Postnatal oxytocin alleviates adverse effects in adult rat offspring caused by maternal malnutrition

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Submitted 8 July 2002; accepted in final form 2 October 2002

Olausson, Hanna, Kerstin Uvnås-Moberg, and Annica Sohlström. Postnatal oxytocin alleviates adverse effects in adult rat offspring caused by maternal malnutrition. Am J Physiol Endocrinol Metab 284: E475–E480, 2003; 10.1152/ajpendo.00297.2002.—Repeated oxytocin administration to adult rats causes a long-term decrease of plasma levels of corticosterone and blood pressure and stimulates growth and fat retention. Maternal undernutrition increases blood pressure and plasma corticosterone in adult offspring. We hypothesized that oxytocin treatment early in life would alleviate adverse effects of intrauterine food restriction. Male pups from ad libitum-fed and food-restricted (fed 60% of ad libitum intake) dams were injected with oxytocin or saline in days 1–14 after birth. At 4 mo, blood pressure, plasma levels of corticosterone, and adiposity were assessed. Oxytocin treatment decreased blood pressure independently of nutrition, whereas the increased plasma levels of corticosterone were lowered to normal levels in food-restricted offspring. Blood pressure and adiposity were not affected by in utero food restriction, whereas birth and adult weight were. In conclusion, postnatal events may alleviate adverse effects caused by in utero food restriction. In contrast to more severe food restriction, a moderate general food restriction during gestation had no effect on blood pressure in the offspring.

A NUMBER OF STUDIES have shown that the nonapeptide oxytocin has physiological anti-stress and metabolic effects in the body (3, 42), in many cases resembling those of sensory stimulation (8, 37). Oxytocin is released from neurons in the hypothalamus in response to nonnoxious sensory stimuli, such as stroking, warm temperature, touch, and light pressure (2, 41, 45). Repeatedly administered oxytocin to adult male rats decreases plasma levels of corticosterone (30) and blood pressure (29) and stimulates anabolism (5, 43) in the long term. Oxytocin administered postnatally has been shown to have life-long effects, resulting in increased body weight and body fatness in adulthood (44). Furthermore, oxytocin administered early in life to offspring from food-restricted mothers stimulates postnatal weight gain and decreases the elevated levels of plasma corticosterone (40). Because oxytocin exhibits these long-term anti-stress and growth-promoting effects, it was hypothesized that the peptide is able to alleviate adverse effects of intrauterine stress caused by maternal food deprivation.

Intrauterine stress, resulting in impaired fetal and postnatal growth, is associated with an increased risk of developing diseases later in life, such as cardiovascular diseases, non-insulin-dependent mellitus, and high blood pressure (4). This relationship was first seen in epidemiological studies (4) and was confirmed in many experimental animal studies. For example, rat offspring from dams subjected to severe protein food restriction (17) or extreme general food restriction (48) had a lower birth weight and higher blood pressure in adulthood than well-nourished counterparts. Furthermore, nutrition in early postnatal life influences the cardiovascular system, resulting in higher blood pressure in adulthood (23). One proposed mechanism, explaining how poor nutrition in fetal or early postnatal life can cause hypertension in adult life, is that life-long changes in the fetal or newborn brain, for example, in the hypothalamic-pituitary-adrenal (HPA) axis, would reset the homeostatic mechanisms controlling blood pressure (9).

So far, most experimental studies have focused on effects of intrauterine stress and life-long consequences. Few studies have investigated whether the adverse effects caused by intrauterine stress may be ameliorated by postnatal events. However, administration of antihypertensive drugs (38, 39) early in life has been shown to prevent the development of hypertension programmed by intrauterine exposure to a low-protein maternal diet. Furthermore, increased maternal care early in life decreases HPA axis responsiveness to stress in adult rats (21, 24–26) and boars (46). Massage (1, 16, 22, 37) and kangaroo care (8) given to rats and premature babies have positive physiological anti-stress, metabolic, behavioral, and growth effects. The underlying mechanisms behind these effects are fairly unknown, but they are likely to

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be induced by stimulation of somatosensory nerves, which leads to a change in the activity of the HPA axis and in the balance of the autonomic nervous tone in favor of parasympathetic nervous tone. Interestingly, the anti-stress effects of massage and kangaroo care in many cases resemble those of oxytocin. Thus we hypothesized that oxytocin administered early in life may ameliorate the long-term adverse effects caused by food restriction in utero.

The aims of this study were to use oxytocin as a model to study whether postnatal events would have ameliorating effects on blood pressure and levels of corticosterone in male offspring from ad libitum-fed and food-restricted dams.

**METHODS**

**Experimental design.** Fourteen female Sprague-Dawley rats (280–320 g) were mated overnight, after 1 wk of adjustment in the animal department. Vaginal smear was taken every morning, and when sperms were found they were considered to be pregnant (day 1 of gestation). On this day, the females were divided into two dietary treatment groups. One control group was given food (R36; Ewos, Södertälje, Sweden) ad libitum throughout gestation, and the other group received 60% of the average food intake consumed by the ad libitum-fed control group until the day of parturition (day 22 of gestation). The rats had free access to water during the whole experiment. Animals were housed individually, and food intake was measured in the ad libitum fed-group three times per week. The day after parturition was considered as day 1 of lactation. From this day, food was available ad libitum in both groups. Litter size was adjusted to eight pups per mother on day 1 of lactation by random selection, keeping the mean body weight of the whole litter unchanged. In each litter, four pups were injected one time per day, subcutaneously, with oxytocin (1 mg/kg body wt), and the other four pups were injected with saline (0.9% NaCl) during days 1–14 after birth. A total of 112 pups were left after culling, of which 58 were males. Forty male pups were used in this study for measuring blood pressure, heart rate, levels of plasma corticosterone, and adiposity at 4 mo of age. The number of pups in each group (ad libitum/NaCl, ad libitum/oxytocin, food restricted/NaCl, food restricted/oxytocin) is shown in Table 1. The females and the remaining males were kept for other experiments not described in this paper. On day 21 of lactation, the pups were separated from their mothers, and females and males were divided and housed four in each cage. The body weight of the pups was recorded on days 1, 5, 10, 15, and 20 of lactation and weekly thereafter until an age of 4 mo. The rats were killed by decapitation.

The rats were kept in their cages in another room than the room of killing, and they were removed one by one from the cage and carried to the place of decapitation. The animals were killed within 20 s after removal from cages. Thereafter, blood was collected immediately, mixed with heparin [10 International Units (IU)/ml] and aprotinin (500 IU/ml), and kept on ice until centrifugation, whereupon plasma was harvested and frozen until further analyses. The killing was performed between 9:00 and 12:00 AM. Adipose tissues from inguinal and retroperitoneal regions were dissected out and weighed. The study was approved by the Stockholm ethical committee for experiments in animals.

**Blood pressure and heart rate.** Systolic and diastolic blood pressure and heart rate were measured on awake rats by placing a cuff (Kent RTBP-002) and a microphone on the base of the tail. The cuff and the microphone were connected to a Grass 7P8DC sphygomanometer and a Grass 7P8DC amplifier with a printer. The rat was kept on a heating pad and beneath an infra-red lamp with the purpose to increase the vasodilatation of the vessels in the rat tail. The measurement could start as soon as the heart rate was clearly shown and the rat was lying still. To make this possible, the rat had to be trained during ~2 wk before the actual blood pressure values, and the heart rates were recorded. Three to five measurements were taken, and averages of the pressure and the heart rate were calculated.

**Analysis of plasma corticosterone.** Corticosterone was measured quantitatively by a solid-phase RIA (Count-a-count Rat Corticosterone; DPC, Los Angeles, CA) in which 125I-labeled rat corticosterone competes for a fixed time with corticosterone in the sample for antibody sites. The antibody-bound fraction in each sample was counted in a gamma counter, and the yielded number was converted to corticosterone concentration in a calibration curve of known samples provided in the kit.

**Statistical analysis.** Results presented are means ± SD. The effect of food restriction in utero on litter size and birth weight was assessed with Student’s unpaired t-test. The effects of food restriction and oxytocin treatment on body weight, adipose tissues, systolic and diastolic blood pressure, heart rate, and plasma levels of corticosterone were assessed by a two-way ANOVA, having treatment and nutrition as components of variation. When interactions were found, the following planned comparisons were made: ad libitum/NaCl vs. food restricted/NaCl, ad libitum/NaCl vs. ad libitum/oxytocin, food restricted/NaCl vs. food restricted/oxytocin, and ad libitum/oxytocin vs. food restricted/oxytocin. Levene’s test for equal variances was performed, but no significances were found. Differences were considered statistically significant at P < 0.05.

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**Table 1. Birth weight, body weight, and inguinal and retroperitoneal adipose tissues in male rats at 4 mo of age coming from ad libitum-fed or food-restricted dams and being treated with oxytocin or NaCl during the first 14 days of life**

<table>
<thead>
<tr>
<th></th>
<th>Ad Libitum</th>
<th>Food Restricted</th>
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<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>Oxytocin</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>7.3 ± 0.5*</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>568 ± 43†</td>
<td>577 ± 41†</td>
</tr>
<tr>
<td>Inguinal adipose tissue, % of body wt</td>
<td>1.74 ± 0.36</td>
<td>1.86 ± 0.37</td>
</tr>
<tr>
<td>Retroperitoneal adipose tissue, % of body wt</td>
<td>1.62 ± 0.42</td>
<td>1.80 ± 0.39</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 rats in each group. *Significant effect of nutrition (P < 0.000) by Student’s unpaired t-test. †Significant effect of nutrition (P = 0.016).

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RESULTS

Body weight and adiposity. Litter size was not affected by food restriction during gestation (ad libitum fed 11.1 ± 4.1 pups/dam and food restricted 11.3 ± 2.4 pups/dam). However, in utero food-restricted pups were significantly smaller at birth than offspring from ad libitum-fed dams, and they remained smaller throughout the study (Table 1). However, there was no effect of in utero food restriction or postnatal oxytocin treatment on adiposity at adult age (Table 1).

Blood pressure and heart rate. Oxytocin treatment early in life had long-term effects, decreased systolic pressure, and tended to decrease diastolic (P = 0.067) blood pressure in adulthood, independent of in utero nutrition (Fig. 1, A and B). However, food restriction in utero had no effect on systolic or diastolic blood pressure in the adult offspring (Fig. 1, A and B). Mean heart rate for all animals during blood pressure measurements was 422 ± 32 beats/min. No difference in pulse between the groups was seen.

Plasma levels of corticosterone. Oxytocin treatment early in life decreased the levels of corticosterone in adult offspring from food-restricted dams to the same levels as those of offspring from the ad libitum-fed dams (Fig. 2). Food restriction in utero increased the levels of corticosterone in the adult offspring treated with NaCl (Fig. 2).

DISCUSSION

Early postnatal oxytocin treatment induced life-long decreasing effects on arterial blood pressure. Furthermore, oxytocin decreased the otherwise high levels of corticosterone observed in the in utero food-restricted offspring. Food restriction in utero did, however, not affect blood pressure in adulthood. Seemingly, certain early postnatal treatments, such as oxytocin administration, may alleviate life-long perturbations caused by in utero food restriction.

Mean values of blood pressure in this study were 10–15 mmHg higher than in previous studies using the same equipment (29, 32). The rats in this study were, however, 2 mo older than the rats in previous studies. Moreover, the measurements were performed manually by different observers in the different studies, which also may explain the differences in mean values of blood pressure. Mean heart rate in this study was 422 ± 32 beats/min, which is within the range of normal values for rats (313–493 beats/min; see Ref. 13). Because the rats were habituated to the entire test procedure during 2 wk before the start of the experiment, we considered these rats to be relatively unstressed during the time of measurement. However, it is conceivable that the tail-cuff method imposes some stress to the animal discussed by Fraser et al. (11). The levels of corticosterone in this study were within the

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Fig. 1. Systolic (A) and diastolic (B) blood pressure in adult male rat offspring treated with oxytocin or NaCl during the first 14 days of life, coming from ad libitum-fed or food-restricted dams. No. of animals in each group is shown in Table 1. *Significant effect of oxytocin treatment on systolic blood pressure (P = 0.026). Values are means (small boxes), SD (bars), and SE (large boxes).

Fig. 2. Plasma levels of corticosterone (ng/ml) in adult male rat offspring treated with oxytocin or NaCl during the first 14 days of life, coming from ad libitum-fed or food-restricted dams. No. of animals in each group is shown in Table 1. *Significant difference between adult offspring treated with NaCl, coming from ad libitum-fed and food-restricted dams (P = 0.006). **Significant difference between adult offspring treated with NaCl or oxytocin, coming from food-restricted dams (P = 0.002). Values are means (small boxes), SD (bars), and SE (large boxes).
normal interval according to the manufacturer’s guidelines for adult male rats during this time of the day.

This study shows for the first time that oxytocin treatment early in life can cause a life-long decrease in arterial blood pressure. In earlier studies of adult rats, the most sustained reported effect of oxytocin administration to rats is a decrease of arterial blood pressure lasting up to 10 days after the last injection (29–31, 33). However, in this study, the effect of postnatal oxytocin administration on blood pressure lasted at least up to the age of 4 mo. Furthermore, oxytocin treatment early in life normalized the increased plasma levels of corticosterone in the adult offspring from food-restricted dams. Similar results were observed by Sohlström et al. (40) in a previous study on 2-mo-old rats, indicating that oxytocin administered early in life can ameliorate certain adverse effects of in utero-induced alterations caused by food restriction.

As the brain develops rapidly in the rat pup during the first weeks after birth, it is sensitive to environmental influences. Because ~0.2% of the oxytocin administered subcutaneously passes through the blood–brain barrier (15), sufficient amounts should have reached the central nervous system to induce central effects. Interestingly, autoradiographic studies of the nucleus of the solitary tract and hypothalamus in the brains from the rats in this study indicate a sustained enhancement of \( \alpha_2 \)-adrenoceptor function in the offspring treated with oxytocin early in life (Diaz-Cabiale Z, Olausson H, Sohlström Å, Angel Narvæå J, Uvnäs K, and Fuxe K, unpublished observation). These persisting modulations of \( \alpha_2 \)-adrenoceptors in the central regions, of importance for the control of the autonomic nervous system and the HPA axis (10), may be linked to the recorded long-lasting effects of postnatal oxytocin treatment on blood pressure and corticosterone levels. This may therefore be one mechanism explaining the long-lasting antistress action of oxytocin. Another possible explanation may be that oxytocin administered postnatally would reset the HPA axis via other mechanisms, such as an influence on glucocorticoid receptor. This axis is known to be subjected to programming by events during gestation (40) or the postnatal period (24–27). Possibly, massage and maternal care decrease blood pressure (16, 22) and plasma levels of corticosterone in response to stress (21), respectively, through the same mechanism as administration of exogenous oxytocin, since endogenous oxytocin is released in response to warmth, touch, and stroking (2, 41, 45).

Maternal food restriction during gestation resulted in smaller offspring, which is in agreement with previous data published using the same model (40). This shows that, although the food restriction was of a general moderate type, it is valid in its purpose to affect the females and the offspring. However, adult blood pressure in the offspring was unaffected by this type of food restriction in utero. Earlier studies with protein restriction before and during gestation (17) and severe food restriction (30% of ad libitum intake) during gestation (48), possibly implicating protein restriction too, results, however, in adult rat offspring with elevated blood pressure. Comparisons of the effect on the offspring's blood pressure of two different low-protein-diet manipulations in rat pregnancy show that the balance of protein and other nutrients may be a critical determinant (18). Milder food restrictions have in some studies been shown to increase blood pressure (28) while others, including the present, have been unable to see such effects (14). Only a few human studies have related maternal nutritional status or food intake during pregnancy to blood pressure in adult offspring (6, 12, 20, 36). Composition rather than the quantity of the pregnant woman’s diet seems to be of importance for her child’s blood pressure in later life (35). Taken together, both human and animal studies indicate that maternal food intake during pregnancy may be associated with the blood pressure of the offspring. However, the degree of malnutrition, composition of the diet, and the timing of malnutrition are probably factors of importance for this complex and not completely understood relationship.

Food restriction in utero increased, however, the plasma levels of corticosterone in male offspring, which confirm previous data from a study with similar design but performed in younger rats (40). The results indicate that food restriction during pregnancy induces maternal stress, which in some way gives rise to permanently altered activity of the HPA axis in the adult offspring, as previously described by Langley-Evans et al. (19). In agreement with animal studies, low human birth weight, as a proxy for undernutrition in utero, is associated with increased urinary glucocorticoid secretion in 9-yr-old children (7) and increased plasma levels of glucocorticoids in adult men (34). Not only maternal food restriction but also other types of maternal stress during pregnancy have been shown to induce long-lasting changes in the HPA axis in the offspring (47).

Seemingly, a moderate general food restriction, as applied in this study, affected the plasma levels of corticosterone rather than blood pressure in male rats, indicating that various biological systems do not exhibit the same sensitivity to undernutrition. Results to be published from this study, as mentioned above (Diaz-Cabiale et al., unpublished observation), also showed that food restriction in utero actually resulted in changes in the \( \alpha_2 \)-adrenoceptors in the nucleus tractus solitarius, which may protect against hypertension. Thus the effect of nutrition on blood pressure is complex since both factors that may elevate and/or lower the blood pressure can be influenced.

In conclusion, an early postnatal event, such as oxytocin treatment, induced long-lasting effects on arterial blood pressure and exhibited ameliorating effects on increased levels of plasma corticosterone induced by maternal moderate food restriction. Thus perturbations in utero may be alleviated postnatally. A moderate maternal food restriction in utero did not affect blood pressure but elevated the levels of corticosterone, indicating that the relationship between maternal nutrition and health of the offspring is complex and that
various regulatory systems may be affected in different ways by undernutrition.

Ferring (Malmö, Sweden) is acknowledged for generously supplying oxytocin, and Dr. Maria Petersson for introducing us to the tail-cuff method.

This work was supported by Ake Wiberg’s Foundation, Magnus Bergvall’s Foundation, and Tore Nilsson’s Foundation.

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