Maternal Exposure to Atrazine during Lactation Suppresses Suckling-Induced Prolactin Release and Results in Prostatitis in the Adult Offspring

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Received March 3, 1999; accepted June 1, 1999

The availability of prolactin (PRL) to the neonatal brain is known to affect the development of the tuberoinfundibular (TIDA) neurons and, as a consequence, lead to alterations in subsequent PRL regulation. Without early lactational exposure to PRL (derived from the dam’s milk), TIDA neuronal growth is impaired and elevated PRL levels are present in the prepubertal male. These observations, combined with the finding that alterations in PRL secretion (i.e., hyperprolactinemia) in the adult male rat have been implicated in the development of prostatitis, led us to hypothesize that early lactational exposure to agents that suppress suckling-induced PRL release would lead to a disruption in TIDA development, altered PRL regulation, and subsequent prostatitis in the male offspring. To test this hypothesis, suckling-induced PRL release was measured in Wistar dams treated twice daily with the herbicide atrazine (ATR, by gavage, on PND 1–4 at 0, 6.25, 12.5, 25, and 50 mg/kg body weight), or twice daily with the dopamine receptor agonist bromocriptine (BROM, sc, at 0.052, 0.104, 0.208, and 0.417 mg/kg). BROM is known to suppress PRL release. Similarly, atrazine has also been reported to suppress PRL in adult females. Serum PRL was measured on PND 3 using a serial sampling technique and indwelling cardiac catheters. A significant rise in serum PRL release was noted in all control females within 10 min of the initiation of suckling. Fifty-mg/kg ATR inhibited suckling-induced PRL release in all females, whereas 25 and 12.5 mg/kg ATR inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of ATR was without effect, BROM, used here as a positive control, also inhibited suckling-induced PRL release at doses of 0.104 to 0.417 mg/kg, with no effect at 0.052 mg/kg. To examine the effect of postnatal ATR and BROM on the incidence and severity of inflammation (INF) of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of ATR-treated dams (25 and 50 mg/kg). The 12.5 mg/kg ATR and the two highest doses of BROM increased the incidence, but not the severity, of prostatitis. Combined treatment of ovine prolactin (oPRL) and 25 or 50 mg/kg ATR on PND 1–4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in INF, seen after ATR alone, resulted from the suppression of PRL in the dam. To determine whether or not there is a critical period for these effects, dams were dosed with 25 and 50 mg/kg on PND 6–9 and PND 11–14. Inflammation was increased in those offspring from dams treated on PND 6–9, but this increase was not significant. Dosing on PND 11–14 was without effect. These data demonstrate that ATR suppresses suckling-induced PRL release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND 1–9.

Key Words: atrazine; prolactin; tuberoinfundibular neuron; bromocriptine; prostatitis.

Normal development of reproductive function depends on the presence or absence of naturally occurring steroid hormones during certain prenatal and postnatal periods. It has been shown in laboratory and wildlife species that disturbances in this maturational process can lead to permanent alterations in the neuroendocrine control of reproduction. Many of these changes may not be fully expressed until the offspring reaches maturity or even middle age. For example, it has been well documented that administering an estrogenic or anti-estrogenic compound during late gestation or early postnatal days (PND) can permanently affect sexual differentiation of the brain. In contrast to these “organizational” effects, exposure to these agents in adulthood will transiently bring about “activational” changes in the neuroendocrine regulation of reproduction (Review: Arnold and Breedlove, 1985).

In addition to the organizational effects of steroids on reproductive processes, other key developmental changes are dependent upon exposure to peptide hormones. For example, the presence or absence of milk-derived proteins and of hor-
mones such as prolactin (PRL) have been reported to be critical for the normal development of neuroendocrine function (Shyr et al., 1986). It has been shown that prolactin accumulates rapidly in the milk of lactating rats and approximately 16% of the milk prolactin ingested by the rat pups passes into their systemic circulation. Approximately 300 ng/24 h are delivered via the milk in early lactation (Shah et al., 1989). High PRL biological activity is detected in serum during PND 2–5 of life (Kacsoh et al., 1993), although endogenous production of PRL by the rat pituitary does not occur until PND 5 of life (Hoeflffler et al., 1985).

Shyr et al. (1986) identified a critical role for milk-derived prolactin in the postnatal development of the tuberoinfundibular dopaminergic system (TIDA). In the adult, these neurons have an important inhibitory effect on prolactin secretion from the anterior pituitary. Shyr et al. dosed lactating dams with bromocriptine on PND 2–5 to block the release of maternal prolactin and found that the offspring had a decreased steady-state dopamine (DA) concentration and turnover rate in the median eminence on days 33–35 that resulted in hyperprolactinemia. When they delayed maternal bromocriptine treatment until PND 9–12, these effects were not evident, suggesting that PND 2–5 was a sensitive period for neuroendocrine development of prolactin regulation in the offspring. Earlier studies demonstrated that the organization of the TIDA neurons (onset of DA synthesis and release) occurs primarily during the first postnatal week in the rat (Loizou, 1971; Ojeda and McCann, 1974).

There is evidence that the hyperprolactinemia, such as that observed by Shyr et al., could lead to adverse effects in adulthood. For example, increased prolactin levels have been shown to play a major role in the induction of lateral prostate inflammation in the adult rat (Tangbanluekal and Robinette, 1993). These investigators found that estradiol could induce hyperprolactinemia and lateral prostate inflammation. However, when they co-treated with estradiol and bromocriptine, and thus lowered serum prolactin, the prostatitis was absent, indicating that elevated prolactin was involved in the induction of prostatitis. To further characterize this effect, the investigators also found a dose-response relationship between the administration of exogenous PRL and the severity of the inflammation that was induced. In humans, nonbacterial prostatitis with undefined etiology has become a significant clinical problem (Uehling, 1989), and it is unknown at this time whether hormonal status is involved in this condition. The presence of inflammation has been noted by histological examination in other prostate diseases as well, such as benign hyperplasia and adenocarcinoma (McClinton et al., 1990).

Many environmental agents have been shown to alter the secretion of prolactin by various mechanisms (i.e., Blake and Boocoffor, 1997; Steinmetz et al., 1997). The consequences of these effects on reproductive health are poorly understood. For example, the widely used chlorotriazine herbicide, atrazine has been shown recently to suppress prolactin release in ovariec-
tomized, estradiol-implanted females (Cooper et al., 1996). Therefore, to investigate the possibility of effects of alterations of TIDA development by environmental exposures such as atrazine, we hypothesized that a lactational exposure to atrazine during early postnatal development could suppress suckling-induced prolactin release in the dam and possibly result in adverse effects such as prostatitis in adulthood.

**MATERIALS AND METHODS**

**Animals.** Timed-pregnant female Wistar rats were purchased from Charles River in Raleigh, NC. Rats arrived on gestation day 13 and were housed one per cage in an AAALAC (American Association for the Accreditation of Laboratory Animal Care)-accredited facility at 22°C. All animals were maintained on a 12h:12h light:dark cycle (on 0500 h, off 1700 h) and were provided with food and water ad libitum. The day of delivery was designated postnatal day (PND) 0. On PND 1, the pups were culled to 10 per litter. In the suckling-induced prolactin experiment, the pups were taken from the mother prior to surgery and weighed prior to placing them back with the mother four h later. The pups were weighed again after the 45-min suckling period. For all studies, the offspring were weaned at 25 days-of-age and placed in individual cages. All pups were weighed at 29 days-of-age and the day prior to sacrifice at either 90 or 120 days-of-age. All males were decapitated within 15 s of removal from their cage at approximately 0900 to 1000 h. Each treatment group consisted of males from at least 5 different litters.

It should be mentioned that Wistar rats were selected because of their age-related development of prostatitis. Naslund et al. (1988) compared the incidence of spontaneous prostatitis in several strains of rat, and they reported that the Wistar strain showed no detectable spontaneous prostatitis in young and 27% in old rats. Therefore, the Wistar rat was an appropriate rat model for this study to examine the incidence of prostatitis in the young adult to middle-aged rat, with a low incidence in the younger animal and a moderate sensitivity for the development of prostatitis.

**Dosing.** Animals were dosed twice a day by gavage or subcutaneous (sc) injection at a volume of 0.1 cc per 100-g body weight. Atrazine (Novartis, Greensboro, NC, 98% purity) was administered as a suspension in a 1% solution of methylcellulose (Sigma Chemical, St. Louis, MO) by gavage. Doses of atrazine used were 6.25, 12.5, 25, or 50 mg/kg body weight on PND 1–4, 6–9, or 11–14. Control females received the 1% solution of methylcellulose only. Bromocriptine (2-bromo-a-ergocryptine methane sulfonate; Sigma Chemical, St. Louis, MO) was dissolved in saline and administered sc in the nape of the female’s neck at doses of 0.052, 0.104, 0.208, or 0.417 mg/kg bw for the suckling-induced prolactin release experiment on PND3. For examination of effects of maternal dosing on PND 1–4 and subsequent effects on the prostate at 90 or 120 days-of-age, only the 0.104 and 0.417 mg/kg doses of bromocriptine were used. Control females received saline only. Ovine prolactin (oPRL; Sigma Chemical, St. Louis, MO) was administered as a countermeasure to the control and atrazine-treated dams in a follow-up experiment. The oPRL was injected sc at a dose of 0.3 mg in 0.1 cc of saline (Caron et al., 1994) All doses were administered twice a day at 0900 and 1600 h.

**Suckling-induced prolactin release experiment.** On PND 2, the dams and their pups were moved, in their home cages, to a surgery/observation laboratory that was maintained on a light:dark cycle identical to that of the animal holding room. On the morning of PND3, pups were removed from the dam at approximately 0800 and placed in fresh bedding, then the dams were dosed with vehicle, atrazine, or bromocriptine. The dams were anesthetized with halothane anesthesia and fitted with indwelling cardiac catheters by a modification of the method of Goldman and Cooper (1993). Following surgery, the dams were taken off halothane and placed in plastic jars with bedding, and the catheter with needle and syringe attached were hung out of a hole in the lid of the jar to allow the dam to move about freely within the jar. The dams were provided food and water ad libitum for the remainder of the experiment. At
least 2 h were required for full recovery. For each blood sample taken, 0.2 cc of blood were drawn and replaced with heparinized saline solution. Baseline blood samples were taken at 1 h, at 30 min, and immediately prior to placing the pups back with the dams at approximately 1200 h. Once the pups were individually weighed and returned to the dam’s cage, blood samples were collected at 10-min intervals over the next 80 min. After 45 min of nursing, the pups were removed and weighed again. The behavior of the dams and the nursing activity of the pups were monitored during this period. After the last blood sample was drawn at 80 min, the pups were returned with the dams, in their original cages. Because of possible effects of the surgical procedure, the male offspring of catheterized dams were not used in the evaluation of the prostate in adulthood.

Radioimmunoassays. Serum and anterior pituitary prolactin were quantified by radioimmunoassay. The assay was performed using the following materials, supplied by the National Hormone and Pituitary Agency: iodination preparation I-6, reference preparation RP-3, and antiserum S-9. Iodination material was radiolabeled with 125I (Dupont/New England Nuclear) by a modification of the chloramine-T method of Greenwood et al. (1963). Labeled PRL was separated from unreacted iodide by gel filtration chromatography as described previously (Goldman et al., 1986).

Sample serum and pituitary homogenate were pipetted with appropriate dilutions to a final assay volume of 500 μl with 100-mM phosphate buffer containing 1% bovine serum albumin (BSA). Standard rPRL was serially diluted for standards of 0.313 to 10 ng/ml. Two hundred μl of primary antiserum, at a dilution of 437,500 in 100 mM potassium phosphate, 76.8 mM EDTA, 1% BSA, and 3% normal rabbit serum were pipetted into each assay tube, vortexed, and incubated at 5°C for 24 h. One hundred μl of 12I Prl containing 20,000 CPM were added to each tube, vortexed and incubated for 24 h. Second antibody (Goat Anti-Rabbit Globulin, Calbiochem, at a dilution of 1 unit/100 μl) was then added, vortexed and incubated for 24 h. The samples were then centrifuged at 1260 g for 30 min, the supernate was aspirated, and the sample tube with pellet was counted on a gamma counter. Intra- and interassay coefficients of variation for the PRL assays were 2.86% and 9.1%, respectively.

Necropsy and examination of prostates. At 90 or 120 days-of-age, within the same experiment, the male offspring were killed by decapitation for collection of blood, pituitary, brain, testes, and prostate. Body weights were taken the day prior to sacrifice. The clotted blood was centrifuged at 1260 g for 30 min and the serum was then harvested and stored frozen at –80°C for the prolactin assay. The brains and anterior pituitaries were removed, frozen on dry ice, and stored at –80°C for subsequent hormonal analyses. The extent of inflammation in the ventral and lateral lobes of the prostate was visually inspected and given a subjective inflammation score (0–3; 0 = none, 1 = mild, 2 = moderate, 3 = severe). This score was used to determine the dilutions for the DNA assay. The lateral lobes were carefully separated from the dorsal lobe. The left lateral and ventral prostates were removed, weighed, immediately frozen on dry ice, and stored at –80°C until analyzed for myeloperoxidase (MPO) and DNA. The right lateral prostate was fixed in 10% neutral buffered formalin for 24 h and then transferred to 70% ethanol for later histological examination.

DNA assay. Total DNA was performed by a modification of the method of Labarca and Paigen (1980). The left lateral prostates were homogenized with an Ultra-turrax T25 (IKA-Labortechnik, Staufen, Germany) in a final volume of 10 ml, with a phosphate buffer containing 0.05-M sodium phosphate and 2-M sodium chloride at a pH of 7.4. Samples were vortexed for 5 min and centrifuged at 1260 g for 15 min. A 600-μl sample of the supernate was removed and appropriate aliquots (50–200 μl depending on visual inflammation score) were brought to a final volume of 4 ml with DNA assay buffer containing 0.8 μg bisbenzimide (Sigma, St. Louis, MO). Samples were then read on a DyNA Quant 200 fluorometer (Hoefer Pharmacia, Inc., San Francisco, CA) at an excitation wavelength of 350 nm and an emission wavelength of 456 nm. Calf thymus DNA standards (Sigma), ranging from 0–9.6 μg/tube, were used to generate a standard curve for determination of unknown sample amounts.

Myeloperoxidase assay. A myeloperoxidase (MPO) assay was performed on all left lateral prostates. Myeloperoxidase is an enzyme secreted by neutrophils, and is also found at lower concentrations in monocytes and macrophages. This enzyme catalyzes the oxidation of electron donors by hydrogen peroxide. The myeloperoxidase activity from this assay is directly proportional to the number of neutrophils seen in histologic sections (Krawisz et al., 1984). It has advantages over histological examination in that it provides a readily quantifiable assessment of neutrophil infiltration at the whole-organ level.

Once the sample of supernate was taken for the DNA assay as described above, 600 μl of 8.33% hexadecyltrimethylammonium bromide (HTAB) buffer (50 mM sodium phosphate, pH 5.4) were added to the tube containing the remaining DNA supernatant and the pellet cell fragments, followed by vortexing for 2 min. HTAB is a detergent that releases MPO from the primary granules of the neutrophil (Schultz and Kaminker, 1962). The suspension was then centrifuged at 1260 g for 15 min and 1 ml of the supernate was diluted with 1.5 ml of 0.5% HTAB. Two hundred μl of the diluted supernatant was brought up to 1580 μl with 100-mM sodium phosphate buffer. Two hundred μl of 16-mM 3,3′,5,5′-tetramethyl-benzidine (Sigma, St. Louis, MO) and 20 μl of 30-mM hydrogen peroxide solution (Sigma, St. Louis, MO) were added to the sample in a cuvette and read at a wavelength of 655 nm on a Beckman DU 650 Spectrophotometer for 2 min at 30-s intervals. The rate was then used to calculate the final concentration of MPO (MPO units per mg) in each sample. The small amount of MPO activity present in the 600-μl aliquot removed for DNA determinations was similarly assayed and added to the amount found in the residual supernatant/cell-debris fraction, to calculate a total MPO/rng tissue.

Histology. The right lateral prostate was placed in 10% formalin at necropsy and 24 h later was transferred to a 70% ethanol solution. The tissue was then submitted to Experimental Pathology Laboratory (EPL), Inc. for processing and histopathological examination. Following paraffin embedding, the first complete 10-μm section from the flat (urethral) side of the prostate tissue was stained with hematoxylin and eosin for evaluation. The sections were examined and scored for the relative degree of severity of inflammatory, degenerative, and proliferative changes and graded on a scale from 1 to 5 (1 = minimal, 2 = slight/mild, 3 = moderate, 4 = severe, 5 = severe/high). Cellular infiltration in the prostate was separated by cell type (mononuclear or polymorphonuclear) as well as location (interstitial or luminal).

Statistics. Tissue weights and prolactin and DNA data were analyzed for statistical significance by analysis of variance (ANOVA), and comparisons among individual treatment conditions within each time period were further examined by Dunnett’s t test for multiple comparisons using INSTAT (San Diego, CA). The incidence of inflammation in the right lateral prostates examined by histology and MPO assay was analyzed using Fisher’s Exact Test of Probability. The severity of prostate inflammation, with comparisons of treatment with control, was analyzed using Welch’s alternate t-test.

RESULTS

Suckling-Induced Prolactin Release

Figure 1 (top) shows the suckling-induced prolactin release in ATR-treated dams on PND 3. Baseline levels of PRL (B60–A0) in the treated dams were not different from controls. Fifty mg/kg ATR completely inhibited suckling-induced prolactin secretion (p < 0.01). In the 12.5- and 25-mg/kg group, the effects on the suckling-induced prolactin surge showed female-to-female variation. In the 25-mg/kg group, the suckling-induced surge was completely suppressed in 60% of the dams, while the remaining 40% had PRL levels well within the control range. In the 12.5-mg/kg group, the PRL surge was
blocked in 40% of the dams, while the others had normal PRL surges. Therefore, there appeared to be an all-or-none effect in these atrazine-treated dams. Still, comparing the overall means of the 12.5- and 25-mg/kg ATR groups with control, serum PRL levels observed during suckling were significantly reduced ($p < 0.05$). The 6-mg/kg dose of ATR was without effect on the dams’ serum PRL concentrations.

Figure 1 (bottom) also shows suckling-induced prolactin release in the bromocriptine-treated dams. Doses of 0.417, 0.208, and 0.104 mg/kg of bromocriptine completely suppressed suckling-induced PRL release ($p < 0.05$), while PRL levels in the 0.052-mg/kg group were not significantly different from controls. Only the 0.104- and the 0.417-mg/kg bromocriptine groups were used in the subsequent experiments that examined the prostates at 120 days.

The mean body weights of the offspring of the ATR- or BROM-treated dams prior to the suckling period were not significantly different from the controls. All of the treatment-group dams displayed normal nursing behavior and all of the pups nursed constantly during the 45-min suckling period. Following that, there was a dose-related trend for a decrease in mean weight gain per litter in the offspring of the high dose atrazine- and high dose bromocriptine-treated dams as compared to the controls; however this difference was not statistically significant (data not shown). At 29 days of age, the body weights of treated and control litters were similar.

Prostate Examinations

Weights. There were no differences in body and tissue weights at 90 days-of-age or body weights at 120 days of age in any of the treated dams’ offspring when compared to controls.
Table 1 shows tissue weights for the 120-day-old males. ATR or BROM affected none of these measures with the sole exception of the ventral prostates of the 6.25-mg/kg ATR males, which were significantly greater than those of controls. However, when analyzed as a percent of body weight, this difference in the 6.25 mg/kg ATR group was no longer significant. Treating the dams with oPRL on PND 1–4 resulted in a significant increase in the ventral and lateral prostate weights compared to control males. It is interesting to note that the mean ventral prostate weight of the offspring in the oPRL plus ATR-50 mg/kg groups was similar to controls, yet was significantly smaller than those from the oPRL-treatment-only group. There was a significant increase in absolute, but not relative, testis weights in the ATR 50-oPrl group when compared to the appropriate control.

Table 1: Accessory Tissue Weights and Prolactin Levels at 120 Days of Age

<table>
<thead>
<tr>
<th></th>
<th>VP (g)</th>
<th>LP (g)</th>
<th>Testis (g)</th>
<th>sPRL (ng/ml)</th>
<th>pPRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND 1–4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.580 ± 0.021</td>
<td>0.099 ± 0.003</td>
<td>1.807 ± 0.033</td>
<td>4.440 ± 0.670</td>
<td>2.781 ± 0.126</td>
</tr>
<tr>
<td>ATR 6.25</td>
<td>0.675 ± 0.029*</td>
<td>0.094 ± 0.003</td>
<td>1.779 ± 0.089</td>
<td>2.936 ± 0.777</td>
<td>2.707 ± 0.224</td>
</tr>
<tr>
<td>ATR 12.5</td>
<td>0.581 ± 0.029</td>
<td>0.108 ± 0.004</td>
<td>1.759 ± 0.022</td>
<td>5.367 ± 0.844</td>
<td>3.507 ± 0.247</td>
</tr>
<tr>
<td>ATR 25</td>
<td>0.592 ± 0.020</td>
<td>0.100 ± 0.005</td>
<td>1.781 ± 0.027</td>
<td>4.452 ± 1.216</td>
<td>2.850 ± 0.147</td>
</tr>
<tr>
<td>ATR 50</td>
<td>0.577 ± 0.024</td>
<td>0.101 ± 0.004</td>
<td>1.885 ± 0.029</td>
<td>4.020 ± 0.551</td>
<td>2.341 ± 0.180</td>
</tr>
<tr>
<td>BROM 0.03</td>
<td>0.602 ± 0.055</td>
<td>0.089 ± 0.005</td>
<td>1.785 ± 0.085</td>
<td>4.779 ± 2.04</td>
<td>2.766 ± 0.624</td>
</tr>
<tr>
<td>BROM 0.125</td>
<td>0.636 ± 0.029</td>
<td>0.095 ± 0.006</td>
<td>1.694 ± 0.039</td>
<td>3.452 ± 0.620</td>
<td>2.418 ± 0.168</td>
</tr>
<tr>
<td>oPRL</td>
<td>0.684 ± 0.036*</td>
<td>0.116 ± 0.007*</td>
<td>1.851 ± 0.037</td>
<td>3.524 ± 1.07</td>
<td>2.654 ± 0.125</td>
</tr>
<tr>
<td>ATR25+oPRL</td>
<td>0.618 ± 0.031</td>
<td>0.108 ± 0.006</td>
<td>1.872 ± 0.039</td>
<td>2.862 ± 0.724</td>
<td>2.839 ± 0.321</td>
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<tr>
<td>ATR50+oPRL</td>
<td>0.561 ± 0.027***</td>
<td>0.100 ± 0.004</td>
<td>1.936 ± 0.035*</td>
<td>0.572 ± 0.109**</td>
<td>2.654 ± 0.185</td>
</tr>
<tr>
<td>PND 6–9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.610 ± 0.027</td>
<td>0.104 ± 0.003</td>
<td>1.814 ± 0.026</td>
<td>3.618 ± 0.509</td>
<td>2.541 ± 0.134</td>
</tr>
<tr>
<td>ATR 25</td>
<td>0.603 ± 0.029</td>
<td>0.110 ± 0.005</td>
<td>1.902 ± 0.042</td>
<td>3.495 ± 0.463</td>
<td>3.025 ± 0.211</td>
</tr>
<tr>
<td>ATR 50</td>
<td>0.605 ± 0.025</td>
<td>0.107 ± 0.004</td>
<td>1.812 ± 0.028</td>
<td>2.774 ± 0.560</td>
<td>2.745 ± 0.156</td>
</tr>
<tr>
<td>PND 11–14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.598 ± 0.027</td>
<td>0.098 ± 0.005</td>
<td>1.884 ± 0.014</td>
<td>7.093 ± 2.50</td>
<td>2.812 ± 0.137</td>
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<tr>
<td>ATR 25</td>
<td>0.584 ± 0.019</td>
<td>0.094 ± 0.004</td>
<td>1.962 ± 0.044</td>
<td>2.626 ± 1.53</td>
<td>2.456 ± 0.166</td>
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<tr>
<td>ATR 50</td>
<td>0.640 ± 0.018</td>
<td>0.101 ± 0.006</td>
<td>1.770 ± 0.044</td>
<td>2.718 ± 0.87</td>
<td>2.832 ± 0.191</td>
</tr>
</tbody>
</table>

Note. Values are mean ± SEM. sPRL = serum prolactin, pPRL = pituitary prolactin.  
* p < 0.05 when compared to control in each dosing interval; ** p < 0.01.  
*** p < 0.05 as compared to oPRL group.

(data not shown). Table 1 shows tissue weights for the 120-day-old males. ATR or BROM affected none of these measures with the sole exception of the ventral prostates of the 6.25-mg/kg ATR males, which were significantly greater than those of controls. However, when analyzed as a percent of body weight, this difference in the 6.25 mg/kg ATR group was no longer significant. Treating the dams with oPRL on PND 1–4 resulted in a significant increase in the ventral and lateral prostate weights compared to control males. It is interesting to note that the mean ventral prostate weight of the offspring in the oPRL plus ATR-50 mg/kg groups was similar to controls, yet was significantly smaller than those from the oPRL-treatment-only group. There was a significant increase in absolute, but not relative, testis weights in the ATR 50-oPrl group when compared to the appropriate control.
The results of the present study demonstrate that a brief postnatal toxicant exposure to the rat dam during the first 4 days of lactation leads to an increased incidence of prostate inflammation in the male offspring. Although many studies have examined the effects of steroidal compounds on the development of the male sex accessory organs, to our knowledge this is the first observation that non-steroidal compounds produced identifiable pathologic changes in the prostate. Three observations support our hypothesis that there is a functional link between the decreased amount of prolactin in the treated dams’ milk during early lactation and the increased incidence of lateral prostate inflammation in the adult offspring: (1)
prolactin in the serum of the dam was suppressed by the same doses of atrazine and bromocriptine that were shown to induce inflammation; (2) doses of atrazine and bromocriptine administered to the dam were effective only during the first 4 days of lactation; and (3) cotreatment of ovine prolactin reduced the incidence of inflammation observed in the offspring to control level. Therefore, we conclude that a decrease in maternal prolactin during early lactation resulted in an alteration in the offspring that increased its susceptibility for lateral prostate inflammation.

Although prolactin has been found in the milk of several species including cows, goats, sheep (Malven and McMurry, 1974), rats (Grosvenor and Whitworth, 1976), and humans (Gala et al., 1975), there are relatively few studies examining the role of this milk-derived hormone on the developing offspring. Although many have viewed milk hormones simply as

FIG. 4. Lateral prostate of a representative control offspring at 120 days of age (upper panel) and a representative lateral prostate of an atrazine-50 offspring at 120 days of age (lower panel) whose mothers were dosed on PND 1–4. No inflammatory cells are observed in the control, and both neutrophils in the lumen and mononuclear cells in the stromal areas were observed in the atrazine-50 offspring. Bar on bottom right represents 30 μm.
byproducts of milk secretion, others have suggested that their abundance in early milk, in both humans and rodents, may be important in the development of the functionally immature neonatal organ systems (Ellis et al., 1997; Nagasawa, 1991; Polk, 1992). Several studies have suggested that milk PRL is involved in the development of the neonatal immune system (Ellis et al., 1997; Gala and Shevach, 1993; Grove et al., 1991). Neonates ingesting PRL-poor milk in early lactation had reduced splenocyte numbers on PND 5 (Gala and Shevach, 1993), while proliferation of splenocytes and thymocytes increased on PND10 of life (Grove et al., 1991). Others have suggested a role for milk-derived prolactin in the postnatal development of the TIDA neuronal system in the hypothalamus of rodents (Shyr et al., 1986). These studies have described PND 1–5 as the sensitive or critical period for TIDA neuronal development and correlated a decrease in milk-derived PRL with hyperprolactinemia in the offspring when measured at 30–35 days of age.

This type of increased prolactin secretion in the offspring due to abnormal TIDA development may be related to the effects on the prostate seen in the present study, since other studies using adult male rats have shown that periods of hyperprolactinemia, induced with either estradiol or ovine prolactin, can result in lateral prostate inflammation (Robinette, 1988; Tangbanluekal and Robinette, 1993). The ability of estradiol to induce inflammation was reversed if the rats were simultaneously treated with bromocriptine. They also demonstrated that the severity of inflammation paralleled increasing doses of oPRL. The inflammation induced by increases in PRL in the adult rat appeared to be similar to the spontaneous prostatitis seen in the lateral prostate of aging Lewis, Copenhagen, and Wistar rats (Aumüller et al., 1987; Müntzing et al., 1979). In addition, this type of inflammation has been shown to precede fibromuscular growth (Bunning et al., 1986) and the synthesis of collagen (Chen-Kiang et al., 1978). Inflammation is noted frequently in human BPH specimens, with one study reporting inflammation in 98% of the specimens analyzed (Kohnen and Drach, 1979), but no correlation has been made between the inflammatory cells and the stromal proliferation.

The type of lateral prostate inflammation that we observed histologically in the 12.5-, 25-, and 50-mg/kg atrazine and high-dose bromocriptine groups of offspring at 120 days of age was similar to that seen in the estradiol and oPRL-induced adult model. It was distinguished by a focal polymorphonuclear leukocyte (neutrophil) infiltrate in the lumen and focal mononuclear cells located in the fibromuscular stromal areas, which typically indicates more chronic inflammatory cells including lymphocytes, plasma cells, and macrophages. The MPO activity in these groups was increased markedly corresponding to an increased neutrophil infiltration in the lumen of the lateral prostate glands.

There was a strong trend for an increase in total DNA and

### TABLE 2

<table>
<thead>
<tr>
<th>Location and type of inflammation</th>
<th>Control</th>
<th>ATR 25</th>
<th>ATR 50</th>
<th>BROM 0.417</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, M, F</td>
<td>11.9</td>
<td>37.0*</td>
<td>32.1*</td>
<td>28.6*</td>
</tr>
<tr>
<td></td>
<td>(1.2)</td>
<td>(1.3)</td>
<td>(1.5)</td>
<td>(1.5)</td>
</tr>
<tr>
<td>L, P, F</td>
<td>16.7</td>
<td>33.3*</td>
<td>42.9*</td>
<td>35.7*</td>
</tr>
<tr>
<td></td>
<td>(1.2)</td>
<td>(2.1)*</td>
<td>(1.7)</td>
<td>(1.6)</td>
</tr>
</tbody>
</table>

Note. Percent of prostates with inflammation type indicated. Number in parenthesis shows severity of inflammation: 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, and 5 = severe/high. I, Interstitial; L, Lumenal; M, Mononuclear; P, Polymorphonuclear; and F, Focal.

* p < 0.05 when compared to control.

*FIG. 5.* Incidence of lateral-prostate inflammation at 120 days of age in offspring of dams treated with vehicle or 25 or 50 mg/kg of atrazine with and without oPRL on PND 1–4; **p < 0.05 when compared to treatment control.

*FIG. 6.* Incidence of lateral prostate inflammation at 120 days of age in offspring of dams treated with 25 or 50 mg/kg of atrazine on PND 1–4, 6–9, or 11–14. **p < 0.05 when compared to control correlating with days of dosing.
FIG. 7. Mean Total DNA (μg DNA/left lateral prostate) in lateral prostates at 120 days of age in the atrazine and bromocriptine groups (upper panels). Values represent mean ± SEM; **p < 0.01 as compared to control. Mean DNA concentration (μg DNA/mg wet tissue weight) of lateral prostate tissue at 120 days of age in the atrazine and bromocriptine groups (lower panels). Values represent mean ± SEM.
DNA/mg of lateral prostate tissue in the 25- and 50-mg/kg ATR groups and the 0.417-mg/kg bromocriptine group at 120 days of age, which reached statistical significance at the 25-mg/kg ATR dose. This effect correlated well with the effects on lateral prostate inflammation that we observed at the same doses at 120 days of age, since an increase in the neutrophil and lymphocytic infiltrate would cause a concurrent increase in the DNA levels.

From the current results, the critical period for the effects of a suppression of maternal prolactin secretion on the adult offspring’s prostate appears to be confined to PND 1-9 in the rat, with the most sensitive period being PND 1–4. Significant inflammation was observed when the dams were dosed with 25 or 50 mg/kg ATR on PND 1-4, but there was also an increase in lateral prostate inflammation in the 25-mg/kg ATR group when dams were dosed from PND 6–9 that approached statistical significance. We did not examine ATR or BROM exposure throughout PND 1–9, but it is possible that such prolonged exposure may have resulted in a greater incidence and/or severity of prostate inflammation. It is not understood why the 50-mg/kg atrazine group only showed a small incidence of prostatitis, which was comparable to controls when dosed PND 6-9. In contrast, both doses of atrazine treatment to the dams from PND 11-14 were without effect on the offspring prostate. It is interesting that a similar time course was noted by Shah et al. (1989) for the role of PRL in the development of the TIDA neurons in which BROM treatment to the dam on PND 2–5 led to hyperprolactinemia, but treatment on PND 9–12 was without effect. Both of these observations take place during a period in which the offspring are able to absorb prolactin from the intestines, prior to PND 12 in rat pups (Clarke and Hardy, 1969; Whitworth and Grosvenor, 1978), and correlate with the organization of the TIDA neurons during the first week postnatally in the rat (Loizou, 1971; Ojeda and McCann, 1974).

Lateral prostate inflammation was absent in the offspring from dams receiving 25 and 50 mg/kg of atrazine in combination with ovine prolactin on PND 1–4. This observation further suggests that the effects of atrazine on PND 1–4 on offspring prostate is indirect and somehow mediated by the decrease of maternal prolactin release, rather than being a direct action of atrazine on the pup prostates. Curiously, the group of dams receiving only oPrl showed an increased incidence of lateral prostate inflammation at 120 days of age (in approximately 59% of the offspring tested from 5 litters). We assume that these females had normal suckling-induced prolactin surges. Thus, the additional prolactin may have been additive with the dam’s own prolactin to somehow affect the male offspring’s TIDA development and prolactin release, which then affected the prostate weight and inflammation in adulthood. The proper amount of prolactin may be required for the TIDA neurons, in which too little or too much during this critical period affects their normal development. It is also possible that the oPRL stimulated an upregulation of DA receptors or DA release, and thus suppressed the dam’s normal prolactin release. The mechanism of this effect of oPRL will have to be determined.

In addition to inflammation, there was also an increase in ventral and lateral prostate weights in the oPRL group. This increase could also be attributed to additional prolactin in the maternal milk above the normal suckling-induced prolactin release and thus, an additional amount transferred to the pups. Many reports have described the tropic effects of prolactin on the prostate (Grayhack, 1963; Negro-Vilar et al., 1977). In the immature rodent, prolactin plays a role in the development of the prostate independent of androgens, while in the adult the trophic effects of prolactin appear to become more tissue-specific, in that the lateral prostate is selectively responsive (Grayhack and Lebowitz, 1967; Prins and Lee, 1982a,b). Therefore, this could explain why we see changes in both the ventral and lateral tissues in adulthood, with lactational exposure to oPRL, but this remains to be determined.

Although we saw no differences in the weights of the lateral prostate at 90 or 120 days of age in our other treatment groups, a number of studies have reported a direct PRL stimulation of the growth of the lateral prostate (Kolbusz and Grayhack, 1982; Prins, 1987), in the adult animal. One study did report a biphasic effect of prolactin on the growth of the lateral prostate, with low to moderate elevations in prolactin having a trophic effect, which diminishes with higher levels of the hormone (Prins and Lee, 1983). However, these studies were conducted in adult animals and did not examine the possible effects of transient periods of increased prolactin levels during developmental periods, such as puberty, on prostate growth. This possibility cannot be addressed until the exact sequelae of changes in the offspring prolactin regulation resulting from suppression of the dam’s prolactin release are identified.

Recently, we have shown that a transient prepubertal increase in prolactin induced by administration of pimozide, a dopamine receptor antagonist, was correlated with lateral prostate inflammation at 120 days in over 80% of the males (Stoker et al., 1999). Also, it has been observed that once an adult rat is made chronically hyperprolactinemic with an estradiol implant, the lateral prostate inflammation that is induced cannot be reversed by removing the implant or by normalizing prolactin levels with bromocriptine (Robinette, unpublished data). This result would imply that once the inflammatory reaction is triggered in response to prolactin it continues, even if the prolactin levels in the neonate return to normal. Studies are currently in progress to determine the chronology of any changes in prolactin concentrations present in the male offspring of the high-dose, atrazine-treated dams (exposed on PND 1–4) to assess the correlation between prolactin levels and the lateral prostate inflammation at 120 days. In addition, the long-term effects on the offspring need to be examined beyond 120 days of age.

If there is a transient period of hyperprolactinemia in the offspring, there also exists the possibility that such an alteration prior to or during puberty could be advancing the age-
related changes associated with the spontaneous development of prostatitis in this strain of rats. As mentioned earlier, Wistar rats spontaneously develop a 27% incidence of prostatitis between 10 and 13 months of age (Naslund et al., 1988).

It is also possible that the inflammatory responses seen in the lateral prostate of the adult rat in this study are related to an altered immune function due to milk prolactin deficiency on PND 1–4. As mentioned previously, a milk prolactin deficiency can have a lasting influence on immune function, and studies have shown that hyperprolactinemia or manipulation of prolactin in adults can either enhance or suppress humoral and cellular immune responses (Berczi, 1986). Also, it has been shown that the bro-mocriptine-induced milk prolactin deprivation on PND 2–5 resulted in an enhanced mitogen responsiveness and altered expression of cell-surface antigens in the offspring on days 10 and 15, but not on day 21 following intestinal closure to absorption of macromolecules (Grove et al., 1991).

In summary, the results of the present study demonstrate that a brief postnatal exposure to atrazine during the first 4 days of lactation suppressed maternal suckling-induced prolactin release and led to an increased incidence of lateral prostate inflammation in the adult offspring. The effects of this exposure to the dam were reversed by administration of exogenous ovine prolactin to the dam. This supports the hypothesis that decreased maternal prolactin results in the development of prostate inflammation in the offspring. The critical period for the effects of this suppressed maternal prolactin appeared to be confined to PND 1–9, with PND 1–4 being the most sensitive. Since the rat’s dorsal and lateral prostates are said to be the most homologous to the human prostate (Price, 1963) and the lateral prostate is most sensitive to prolactin, the rat is an appropriate model for studying effects of prolactin on the prostate. Since humans are exposed to atrazine in surface drinking water and by dietary exposure (Simoneaux et al., 1998), the effects of such chemicals on the hormones normally present in the milk and subsequent effects on the offspring need to be determined. It is thought that the development of the TIDA neurons takes place during late gestation in the human, yet it remains controversial whether hormonal proteins found in human milk may influence developmental functions in the offspring (Grosvenor et al., 1992; Polk, 1992). If the TIDA development does occur during late gestation, proteins such as prolactin in the milk would be most important in human infants born premature, before such systems have fully developed.

ACKNOWLEDGMENTS

The authors express their gratitude to the National Hormone and Pituitary Agency for the gift of prolactin radioimmunoassay materials. We would also like to thank Keith McElroy, Joy Hein, and Ben Britt for their technical contributions.

REFERENCES


