Ontogeny of hypertonic preabsorptive inhibitory control of intake in neonatal rats

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Weller, Aron, Ludmila Tsitolovskya, Iris H. Gispan, and Gerard P. Smith. Ontogeny of hypertonic preabsorptive inhibitory control of intake in neonatal rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R44–R49, 2000.—The ontogenetic development of postingestive inhibitory control of ingestion by the osmotic load of a preload was examined in rats. On postnatal days 6 (P6) and 12 (P12), pups were deprived for either 6 or 24 h. Gastric preloads (5% body wt) of water, mannitol (a sugar alcohol that is not absorbed) in six concentrations [from 0.125 M (hypotonic) to 1.0 M (hypertonic)], or sham preloads were administered 5 min before a 30-min intake test. Compared with sham treatment, isotonic mannitol (0.25 M), a probe of volumetric control, significantly decreased intake on P12, but not on P6. Compared with isotonic mannitol, the three highest hypertonic concentrations (0.5, 0.66, and 1.0 M) significantly decreased intake on P12, at both levels of deprivation. On P6, 0.66 and 1.0 M mannitol reduced intake after 24 h, but not after 6 h, of deprivation. Thus, on P6, the hypertonic control was detectable only after prolonged deprivation and the volumetric control was not present. On P12, both controls were observed and the hypertonic control was more potent than on P6.

methodology

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METHODS

Animals. Sprague-Dawley rat pups of both sexes were tested on P6 and P12. Each pup was tested only once to avoid
the effects of prior preload experience. Siblings within a litter were allocated to different treatment groups; in all the experiments, no more than two siblings from a litter were allocated to the same treatment group (1). The pups were derived from our breeding colony at the Developmental Psychobiology Laboratory in the Psychology Department at Bar Ilan University. Lights in the colony were on from 0500 to 1700, and temperature was maintained between 21°C and 25°C. Each litter was housed in a polycarbonate cage (38 × 21 × 18 cm) with a stainless steel wire lid and wood shavings as bedding material. Chow and tap water were continuously available in the cage top. Newborn litters found between 0800 and 1200 were designated as born on that day (P0) and culled to 10 pups on P1 or P2.

Test procedure. All 10 pups from each litter were taken from the nest and dam in the morning (0800–0900 h for short deprivation) or afternoon (1300–1500 h for long deprivation). Each pup was weighed, marked, and then placed with its siblings in a small container in a humid and warm (33°C) incubator. Either 5–6 h (referred to as 6 h throughout the rest of this paper) or 24 h later, each pup was removed from the container and weighed, and an intraperitoneal injection [2 ml/kg of a vehicle solution of 9.5% ethyl alcohol and 4.5% carboxymethyl cellulose (Sigma) in distilled water] was administered to equate conditions with those of our previous work (21). Twenty-five minutes later excretion was stimulated by a cotton swab, and the anogenital region was sealed with cyanoacrylate glue. Next, each pup was reweighed and received a specific preload (see below) into the stomach via a polyethylene tube (PE-50, Becton-Dickinson) and then was isolated in a separate container for 5 min.

Next, each pup was weighed again and placed into a plastic beaker (500 ml, Azlon, Stone, UK) that had 2 ml of high-fat milk (UHT Longlife cream, 10% fat, Tnuva Dairy, Rehovot, Israel), sweetened by addition of sucrose to make a 10% solution, warmed to 38°C and spread equally over heavy tissue paper (two layers) cut to fit the bottom of the beaker. The beaker was located in a humidified incubator maintained at 38°C.

After 30 min, each pup was removed from the incubator, wiped dry with tissue paper, and weighed. Intake was measured by the change in body weight that occurred during the 30-min test and is reported as percent body weight gain (%BWG).

Preloads. The volume of all preloads was calculated to be 5% of body weight measured on the morning of the test day. The treatments were sham preload (tube insertion into stomach, no preload) or preloads containing distilled water or mannitol (Sigma, dissolved in distilled water). Six mannitol solutions were used to provide a range of osmotic concentrations: 0.125 M (hypotonic), 0.25 M (isotonic in the 6- to 12-day-old rat, according to calculations based on Ref. 17), 0.33 M, 0.50 M, 0.66 M, and 1.0 M (hypertonic).

Statistical analysis. No significant between-group differences were found in baseline body weight and sex distribution of the pups. Thus data from the two sexes were pooled for further analyses. The data were analyzed by two-way analysis of variance (ANOVA), with two between-subject factors: treatment (preload) and deprivation (6 or 24 h). The one independent measure was %BWG. These analyses were performed separately at the two ages (6 and 12) because baseline differences in level of intake were anticipated. The main effect of major interest was that of the preload. The interaction between preload and deprivation was of secondary interest. Significant main effects were assessed by Duncan’s multiple range test (P < 0.05), and interactions were assessed by simple main effects (24).

Three issues were examined by separate analyses. First, the effect of isotonic mannitol preload was compared with sham preload, to evaluate the effect of preload volume. Second, intake after the four hypertonic (0.25, 0.50, 0.66, and 1.0 M) concentrations of mannitol was compared with intake after isotonic (0.25 M) mannitol, to evaluate effects of hypertonicity. This analysis was followed by regression analysis, predicting %BWG from mannitol concentration. Third, intake after the hypotonic preloads (water and 0.125 M mannitol) was compared with intake after isotonic and sham preloads, to evaluate the effects of hypotonicity.

RESULTS

Figure 1 depicts the effects of the various preloads on P6, presenting the data according to the two levels of deprivation. At this young age control of intake by the volume of the preload was not evident, because the isotonic preload of 0.25 M mannitol did not significantly reduce intake compared with the sham preload [F(1,51) = 1.69, P > 0.198]. There was a significant effect of deprivation [F(1,51) = 7.32, P < 0.01] in this analysis, but the preload × deprivation interaction [F(1,51) = 0.01, P > 0.94] was not significant. Overall,
the pups deprived for 24 h ate significantly more than those deprived for 6 h.

ANOVA using the isotonic and hypertonic concentrations of mannitol yielded a significant interaction \[ F(4,121) = 2.71, P < 0.05 \], in addition to a main effect of preload concentration \[ F(4,121) = 3.73, P < 0.01 \]. The main effect of deprivation was not significant. Post hoc comparisons showed the following pattern of results (see Fig. 3). 1) Intake of the groups that received preloads of isotonic mannitol did not differ over the two levels of deprivation. 2) Among the groups tested after 6 h of deprivation, none was significantly different from the group that received the isotonic mannitol preload. 3) Among the groups tested after 24 h of deprivation, intake was significantly less in the groups that received 1.0 M \( (P < 0.01) \) and 0.66 M \( (P < 0.05) \) mannitol compared with the group that received isotonic mannitol. This observation is supported by regression analysis, which showed no significant linear relation after 6 h of deprivation \[ R^2 = 0.0001, F(1,61) = 0.003, P > 0.95 \] but did show a significant linear regression after the longer level of deprivation \[ R^2 = 0.30, F(1,66) = 28.405, P < 0.0001 \].

In the third analysis \[ F(3,102) = 3.41, P < 0.05 \], Duncan’s test showed that the hypertonic preloads (water and 0.125 M mannitol) had no significant effect on intake on P6 compared with the isotonic preload of 0.25 M mannitol (Fig. 1). Compared with the sham preload, however, the water, but not the 0.125 M mannitol, preload decreased intake significantly \( (P < 0.05) \). In this analysis, as in the first one, 24-h deprivation produced greater intake compared with 6-h deprivation \[ F(1,102) = 10.87, P < 0.01 \], but there was no significant interaction \[ F(3,102) = 0.21, P > 0.88 \].

On P12, the isotonic preload of 0.25 M mannitol reduced intake compared with sham preload [Fig. 2; \( F(1,58) = 29.01, P < 0.001 \)]. The main effect of deprivation \[ F(1,58) = 0.95, P > 0.33 \] and the preload \( \times \) deprivation interaction \[ F(1,58) = 0.11, P > 0.73 \] were not significant. Thus the volume of the preload inhibited intake at this age irrespective of deprivation level.

Comparison of the intake of the groups that received isotonic and hypertonic concentrations of mannitol (Fig. 3) showed a main effect of preload concentration \[ F(4,146) = 20.06, P < 0.0001 \], revealing that on P12 the three higher concentrations of mannitol \( (0.5, 0.66, \) and \( 1.0 \) M) but not the lower concentration \( (0.33 \) M), significantly reduced intake compared with the isotonic preload (Duncan’s test, \( P < 0.05 \)). Regression analysis further showed significant linear regressions after 6 h of deprivation \[ R^2 = 0.20, F(1,95) = 24.45, P < 0.0001 \] and after 24 h of deprivation \[ R^2 = 0.46, F(1,57) = 47.76, P < 0.0001 \]. The ANOVA showed that whereas the main effect of deprivation was not quite significant \[ F(1,146) = 3.78, P > 0.053 \], the concentration \( \times \) deprivation level interaction \[ F(4,146) = 0.14, NS \] was not significant. This is in accordance with the data in Fig. 3 that suggest similar intake-inhibitory functions of the hypertonic preloads at the two levels of deprivation.

![Figure 2](https://example.com/image2.png)

**Fig. 2.** Effects of gastric preloads (5% body wt) administered 5 min before a 30-min meal on intake (expressed as % body wt gain) in 12-day-old rats. A: data after 6 h of deprivation; \( n = 19–21 \) for each preload group. B: data after 24 h of deprivation; \( n = 11–12 \) for each preload group. Preloads were sham, isotonic \( (0.25 \) M mannitol), hypertonic \( (0.33–1.0 \) M mannitol), or hypotonic \( (\text{water or } 0.125 \) M mannitol). At this age all preloads decreased intake compared with sham preload. Some hypertonic preloads of mannitol \( (0.50, 0.66, \) and \( 1.0 \) M, but not \( 0.33 \) M) reduced intake compared with isotonic mannitol. The hypertonic preloads did not reduce intake compared with isotonic mannitol but did reduce intake compared with sham preload \( (P < 0.05) \). Intakes after preload of 0.25 M mannitol was significantly less than intake after sham preload, \( P < 0.05 \). Intakes after \( 0.5, 0.66, \) and \( 1.0 \) M mannitol were significantly less than intake after 0.25 M mannitol, \( P < 0.05 \).

In the third analysis \[ F(3,116) = 9.06, P < 0.001 \], Duncan’s test showed that the intake after hypotonic \( (\text{water and } 0.125 \) M mannitol) and isotonic \( (0.25 \) M mannitol) preloads was not significantly different. Compared with sham preloads, however, both hypotonic preloads significantly reduced intake. The main effect of deprivation \[ F(1,116) = 0.16, P > 0.69 \] and the interaction between deprivation and preload treatment \[ F(3,116) = 2.00, P > 0.11 \] were not significant in this analysis. Thus hypotonic preloads inhibited intake compared with sham preload at this age, irrespective of deprivation level.

**DISCUSSION**

Previous research has shown that hypertonic preloads of glucose, maltose, and 2-DG reduce intake in 12-day-old rats to a similar extent \( (20, 21) \). This suggests that the preabsorptive osmotic load of the food in the gastrointestinal tract controls feeding at this age. Use of mannitol, which is not absorbed or metabolized,
allowed us to establish the existence of this osmotic inhibitory control on P12. The relationship between mannitol’s hypertonic concentration and %BWG is dose dependent ($R^2 = 20\%$ and $46\%$ at the 2 deprivation levels, respectively), as is evident from Fig. 3. This demonstrates the sensitivity of the rat’s feeding system to the osmotic property of the preload on P12. In contrast, this osmotic control appears to be much less potent on P6. At this younger age, after 6 h of deprivation, no effect of the mannitol preload was evident at any of the concentrations examined. A dose-response curve appeared only after depriving the pups for 24 h ($R^2 = 30\%$; Fig. 3). Analysis of the data in Fig. 3 showed that the threshold concentration of mannitol that reduced intake significantly in the younger pups after 24 h of deprivation was 0.66 M, whereas at both levels of deprivation the 12-day-old pups showed a lower threshold concentration of 0.50 M. We conclude that postigestive inhibitory control of intake 5 min after hypertonic preloads of mannitol is age dependent. This control begins to emerge on P6, particularly after 24 h of deprivation, and is clearly present on P12. The dose-response function and the threshold dose on P12 were similar after 6 and 24 h of deprivation.

The mechanism by which mannitol produces inhibition of intake in pups is not known. However, it seems reasonable to assume that the hypertonic preloads of mannitol exert an osmotic gradient that draws body fluids into the small intestine, as has been shown in adult rats (4). Further studies in adults have shown that inhibition of intake (in rats; Ref. 6) and of gastric activity (in cats; Ref. 7) by mannitol are mediated by abdominal vagal nerves, many afferents of which are polymodal. Future studies should examine which type of receptor, osmo- or mechanosensitive, is responding to the hypertonic signal of mannitol in the infant rat. In cats, most of mannitol’s effect is apparently mediated by mechanoreceptors, sensitive to the mechanical stimulation produced by water being drawn into the intestine, because the osmoreceptors studied by Mei and Garnier (Table II of Ref. 13) were apparently insensitive to increasing doses of mannitol (over the range 275–1,100 M, similar to the range of the current study).

In contrast to the effects of the hypertonic preloads, hypotonic preloads did not decrease intake more than the effect of the preload’s volume on P6 or P12. However, in adult rats, when hypotonic mannitol was added to drinking water (containing saccharin and glucose), intake was reduced (3, 4). Furthermore, Garnier et al. (7) reported that perfusion of hypotonic concentrations of mannitol to the small intestine of anesthetized cats decreased gastric activity, as measured by electromyogram recorded in the antrum. Thus, to the extent that the physiological systems are comparable over species, it would seem that hypotonic concentrations of mannitol can exert a physiological effect (reduction in gastric activity) and reduce intake in adults, whereas in neonates these hypotonic concentrations appear ineffective.

In the present study isotonic preloads of 5% body wt volume decreased intake significantly compared with sham preload on P12, but not on P6. The efficacy of the preload’s volume on P12 replicates our previous findings at this age (20, 21). This is consistent with a previous report by Hall and Bruno (Fig. 3A of Ref. 10) that showed that isotonic preloads (5% body wt) did not decrease intake compared with sham preloads in 24-h deprived pups on P6. Thus it appears from this, and from our evidence, that volume per se of the 5% preload is not sufficient to produce postigestive control of intake on P6. Although Hall and Bruno (10) have shown that a larger saline preload (10% body wt) reduced intake significantly on P6, the volume of this preload is almost twice as large as the postigestive volume (5.8 ± 0.2% BWG) measured in 24-h deprived sham-loaded pups on P6 (Table 3 of Ref. 10).

Level of deprivation presented an interesting pattern of effects in the current study. The expectation was for deprivation to provide a metabolic stimulus that increases feeding, and therefore to find that under the 24-h deprivation the preloads are less potent in inhibiting intake. However, the results showed that on P12, when the impact of volume and hypertonicity of the preload were strong, the deprivation manipulation did alter these effects significantly. In the younger P6 pups, however, 24-h deprivation increased intake in controls (sham and isotonic preloads) and allowed the hypertonic control to be apparent.

The reason for this difference in the efficacy of deprivation on P6 and P12 is unknown. The most likely explanation is to relate it to ontogenetic differences in response to dehydration (2). Briefly, rat pups respond to cellular dehydration by increasing the intake of milk or water, on P3, P6, and P10; this is not evident on P15, and by P20 they clearly show the opposite effect, i.e., “dehydration-induced anorexia,” selectively increasing water intake, but decreasing milk intake in response to...
dehydration (2). In the context of our findings, the 24-h deprivation should have dehydrated the pups, and the younger P6 pups responded characteristically by increasing milk intake. In contrast, the P12 pups may have been at a transition age between dehydration-anorexia and dehydration-anorexia, with a net result of no significant deprivation effect.

In summary, the current results demonstrate a moderate effect of the postigestive osmotic load of mannitol on P6 and a clear effect on P12. The initial site of action of this osmotic control is preabsorptive.

Perspectives

The demonstration of a concentration-dependent, preabsorptive, osmotic inhibitory control of intake on P12 in these experiments with preloads of mannitol means that by P12 two postigestive, preabsorptive controls of independent ingestion, volumetric and osmotic, are operating. Although both of these controls can be elicited on P6, they are relatively insensitive at that age. It is interesting that nutrient controls of intake that are prominent in the adult rat, such as those elicited by glucose and oils (8), are not present on P12 (20, 21, 22). The inhibitory effect of preloads of corn oil does not appear until P15 (22). Furthermore, recent experiments show that preloads of glucose given 5 min before an intake test do not inhibit intake more than can be accounted for by the volume and osmolality of the preloads at any preweaning age up to P18, the oldest age tested (20, 23). The lack of inhibitory effect of the primarily preabsorptive stimuli of glucose preloads given 5 min before an intake test contrasts with the significant inhibitory effect observed as early as P9 produced by the postabsorptive actions of preloads of glucose given 2 h before the intake test (9, 14, 19).

The volumetric and osmotic controls present on P12 probably do not have a major learned component because they are effective on the first test in which the pup is eating independently away from the dam. Of course, it is not possible to exclude some associative learning about postigestive stimuli acquired during suckling (or during in utero ingestion of amniotic fluid; Ref. 15) that also acts in independent ingestion.

Because the volumetric and osmotic controls act preabsorptively, they can be classified as direct controls (16). In the adult rat, the metabolic effect of food deprivation is classified as an indirect control because it changes intake by altering the potency of direct controls. The modulatory effect of all indirect controls on direct controls is hypothesized to depend on neural connections between the forebrain and hindbrain (16, 17, 18). In the context of this theory, it is interesting that increasing the duration of food deprivation from 6 to 24 h allowed the 6-day-old rat to show intake control by hypertonic mannitol and that deprivation increased intake overall on P6 in the analyses that included sham, isotonic, and hypotonic preload conditions. This suggests that the neural mechanisms that mediate the indirect control elicited by food deprivation in the adult rat are functional on P6. The fact that increased deprivation did not change the potency of the volumetric or osmotic control on P12 further suggests that these controls become resistant to modulation by deprivation on P12. It is possible that the differential response to deprivation was due to ontogenetic differences in response to dehydration (2). Thus developmental experiments can be used to determine when in ontogeny the neural mechanism of this indirect control is active, when it interacts with other, emerging controls, and whether these effects can be correlated with the maturation of synaptic connections between the forebrain and caudal brainstem.

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