Effects of Chronic Hyperprolactinemia on Tuberoinfundibular Dopaminergic Neurons (44258)

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Abstract. **Prolactin (PRL) secretion is under the inhibitory regulation of the tuberoinfundibular dopaminergic (TIDA) system. Short-term elevation in PRL levels has been shown to increase the activity of TIDA neurons, however, the responsiveness of TIDA neurons to chronically elevated serum PRL levels is controversial. The purpose of this study was to investigate the effects of prolonged elevations of serum PRL on TIDA neuronal activity. Female Sprague-Dawley rats (2–3 months old) were ovariectomized and implanted (sc) with haloperidol (HAL), a dopamine receptor antagonist for 6 or 9 months to produce hyperprolactinemia. Ovariectomized, sham-implanted rats were used as controls. Other groups of intact rats were implanted with HAL or sham-implanted for 9 months and then were implanted with PRL-producing MMQ cells for 6 weeks to further increase circulating PRL levels. TIDA neuronal activity was measured in terms of tyrosine hydroxylase (TH) activity in the stalk-median eminence and was correlated with changes in serum PRL levels. After 6 months of treatment, TH activity in HAL-treated rats was 130% higher than that in the control rats. After 9 months of treatment, TH activity in HAL-treated rats was 81% higher than that in control rats. This increase was significantly less than the increase that occurred after 6 months of treatment. Nine months of HAL-induced hyperprolactinemia followed by implantation of PRL-producing MMQ cells, which resulted in very high levels of PRL, did not increase TH activity in the stalk-median eminence. These results demonstrate that hyperprolactinemia over a prolonged period reduces the responsiveness of TIDA neurons, and these effects vary depending on the duration and intensity of hyperprolactinemia.** [P.S.E.B.M. 1998, Vol 217]

Dopamine (DA) is one of the major neurotransmitters affected by aging. Recently, we reported that the release of DA in the medial basal hypothalamus decreases with advancing age (1). In the arcuate nucleus of old animals the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, and TH messenger RNA levels are also markedly decreased (2, 3). The reasons for this age-related decrease in DA activity are not clear. The activity of hypothalamic dopaminergic neurons is markedly influenced by prolactin (PRL) (4, 5). Acute increases in PRL are known to increase DA activity in tuberoinfundibular dopaminergic (TIDA) neurons (6), but the effects of chronic hyperprolactinemia on DA neuronal activity have not been investigated. However, implantation of pituitary tumors that are known to produce large amounts of PRL have been reported to decrease DA activity in TIDA neurons (7, 8).

The purpose of this study was to investigate the effects of prolonged periods and varying intensities of hyperprolactinemia on TIDA neurons in the brain. TIDA neurons have their cell bodies in the arcuate nucleus, and their terminals reach the median eminence (9). TIDA neuronal activity in the stalk-median eminence (SME) was assessed by measuring TH activity after producing hyperprolactinemia by treatment with haloperidol (HAL), a dopamine receptor antagonist, and PRL-secreting MMQ cells.

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Materials and Methods

Cell Culture. PRL-secreting MMQ cells were maintained in culture as described before (10–12). Briefly, RPMI-1640 with 7.5% horse serum and 2.5% fetal bovine serum (FBS) was used to maintain these cells. The medium was also supplemented with 100 U/ml of penicillin and 100 μg/ml of streptomycin. When the cells reached a concentration of 1×10^6 cells/ml, a small fraction of the cells was used to start a fresh culture. The injection of MMQ cells under the kidney capsule is described under treatment.

Animals. Female Sprague-Dawley rats (2–3 months old) (SASCO, Omaha, NE) were housed in temperature-(23° ± 2°C) and light-controlled (lights on between 0500–1900 hr) animal quarters (Kansas State University, Manhattan, KS) and were fed rat chow and water *ad libitum.*

Treatment. Group 1 consisted of rats that were ovariectomized bilaterally through a midventral incision and were implanted subcutaneously with slow-release pellets of haloperidol (HAL) (36 mg, 60-day pellets or 54 mg, 90-day pellets; Innovative Research America, Sarasota, FL) for a period of 6 months. Rats in Group 2 were treated as the rats in Group 1 except that they were exposed to HAL for a period of 9 months. The control rats in both groups were treated identically except they were not implanted with the HAL pellets.

Rats in Group 3 were treated like the rats in Group 2 but were not ovariectomized. Their estrous cycles were monitored periodically by examining vaginal cytology throughout the treatment period. At the end of 9 months of treatment, HAL-treated rats were implanted with PRL-secreting MMQ tumor cells (1×10^6 cells in 50 μl of RPMI-1640 medium) aseptically under the kidney capsule. This was done to increase serum PRL levels further. Control animals received 50 μl of the medium. Animals were maintained under similar conditions for another 6 weeks after the implantation procedure.

Five to six rats from each group were sacrificed by decapitation. Trunk blood was collected, and the serum was separated and stored at −20°C until they were assayed for PRL. The pituitaries were removed, neural and intermediate lobes were separated, and the anterior pituitaries were weighed and homogenized in 1 ml of 50 m*M* Tris HCl. The homogenates were stored at −20°C until determination of PRL concentrations by RIA.

Measurement of Tyrosine Hydroxylase (TH) Activity. At the end of the treatment period, six to eight rats from each group were injected with m-hydroxybenzylhydrazine dihydrochloride (NSD 1015; 100 mg/Kg BW, ip), an L-aromatic amino acid decarboxylase inhibitor, to prevent conversion of L-dopa to dopamine. Then 30 min after administration of NSD 1015, rats were decapitated, and the stalk-median eminence (SME) was dissected with a pair of fine scissors as described previously (6). The SME was homogenized in 60 μl of 0.1 *M* $HClO_4$ and centrifuged in a refrigerated centrifuge at 13,000 RPM for 10 min. The concentration of L-dopa in the supernatant was determined by HPLC with electrochemical detection (HPLC-EC). The pellet was dissolved in 25 μl of 0.5 *N* NaOH followed by 75 μl of PBS and was stored at −20°C until assayed for protein content. The protein content was estimated by the Bradford method using a BioRad (Hercules, CA) protein assay kit.

HPLC-EC. The details of the HPLC-EC procedure have been described previously (13–15). Briefly, homogenates (50-μl) were thawed at 60°C for 1 min, and 25 μl of internal standard (dihydroxybenzylhydrazine) was injected into the HPLC system for determining the concentrations of L-dopa. The HPLC system consisted of a C18, 5 μm particle size, 250-mm long analytical column (Bioanalytical Systems, West Lafayette, IN), that was maintained at a constant temperature of 37°C in a CTO-6A column oven (Shimadzu, Columbia, MD). The mobile phase consisted of monochloroacetic acid (14.15 g/l), octanesulfonic acid (0.3 g/l), EDTA (0.25 g/l), sodium hydroxide (4.675 g/l) and acetonitrile (3.5%). The mobile phase was prepared in pyrogen-free water and was filtered and degassed using a Milli-Q purification system. An LC-6A pump (Shimadzu) pumped the mobile phase at a flow rate of 1.7 ml/min. The LC-4B amperometric detector (Bioanalytical Systems) had a sensitivity of 1 nA full scale, and the potential of the working electrode was 0.65 V with respect to an Ag/AgCl reference electrode. The chromatograms were analyzed using a C-R4A Chromatopac integrator (Shimadzu). The concentration of L-dopa was expressed in terms of pg/μg protein.

Radioimmunoassay. Double antibody RIA was used to determine serum and pituitary PRL concentrations (13, 16). PRL label was obtained from Hazelton (Vienna, VA) and NEN (Boston, MA), and PRL antibody and standards were obtained from NIDDK (Bethesda, MD). The reference preparation, rPRL-RP-3, had a potency of 30 IU/mg. The assay had a sensitivity of less than 10 pg, an interassay variability of 5.1 ± 0.9% and an intraassay variability of 6.9 ± 1.9%. Pituitary PRL concentrations were expressed as μg/mg pituitary weight and serum PRL concentrations in ng/ml.

Statistical Analysis. The data on body weight, pituitary weight, serum PRL, pituitary PRL and TH activity in the SME were analyzed using a one-way analysis of variance followed by Fisher's least significant difference test.

Results

Body Weight and Pituitary Weight. The changes in body weight and pituitary weight (mean ± S.E.) after HAL treatment are shown in Figure 1. Treatment with HAL decreased body weight significantly to 298.7 ± 7.9 after 6 months when compared to the control group (327.9 ± 5.7, $P < 0.05$). A similar reduction in body weight was observed in the HAL-treated group (320.4 ± 8.2) when compared to the control group (356.5 ± 11.7) after 9 months of treatment ($P < 0.05$). MMQ cell implantation for a period of 6 weeks following 9 months of HAL treatment also reduced body

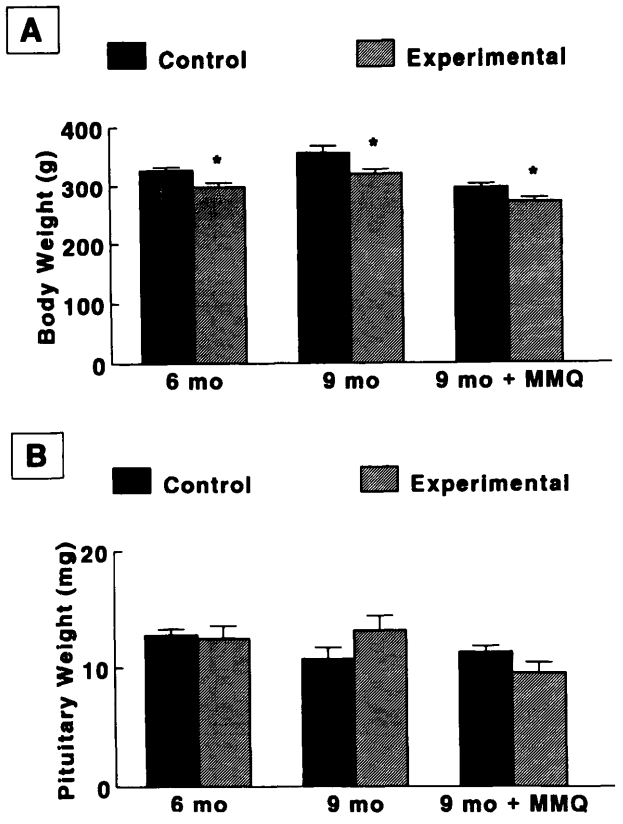

Figure 1. Effects of varying durations and intensities of hyperprolactinemia on body weight and pituitary weight. At the beginning of the treatment, the rats were 3–4 months old. Rats in Group 1 were ovariectomized bilaterally through a midventral incision and were sham-implanted (n = 8) or implanted with haloperidol (HAL) slow-release pellets (n = 7) for a period of 6 months. Rats in Group 2 were treated similarly to those in Group 1 but were treated with HAL pellets for a period of 9 months (n = 6). Rats in Group 3 were not ovariectomized but were sham implanted or implanted with HAL slow-release pellets for a period of 9 months. At the end of 9 months of treatment, they were implanted with 1 × 10^6 prolactin secreting MMQ tumor cells in 50 µl of RPMI-1640 under the kidney capsule to further increase serum prolactin levels (n = 6). Control animals (n = 8) received 50 µl of the medium. Animals were maintained under similar conditions for another 6 weeks after the implantation procedure. *Significantly different (P < 0.05) from their respective controls.

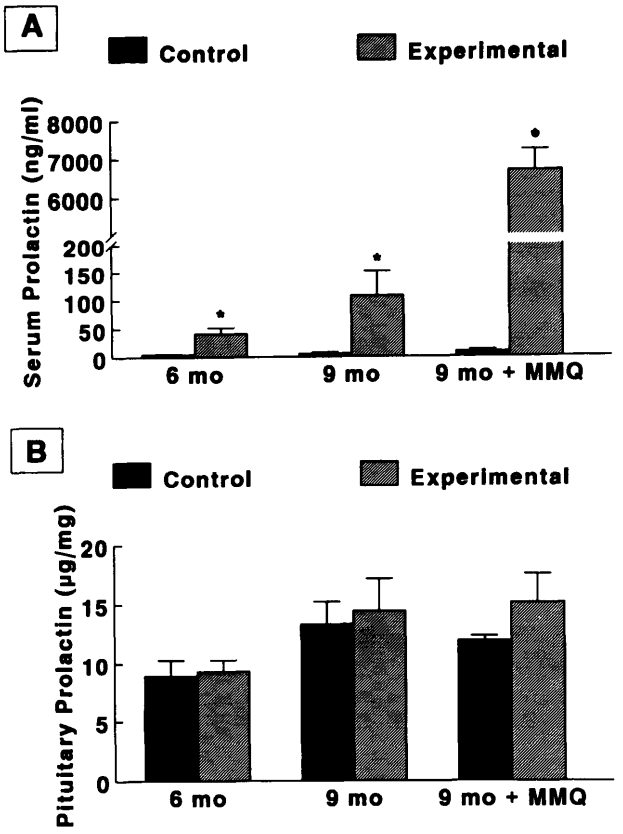

Figure 2. Effects of haloperidol and MMQ cells on serum and pituitary PRL levels. *Significantly different from their respective controls. (See Fig. 1 legend for details).

weight significantly (270.6 ± 7.2) when compared to the control group (296.1 ± 6.6, P < 0.05).

In contrast to its effect on body weight, treatment with HAL for 6 or 9 months did not produce any change in pituitary weight. However, HAL treatment for 9 months followed by exposure to MMQ cells for 6 weeks appeared to decrease pituitary weight (9.5 ± 0.9) when compared to the control group (11.3 ± 0.5). This decrease was not statistically significant (P = 0.06).

Serum and Pituitary PRL. Figure 2 shows the changes in PRL concentrations in the serum (ng/ml) and pituitary (µg/mg). Serum PRL concentrations (mean ± S.E.) increased significantly (41.5 ± 9.7, P < 0.01) when compared to the control group (4.8 ± 1.0) after 6 months of HAL treatment. Similar increases in serum PRL concentrations were observed after 9 months of treatment with HAL (107.2 ± 43.2) when compared to the control group (5.6 ± 2.7, P < 0.01). A dramatic increase in serum PRL concentrations occurred (6674 ± 535) when intact rats were treated for 9 months with haloperidol, and later subjected to MMQ cell implantation, and this was significantly higher (P < 0.001) when compared to the control group (8.4 ± 3.6). In contrast to serum PRL levels, there were no significant changes in pituitary PRL concentrations when rats were subjected to different durations and intensities of hyperprolactinemia.

TH Activity. TH activity (L-dopa pg/µg protein; mean ± S.E.) in the SME of various groups is shown in Figure 3. TH activity increased significantly after 6 months

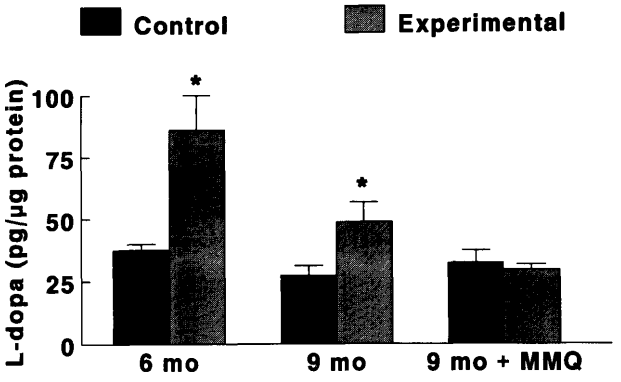

Figure 3. Effects of hyperprolactinemia on TH activity in the stalk median eminence. *Significantly different from the respective control groups and other experimental groups (P < 0.05). (See Fig. 1 legend for details).

of treatment with HAL (86.0 ± 14) when compared to the control group (37.5 ± 2.5) ($P < 0.01$). Similarly, after 9 months of treatment with HAL, TH activity in the SME was significantly different (48.6 ± 8) from that of the control group (27.2 ± 4). However, this increase was significantly less when compared to that after 6 months of treatment. In contrast, treatment with HAL for 9 months followed by exposure to MMQ cells for 6 weeks did not produce any change in TH activity.

Estrous Cycle. Estrous cycles were monitored several times during the treatment period in intact rats (Group 3). Two months after treatment, 75% of the control animals were regular cyclers, the remaining were irregular cyclers. In contrast, in the experimental group, 23% of the rats were regular cyclers, 50% were irregular cyclers, and 27% were in persistent diestrus. When estrous cycles were monitored again, about 7 months after the beginning of treatment, 42% in the control group were regular cyclers, 4% were irregular cyclers, 25% were in persistent diestrus, and 29% were in persistent estrus. In the experimental group, 27% were irregular cyclers, 73% were in persistent diestrus, and there were no regular cyclers. When the estrous cycles were monitored again near the end of the treatment period, a similar profile was observed in control and experimental rats.

Discussion

These results demonstrate that moderate hyperprolactinemia maintained over increasing periods of time gradually decreases the responsiveness of TIDA neurons to PRL. Exposure to moderate hyperprolactinemia for a period of 9 months followed by exposure to very high levels of serum PRL produced by PRL-secreting MMQ cells, resulted in total loss of responsiveness of TIDA neurons to PRL.

PRL is known to be under the inhibitory control of DA. DA released from the terminals of TIDA neurons whose cell bodies are located in the arcuate nucleus is known to inhibit the secretion of PRL from the anterior pituitary (4, 5). Changes in the levels of serum PRL, in turn, are known to regulate the activity of TIDA neurons. In this study, we assessed the activity of TIDA neurons by measuring the activity of TH, the rate-limiting enzyme involved in the biosynthesis of DA. This has been used as a reliable indicator of dopaminergic activity in predominantly dopaminergic areas of the mouse and rat brain (17). We have used this method to measure changes in TIDA activity after inducing acute hyperprolactinemia (6). In the present study, we used HAL, which acts on DA receptors located on the pituitary lactotrophs, to block the inhibitory effect of DA on PRL secretion. The hyperprolactinemia thus produced, stimulates the activity of TIDA neurons (18). Therefore, changes observed in TIDA activity in the SME are related to increases in PRL levels induced by HAL rather than due to a direct effect of HAL on TIDA neurons.

Short-term exposure to high PRL levels in young rats has been shown to increase TH activity in the SME (6, 19). In the present study, hyperprolactinemia for a period of 6 months (Group 1, ovariectomized) resulted in a dramatic increase in TH activity in the SME (130%). When hyperprolactinemia was extended for 3 more months, i.e., for a total of 9 months (Group 2, ovariectomized), TH activity in the SME increased significantly by 81% when compared to the control group. However, this increase was significantly less when compared to the increase that resulted after 6 months of hyperprolactinemia. This indicates that the TIDA neurons became less sensitive to high PRL levels as the duration of hyperprolactinemia increases. Nine months of hyperprolactinemia followed by a further increase in PRL levels for 1½ months by implantation of MMQ cells (Group 3, intact), produced no change in TH activity between the experimental and the control groups, indicating that very high levels of PRL over extended periods render the TIDA neurons unresponsive.

The results from the present study provide a mechanism for the age-related decrease observed in hypothalamic dopaminergic activity (1–3, 16) and increases in serum PRL concentrations (1, 3, 4, 16). It is hypothesized that prolonged exposure to increased levels of PRL, as observed in middle-aged animals, reduces the responsiveness of TIDA neurons to PRL. This results in a further increase in PRL levels, which leads to a further reduction in the responsiveness of TIDA neurons to PRL in old age.

There is evidence that HAL-induced hyperprolactinemia increases DA synthesis in the ME (20). There is also evidence that moderate hyperprolactinemia increases TH activity in the TIDA neurons (6). However, there are no studies available on how TH activity is affected under conditions of prolonged and highly intensified hyperprolactinemia as observed in this study. Hyperprolactinemia produced by implantation of PRL-secreting pituitary tumors has been shown to decrease TIDA function (7). In one study, implantation of M-TW15 tumors, which produced marked increases in PRL levels, resulted in increased DA turnover rates in MBH after 2 weeks but decreased DA turnover after 5 and 8 weeks of exposure (8). In a previous study, under similar experimental conditions as used in the present study, we reported a similar effect on DA concentrations in the ME after prolonged exposure to PRL (11).

The effects of HAL treatment on other dopaminergic systems of the brain are not clear. Acute and chronic HAL treatment have been shown to affect DA turnover and release in the striatum and olfactory bulb (21, 22). Chronic HAL treatment has also been shown to decrease DA release in the striatum and nucleus accumbens (23) while in another study it was found to increase striatal dopaminergic activity (24). In one study, chronic HAL treatment did not have any effect on TH mRNA in the substantia nigra (25). In a previous study, we did not find any change in the concentrations of DA or its metabolite dihydroxyphenylacetic acid in the caudate putamen, substantia nigra, medial preoptic area, or zona incerta after prolonged treatment with HAL indi-

cating that the dopaminergic systems in these areas are not affected by HAL treatment (11).

In summary, the results of this study indicate that prolonged moderate hyperprolactinemia followed by extremely high levels of PRL that mimicks the pattern seen in aging female rats (4), causes the TIDA neurons to lose their responsiveness to PRL. Moderate hyperprolactinemia for 6 or 9 months maintains a high level of TIDA activity although the responsiveness decreases as the duration of hyperprolactinemia increases. This suggests that the decrease in TIDA neuronal activity reported in old female rats may be due to prolonged overstimulation by elevated PRL, eventually leading to neuronal exhaustion. The mechanism for this loss remains to be elucidated.

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