Age-Related Changes in Rates of Basal Secretion of Immunoreactive Vasoactive Intestinal Polypeptide and Dopamine into Pituitary Stalk Blood from the Hypothalamus in Anesthetized Male Rats

Harumi Hotta, Hideki Ito,* Koh Matsuda,** Akio Sato,*** and Hideo Tohgi**

Department of Autonomic Nervous System, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo, 173 Japan

Abstract Age-related changes in prolactin (PRL) in systemic blood plasma, and in secretions of hypothalamic vasoactive intestinal polypeptide (VIP), an important candidate for PRL-releasing factor, and dopamine, a PRL-inhibiting factor, into the pituitary stalk blood were investigated. The experiments were performed on male urethane-chloralose-anesthetized Wistar rats of three different ages, i.e., (1) adult rats 5–8 months old, (2) middle-aged rats 12–15 months old, and (3) aged rats 24–26 months old. The concentration of immunoreactive PRL (iPRL) in systemic blood plasma of the aged rats was significantly higher than that of the adult rats ($p < 0.01$). The secretion rate of hypothalamic immunoreactive VIP (iVIP) into the pituitary stalk blood was unchanged during aging, while that of dopamine was markedly increased in the aged rats in comparison with the value in both adult and middle-aged rats ($p < 0.01$). These results indicate that the basal secretion of hypothalamic VIP is well maintained, while that of hypothalamic dopamine is augmented in aged rats with hyperprolactinemia. It can be assumed that the increase in the pituitary PRL secretion is a primary event during aging in rats, and that a high circulating level of PRL may facilitate the hypothalamic dopamine secretion through the activation of a negative feedback system of the hormone.

Key words: vasoactive intestinal polypeptide, dopamine, pituitary stalk blood, aging, rat.

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* Present address: Department of Endocrinology, Tokyo Metropolitan Geriatric Hospital, Itabashi-ku, Tokyo, 173 Japan.
** Present address: Department of Neurology, Iwate Medical University, Morioka, Iwate, 020 Japan.
*** To whom all correspondence should be addressed.
It has been reported that the concentrations of some pituitary hormones in systemic blood plasma change during aging, and those changes have been suggested to depend on age-related alterations in secretory functions of hypothalamic releasing or inhibiting factors (Timiras, 1982). The age-related increase in plasma prolactin (PRL) in both female and male rats has been well established (Simpkins et al., 1977; Meites et al., 1978; Demarest et al., 1980; Gudelsky et al., 1981; Reymond and Porter, 1981; Meites, 1982; Sarkar et al., 1984; Arita and Kimura, 1986). As dopamine is known as a potent hypothalamic PRL-inhibiting factor (Neill, 1980), an age-related decrease in hypothalamic dopamine secretion into the pituitary stalk blood has been suggested to explain the hyperprolactinemia in aged rats. In fact, it has been demonstrated that the concentration of dopamine in the pituitary stalk blood plasma is significantly decreased in both male (Gudelsky et al., 1981) and female (Reymond and Porter, 1981; Sarkar et al., 1984) aged rats. Recently, vasoactive intestinal polypeptide (VIP) has been suggested to be an important candidate for hypothalamic PRL-releasing factor (Kato et al., 1978; Saïd and Porter, 1979; Shimatsu et al., 1981). Therefore, if the age-related increase in plasma PRL depends on age-related changes in the neurosecretory functions of the hypothalamus, it can be assumed that the hypothalamic VIP secretion into the pituitary stalk blood increases during aging. As yet, no study has been reported in which the hypothalamic VIP secretion into the pituitary stalk blood was measured in aged animals.

The present experiment aimed to examine whether the increased PRL concentration in plasma in aged rats is associated with an increase in hypothalamic VIP secretion together with a decrease in hypothalamic dopamine secretion into pituitary stalk blood.

MATERIALS AND METHODS

The experiments were performed on 21 male Wistar rats. The animals were divided into three groups according to their different ages as follows: (1) adult (5–8 months old) rats (n = 7, 360–440 g), (2) middle-aged (12–15 months old) rats (n = 7, 440–550 g) and (3) aged (24–26 months old) rats (n = 7, 410–530 g). All animals were raised at our institute. The room temperature for animals was kept at 22±2°C, and a daily cycle of a 12 h light/12 h dark photoperiod (lights on 08:00–20:00 h) was regularly maintained. Laboratory food (CRF1, Charles River, Tokyo) and water were given to animals ad libitum.

The animals were anesthetized with urethane-chloralose (500 and 50 mg/kg, respectively, intraperitoneally) followed by additional intravenous administration of 10% of the initial dose if necessary to maintain anesthesia at a relatively constant level as judged by the recorded blood pressure. The animals were artificially ventilated through a tracheal cannula to maintain the end-tidal CO₂ at 3.0–4.0%. The core temperature, as monitored in the rectum, was kept at 37–38°C by means of a direct-current heating pad and an infrared lamp. Systemic arterial blood
pressure was continuously measured through an arterial catheter kept in a femoral artery, and the systolic blood pressure was always kept above 90 mmHg by intravenous injection of 4% Ficoll 70 (Pharmacia Fine Chemicals, Sweden) in saline solution when necessary. Animals macroscopically bearing pituitary tumors, which were observed in 3 out of 10 tested aged rats, were excluded from the present study.

Collecting blood samples. The pituitary stalk blood was collected by the method of Porter and Smith (1967). After exposure of the pituitary gland, the animals were kept under resting conditions without any external stimulations for approximately 30 min, and then 0.5 ml of systemic blood was collected from a femoral artery through a previously inserted polyethylene catheter for the measurement of immunoactive PRL (iPRL) in the systemic arterial blood plasma. After the pituitary stalk was transected and the pituitary gland was removed from the fossa, animals were heparinized and a silicone tube (external diameter 1.5 mm, internal diameter 1 mm, Dow Corning, U.S.A.) was placed over the stump of the stalk. The pituitary stalk blood was collected in a tube immersed in an ice bath. The collection of the pituitary stalk blood for the measurements of immunoactive VIP (iVIP) and dopamine was started at least 15 min after the cutting of the pituitary stalk and continued for 60-90 min. The withdrawal speed was adjusted to the flow rate of pituitary stalk blood. Finally, systemic arterial blood of 1 ml was collected through a catheter inserted into a femoral artery to measure the concentrations of iVIP and dopamine in the systemic blood plasma. Fifty microliters of Aprotinin (10,000 KIE/ml, Trasylol, Bayer, F.R.G.) and 2 mg of EDTA per 1 ml of blood was added to each sample of blood. After centrifugation of the blood for 10 min at 10,000 rpm at 4°C, the plasma was stored at -80°C until assay.

Measurement of concentrations of iPRL, iVIP, and dopamine. The concentration of iPRL was measured by the radioimmunoassay method using the kit supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK) of the National Institutes of Health (U.S.A.). The anti-rat PRL-S-9 was used as the antiserum for PRL and the NIADDK-rat PRL-RP-3 was used as the standard preparation. The concentration of iVIP was measured using the radioimmunoassay method previously described (Ito et al., 1987). The anti-VIP serum (Otsuka Pharmaceuticals, Tokyo) was diluted to 1:100,000 and the synthetic porcine VIP (Peptide Institute, Osaka) was used for the standard. The minimum detectable concentration of iVIP in this assay was about 3 pg/ml. The concentration of dopamine was measured by an electrochemical detector (LC-4B, Bioanalytical Systems Inc., U.S.A.) after separation of dopamine from other substances with high-performance liquid chromatography, as described previously (Ito et al., 1984).

Statistical analysis. Results were given as the mean±S.E. and evaluated statistically by means of the Kruskal-Wallis and Turkey test.
RESULTS

1. Concentration of iPRL in systemic blood plasma

First, the concentration of iPRL in systemic blood plasma was measured to confirm the age-related increase in the PRL concentration in these anesthetized rats. The concentrations of iPRL in systemic blood plasma of the three different age groups were as follows: 3.4±0.9 ng/ml (mean±S.E.) in the adult (5–8 months old) rats (n = 7), 15.8±6.5 ng/ml in the middle-aged (12–15 months old) rats (n = 7), and 53.4±24.4 ng/ml in the aged (24–26 months old) rats (n = 7) (Fig. 1). The concentration of iPRL in the middle-aged rats was higher, but not statistically different from the value for the adult rats. The concentration of iPRL in the aged rats was approximately 16 times as high as that of the value in the adult rats (p < 0.01).

2. Concentrations of iVIP and dopamine in pituitary stalk blood plasma and systemic blood plasma

1) Concentration of iVIP. The concentrations of iVIP in the pituitary stalk blood plasma of the three different age groups were as follows: 28.0±5.0 pg/ml in the adult rats (n = 7), 22.7±7.5 pg/ml in the middle-aged rats (n = 7), and 25.5±5.0 pg/ml in the aged rats (n = 7). There were no significant differences among the

![Graph showing concentrations of iPRL](image)

Fig. 1. Concentrations of iPRL in systemic blood plasma of adult (5–8 months old) rats (n = 7; white column), middle-aged (12–15 months old) rats (n = 7; stippled column), and aged (24–26 months old) rats (n = 7; hatched column). Each column and vertical bar shows the mean ± S.E. *shows significant difference between the values in different age groups (p < 0.01).
concentrations of iVIP in the three different age groups.

The concentration of iVIP was much lower in systemic arterial blood plasma than in pituitary stalk blood plasma. The iVIP in systemic blood plasma was below the minimal detectable level for iVIP (<3 pg/ml) in 11 of 21 rats.

2) Concentration of dopamine. The concentrations of dopamine in pituitary stalk blood plasma of the three different age groups were as follows: 1.08±0.30 ng/ml in the adult rats (n = 7), 0.96±0.27 ng/ml in the middle-aged rats (n = 7), and 5.42±1.15 ng/ml in the aged rats (n = 7). The concentration of dopamine in pituitary stalk blood plasma was markedly increased in the aged rats when compared with that in the adult and middle-aged rats (p < 0.01).

The concentrations of dopamine in the systemic arterial blood plasma of the three different age groups were as follows: 0.22±0.08 ng/ml in the adult rats (n = 7), 0.12±0.02 ng/ml in the middle-aged rats (n = 7), and 0.19±0.02 ng/ml in the aged rats (n = 7). The concentration of dopamine in the systemic blood plasma was 4–20% of that in pituitary stalk blood plasma.

3. Flow rate of pituitary stalk blood plasma

The flow rates of pituitary stalk blood plasma of the three different age groups were as follows: 4.3±0.7 μl/min in the adult rats (n = 7), 5.7±1.0 μl/min in the middle-aged rats (n = 7), and 4.2±0.5 μl/min in the aged rats (n = 7). There were no significant differences among the flow rates in the three different age groups.

Systolic arterial blood pressure during collection of pituitary stalk blood was within the normal physiological range in all of the three different age groups, i.e., 125±10 mmHg in the adult rats, 130±6 mmHg in the middle-aged rats, and 122±9 mmHg in the aged rats.

4. Secretion rates of hypothalamic iVIP and dopamine into pituitary stalk blood

1) Secretion rate of iVIP. The secretion rates of hypothalamic iVIP into pituitary stalk blood were calculated from both the concentration in pituitary stalk blood plasma and flow rate of pituitary stalk blood plasma. The secretion rates of iVIP of the three different age groups were as follows: 0.11±0.02 pg/min in the adult rats (n = 7), 0.11±0.03 pg/min in the middle-aged rats (n = 7), and 0.11±0.03 pg/min in the aged rats (n = 7) (Fig. 2A). There were no significant differences among the secretion rates of iVIP in the three different age groups.

2) Secretion rate of dopamine. The secretion rate of hypothalamic dopamine into pituitary stalk blood was calculated from the differences of concentrations of dopamine in pituitary stalk blood plasma and systemic arterial blood plasma, and from the flow rate of pituitary stalk blood plasma. The secretion rates of dopamine in the three different age groups were as follows: 3.2±0.9 pg/min in the adult rats (n = 7), 4.4±1.3 pg/min in the middle-aged rats, and 21.6±4.5 pg/min in the aged rats (Fig. 2B). The hypothalamic dopamine secretion rate in the aged rats was approximately 6.8 and 4.9 times as high as that in the adult and the middle-aged rats, respectively (p < 0.01).
The present result first confirmed the age-related increase in the concentration of iPRL in systemic blood plasma in urethane-chloralose-anesthetized male rats. This increase was observed in the aged (24–26 months old) rats without any apparent tumors of the pituitary gland. The concentration of iPRL in systemic blood plasma in the present study was in the same range of the previous results in conscious (SIMPKINS et al., 1977), ether-anesthetized (DEMAREST et al., 1980), or urethane-anesthetized (GUDELSKY et al., 1981) rats.

As VIP has been suggested to be a candidate of potent hypothalamic PRL-releasing factor, the hypothalamic neurosecretion of VIP into the pituitary stalk blood was expected to increase in the aged rats. However, the present result
demonstrates that the concentration of iVIP in the pituitary stalk blood plasma and the hypothalamic iVIP secretion rate into the pituitary stalk blood remain unchanged in the aged rats. Therefore, the hypothalamic neurosecretion of VIP is not responsible for the hyperprolactinemia in the aged rats. VIP may partially be produced in the pituitary gland per se (ARNAOUT et al., 1986). It has still been undetermined whether or not the VIP production in the pituitary gland increases during aging.

The concentration of dopamine in the pituitary stalk blood plasma and the hypothalamic dopamine secretion into the pituitary stalk blood plasma were increased in the aged rats. This result contradicts the previously reported results that the concentration of dopamine in the pituitary stalk blood plasma decreased with aging in male (GUDELSKY et al., 1981) and female (REYMOND and PORTER, 1981; SARKAR et al., 1984) rats. One of the possible explanations for this contradiction is strain differences. Wistar rats were used in the present study, while Long-Evans rats (GUDELSKY et al., 1981; REYMOND and PORTER, 1981) or Sprague-Dawley rats (SARKAR et al., 1984) were employed in the previous experiments.

The estimation of the hypothalamic dopaminergic neuronal function in aged rats has also been undertaken using other methods such as measuring the dopamine turnover rate, dopamine synthesis rate, or the activity of tyrosine hydroxylase (TH; the rate-limiting enzyme for the dopamine synthesis) within the median eminence or hypothalamus. ESTES and SIMPKINS (1984) described that the dopamine turnover rate in the median eminence is enhanced during aging in Fischer 344 rats, while it declines during aging in Long-Evans rats. The dopamine synthesis rate was reported to decrease (DEMAREST et al., 1980; REYMOND et al., 1984; Arita and Kimura, 1986) and the basal activity of TH measured in vitro was reported to decrease (REYMOND et al., 1984), remain unchanged (ALGERI et al., 1982; Arita and Kimura, 1986) or increase (Reis et al., 1977) with age. The results described by ESTES and SIMPKINS (1984) with Fischer 344 rats and also by REIS et al. (1977) are in accord with the present result, as they suggest that the hypothalamic dopaminergic neuronal function is enhanced in aged rats. It is of note that in spite of the contradiction in the age-related changes in hypothalamic dopaminergic neuronal functions, age-related increase in the concentration of PRL in the systemic blood is quite an invariable phenomenon. Therefore, it is unlikely that the age-related attenuation of the hypothalamic neurosecretion of dopamine is a primary factor for developing the hyperprolactinemia in the aged rats.

The present findings of the lack of change in the hypothalamic iVIP secretion and the increase in the hypothalamic dopamine secretion into the pituitary stalk blood in the aged rats indicate that the age-related increase of the pituitary PRL secretion cannot be explained either by the augmented VIP stimulation or by reduced dopamine inhibition from the hypothalamus. The sensitivity of PRL-secreting cells to the excitatory control by the hypothalamic VIP may increase and/or that to the inhibitory control by the hypothalamic dopamine may greatly decrease with aging. It has been reported that the inhibitory effect of dopamine on
PRL release from cultured pituitary cells is reduced with age (Kochman et al., 1987). The total number of lactotroph cells increases and release of PRL per cell also increases in old female rats (Chuknyiska et al., 1986; Reymond et al., 1989). In addition, it has been suggested that PRL has a feedback action on the hypothalamic dopamine secretion into the pituitary stalk blood (Gudelsky and Porter, 1980; Gonzalez and Porter, 1988). Therefore, our observation of the increase in hypothalamic dopamine secretion into the pituitary stalk blood in aged rats may be explained by an activation of the negative feedback system stimulated by the increase of PRL secretion during aging.

Some other hypothalamic releasing and inhibiting factors (e.g., thyrotropin-releasing hormone, oxytocin, peptide histidine isoleucine, gonadotropin-releasing hormone-associated peptide, etc.) have been known to influence PRL secretion from the pituitary gland. Therefore, it seems to be necessary to extend the scope of investigation to correlate the present results with age-related changes in these other factors.

REFERENCES


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