

	ODC activity		Blood glucose (mg/dl)
	Vehicle	Isoproterenol	
Pups w/normal mother	100 ± 11	1017 ± 62*	142 ± 5
Pups w/nipple-cauterized mother:			
+ saline	51 ± 7†	99 ± 19†	105 ± 4†
+ glucose	81 ± 15	1177 ± 255*	118 ± 5†
+ galactose	90 ± 18	1045 ± 186*	131 ± 3
+ lactose	74 ± 10	697 ± 92*	140 ± 5
+ sucrose	25 ± 4†	31 ± 5†	110 ± 2†
+ fructose	56 ± 9†	305 ± 41*†	139 ± 3
+ L-glucose	55 ± 7†	158 ± 25*†	88 ± 4†
+ pyruvate and lactate (4:1)	49 ± 6†	53 ± 5†	138 ± 6

Note. Rat pups were placed with control or nipple-cauterized mothers for 2 hr, then injected with isoproterenol or vehicle and sacrificed 4 hr later for assay of ODC and blood glucose. Pups receiving oral solutions were intubated and fed at intervals of 75 min throughout the experiment. ODC activity is expressed as percentage of control (control activity = 3.189 nm CO_2/g-hr). $N \geq 9$ for all groups.

* $P < 0.001$ compared to appropriate vehicle-injected control (same row).

† $P < 0.005$ compared to appropriate vehicle or isoproterenol-injected control with normal mother (same column).

for ODC induction. Alternatively, a physiologic signal triggered by the glucose molecule itself (i.e., insulin secretion, afferent nerve activity in the hepatic vagus, etc.) could mediate these effects. As metabolic products of glucose did not substitute for glucose, it is unlikely that blockade of glucose metabolism or utilization mediates the effects of food deprivation. Our current hypothesis to explain the effects of glucose on liver ODC mechanisms is that glucose or some physiologic response to glucose regulates the cascade of events leading from receptor activation through second messenger generation and eventually to ODC induction (steps 2–4, Fig. 9).

Our next studies concerned identification of the step(s) in the events leading to ODC induction that were affected by food deprivation. First we measured the number of adrenergic receptors in liver of food-deprived and control neonatal rats. As shown in Fig. 10, short-term food deprivation did not affect the number of receptors or the affinity of receptors for the radioligands used (^{3}H-prazosin and ^{125}I-pindolol for α and β receptors, respectively). Therefore, a change in receptor number does not appear to mediate the decreased response to adrenergic agonists observed during food deprivation. This finding

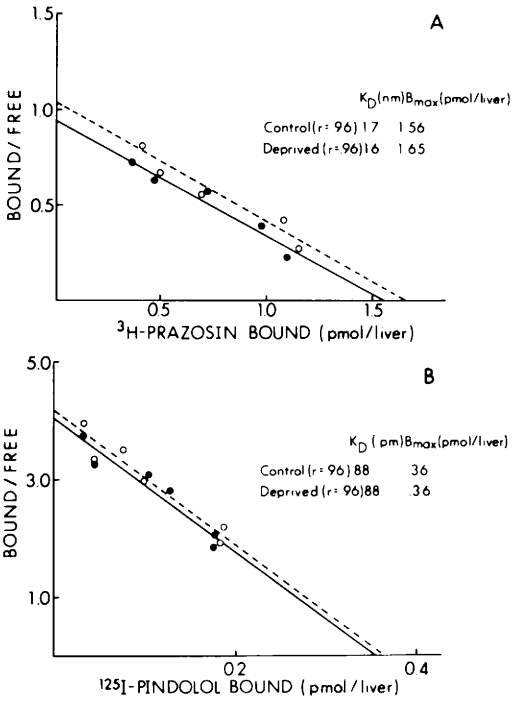

FIG. 10. Scatchard analysis of α-1 (A) and β (B) receptors in livers of control (solid line) and deprived (dotted line) pups. Pups were food-deprived for 4 hr, killed, and livers pooled for analysis of receptors. Each point represents the mean of triplicate determinations. Membrane preparations from control and deprived animals were pooled from at least 10 animals.

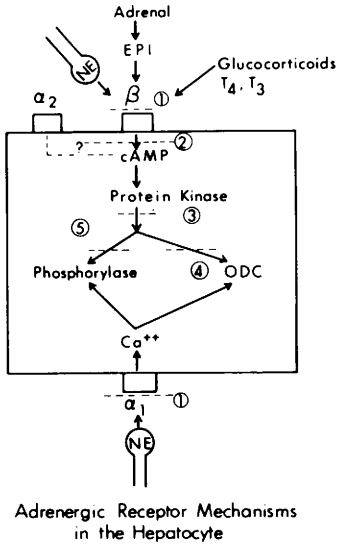

FIG. 9. Schematic analysis of steps leading to hormone action in the hepatocyte.

was not surprising, as the loss of liver responsivity to such a heterogenous group of hormones would not be explained easily by such a mechanism. Similarly, it is unlikely that second messenger generation is affected by food deprivation, as each of these hormones is thought to act through a different second messenger. It is more likely that metabolic effects of these hormones, including ODC induction, involves a common step distal to the receptor–second messenger coupling.

To evaluate this possibility other characteristic effects of these hormones were tested in food-deprived rat pups. The induction of tyrosine aminotransferase (TAT) represents one logical index for such a study, as isoproterenol and glucagon induce this enzyme within the same time period as ODC. As shown in Table XII, both isoproterenol and glucagon caused similar elevations in TAT in

TABLE XII. EFFECT OF NUTRITIONAL DEPRIVATION ON TAT INDUCTION BY ISOPROTERENOL AND GLUCAGON

	TAT activity (% control)	
	Control w/mother	Nutritionally deprived
Vehicle	100 ± 8	109 ± 11
Isoproterenol (1 mg/kg sc)	159 ± 12*	189 ± 15
Glucagon (100 μg/pup)	197 ± 20*	166 ± 14*

Note. Pups were nutritionally deprived for 12 hr, injected sc with vehicle or drug, and killed 5 hr later. Results expressed as percentage control ± SEM. Control TAT activity was 23.1 μM *p*-hydroxyphenylpyruvic acid produced/assay. $N > 5$ for all groups.

* $P < 0.005$ or better relative to appropriate control.

normal and food deprived pups. Furthermore, the ability of the α agonist phenylephrine to activate glycogen phosphorylase and deplete liver glycogen was not significantly affected in food-deprived pups (Tables XIII and XIV).

These findings suggest that ODC induction by phenylephrine, isoproterenol, and glucagon

TABLE XIII. EFFECT OF PHENYLEPHRINE ON LIVER PHOSPHORYLASE-a ACTIVITY IN MATERNALLY DEPRIVED RAT PUPS

	Phosphorylase activity (% control)
Control	100 ± 14 (28)
Deprived	111 ± 24 (20)
Control + phenylephrine, 2 mg/kg	275 ± 28[a] (12)
Control + phenylephrine, 25 mg/kg	372 ± 35[a] (15)
Deprived + phenylephrine, 2 mg/kg	166 ± 26[b] (14)
Deprived + phenylephrine, 25 mg/kg	351 ± 30[a] (15)

Note. Pups were deprived for 2 hr, anesthetized with pentobarbital, then injected with saline or phenylephrine. Pups were killed 30 min after the second injection. Control pups were left with the mother throughout the experiment and injected exactly as deprived animals. Results are expressed as percentage of control ± SEM. Numbers in parentheses, number of rats. Control phosphorylase activity = 3.07 ± 0.43 μmole/min/g of liver.

[a] $P < 0.001$ relative to control.

[b] $P < 0.05$ relative to drug-injected control.

TABLE XIV. EFFECT OF PHENYLEPHRINE ON LIVER GLYCOGEN IN MATERNALLY DEPRIVED RAT PUPS

	Liver glycogen (% control)	
Control	100 ± 4	(8)
Phenylephrine	36 ± 11[a]	(5)
Deprived	65 ± 11[a]	(5)
Deprived + phenylephrine	24 ± 7[a,b]	(5)

Note. Pups were deprived for 2 hr, then injected with saline or phenylephrine (5 mg/kg) and killed 1 hr later. Control pups were left with the mother throughout the experiment and injected at the same time. Results are expressed as percentage of control ± SEM. Numbers in parentheses, number of rats. Control glycogen content = 8.9 ± 0.4 mg of glucose/g of liver.

[a] $P < 0.05$ or better relative to control.

[b] $P < 0.05$ or better relative to deprived.

shares a common metabolic step that is affected by short-term food deprivation, a step that is not involved in other biochemical effects of these three agents. These findings further indicate that the effects of food deprivation and glucose involve some step beyond which the paths leading to ODC induction and effects on other enzymes diverge (Fig. 9).

The finding that FD affects only ODC responsivity also has significant implications in terms of sensory regulation of metabolic phenomena. These results suggest that short-term food deprivation limits growth-promoting processes but preserves certain aspects of cell metabolism like glycogen utilization that are vital for the survival. In this respect, the effects of glucose resemble those of tactile stimulation in their preservation of normal metabolism at the expense of growth.

Although both tactile stimulation of the pups and food share a common effect on ODC or its induction by hormones, both have extremely specific actions that do not overlap. In the presence of adequate nutrition, the actions of adrenergic agonists are normal even if maternal care is absent, while in the presence of adequate maternal care but no food, the actions of growth hormone are normal. These findings suggest that the division of metabolic processes into "growth promoting" and "energy utilizing" is overly simplistic, and that specific environmental cues are involved in regulation of particular aspects of growth and differentiation. These findings further suggest

that growth hormone on one hand and phenylephrine, isoproterenol, and glucagon on the other regulate specific aspects of growth and development that differ significantly. No difference in the consequences of ODC induction within the cell by different hormones has been identified. Therefore, it is likely that each hormone "group" shares as yet unidentified physiologic responses which regulate the division of cell metabolism into "growth" or "maintenance."

The studies reviewed above hopefully have provided new insight into the well-known effects of environmental stimuli on growth and development of mammals. First, they have identified specific physiologic events which could mediate environmental effects on growth. Perhaps more important, they have demonstrated that identification of these specific physiologic and biochemical responses is possible by interdisciplinary study of the interactions of behavior and physiology in the developing neonate.

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